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RESEARCH ARTICLE

Isolating the role of soil resources, defoliation, and interspecific competition on early establishment of the late successional bunchgrass *Festuca campestris*

Steven Tannas[1], Daniel B. Hewins[1,2], Edward W. Bork[1]

Native grasslands are valued for biodiversity and supporting dormant season grazing, but are prone to invasion. In western Canada, revegetation of *Festuca campestris* grasslands may be hindered by *Poa pratensis*, an invasive grass. To determine the competitive interaction of these species during establishment, two greenhouse experiments were conducted where *F. campestris* seedlings were planted in monocultures or mixtures with *P. pratensis*. The first experiment used equal-aged (3-month old) seedlings of both species, while the second experiment used unequal-aged seedlings (4-month-old *F. campestris* and 2-month-old *P. pratensis*). Seedling performance was measured in response to manipulations of water and nitrogen, defoliation, and plant neighbor. While water and nitrogen reduced the biomass and vegetative reproduction (tillering) of *F. campestris*, exposure to *P. pratensis* most strongly limited the growth of *F. campestris* seedlings regardless of other treatments. More frequent and consistent decreases in *F. campestris* due to *P. pratensis* were observed in older *F. campestris* seedlings than younger seedlings. Defoliation also reduced the growth of *F. campestris*, and the added presence of *P. pratensis* during defoliation further enhanced these reductions in younger, equal-aged bunchgrass seedlings. Overall, these results suggest that when restoring native *F. campestris* grasslands, early establishment may be improved by reducing the negative impacts of *P. pratensis*, and avoiding severe defoliation.

Key words: conservation, grazing, integrated land use, invasive species, northern grassland, *Poa pratensis*, restoration

Implications for Practice

- Abundance of invasive *Poa pratensis* should be reduced prior to revegetation with native *Festuca campestris* grasslands in order to maximize *F. campestris* seedling establishment.
- Favorable soil resources (water and nitrogen) increased *F. campestris* seedling growth, but these benefits were negated by the presence of *P. pratensis* seedlings.
- Severe defoliation of *F. campestris* seedlings during establishment should be avoided.

Introduction

Restoration is often hindered by invasive species (D'Antonio & Meyerson 2002). Invasion success can be linked to resource availability (Huenneke et al. 1990; Burke & Grime 1996), which affects complex facilitative and competitive interactions among plants (Callaway & Walker 1997). The fluctuating resource hypothesis proposes that high resource availability facilitates invasion (Davis et al. 2000), and by temporarily increasing resource availability, disturbance may increase community invasibility. Studies suggest that some invasive species accelerate nutrient cycling (Wedin & Tilman 1990), thereby increasing resources and promoting invasion processes (Ehrenfeld 2003; Allison & Vitousek 2004). If suppression of invasive species is possible under low-resource conditions, controlling resource availability may be a tool in limiting and managing invasions during efforts to restore native plants. The study of invasive species responses to resource levels is therefore needed, particularly in comparison to major endemic plant species.

Invasion of *Poa pratensis* (L.) into disturbed *Festuca campestris* (Rydb.) bunchgrass-dominated grasslands has become a widespread problem during restoration efforts in the Foothills Fescue Natural Subregion of southwestern Alberta, Canada (Adams 2008). To investigate restoration strategies for *F. campestris*, we focused on these two species as both are now common aboveground and in the seed bank of contemporary grasslands of the region, even without mechanical soil disturbance (Willms et al. 1985; Willms & Quinton 1995). Moreover, both species are known to have marked influences on community dynamics (White et al. 2013). Conditions capable of shifting competition between these species include fluctuations in soil nutrients (Huenneke et al. 1990; Martin & Chambers

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Author contributions: ST, EB conceived the work; ST conducted research; ST, EB analyzed data; DBH prepared figures and tables; ST, DBH, EB wrote and edited manuscript.

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2001; Gross et al. 2005), soil moisture (Adams 2008), and defoliation during the growing season (Willms et al. 1985; Willms & Fraser 1992).

Previous work suggests that *P. pratensis*, a fast growing perennial, acts as a driver of environmental change because it is adapted to the rapid capture and use of nutrients, and also benefits from increases in soil nitrogen (N) at the expense of slower growing species (White et al. 2013). As a result, disturbed environments with high available N (Huenneke et al. 1990; Lake & Leishman 2004; Hawkes et al. 2005) may provide species such as *P. pratensis* that thrive under high N (Wilson & Tilman 1991) with a competitive advantage (Alpert et al. 2000). Conversely, reduced N may inhibit *P. pratensis* growth (Martin & Chambers 2001) and subsequently aid slow-growing species such as *F. campestris*. Consequently, restoration of perennial grasslands high in N may first require N immobilization to restore the competitive balance among species (Corbin & D'Antonio 2004). Reducing plant available N through carbon (C) addition has been proven effective (Alpert & Maron 2000) while maintaining complex trophic relationships within the soil. Carbon addition allows slower growing endemic species to be more competitive against invasives (Alpert & Maron 2000; Paschke et al. 2000; Blumenthal et al. 2003). This is accomplished by decreasing the growth rate of invasive species below the growth rate of endemics, thereby enabling the latter to gain a competitive advantage (Corbin & D'Antonio 2004).

Drought appears to favor endemic plants over *P. pratensis* in the Foothills Fescue Natural Subregion region (Adams 2008). Established plants of *F. campestris* are thought to be strong competitors under low moisture, perhaps due to its relatively large root system (Johnston 1961; Adams 2008). In contrast, *P. pratensis* has a shallow, rhizomatous root system that makes this species less able to cope with drought (Bookman & Mack 1982; Dong et al. 2011), and therefore susceptible to competition from deep rooted native bunchgrasses. Differences in moisture adaptation could explain the ability of undisturbed late-seral stands of *F. campestris* to limit *P. pratensis* invasion in long-term grazing exclosures across the region, particularly with intermittent drought (e.g. see Willoughby 1997). The corresponding competitive outcome between juveniles of these two grass species, however, is unknown. Previous research indicates that successful establishment of *Festuca hallii*, a close relative to *F. campestris*, is favored by abundant soil moisture (Romo et al. 1991). Moreover, while defoliation can shift competition in favor of *P. pratensis* within mature grasslands (Trottier 1986), its effect on juvenile plants experiencing competition is poorly understood. Responses of young *P. pratensis* and *F. campestris* to defoliation during recolonization may not be the same as in established grasslands, further complicating restoration.

The goal of this study was to determine the role of growing conditions, defoliation, and competition from *P. pratensis*, on the growth of *F. campestris* seedlings, and to better understand the potential for *F. campestris* re-establishment in disturbed soils. Specific objectives included to (1) assess *F. campestris* growth responses to the presence of neighboring *P. pratensis*, (2) evaluate how changes in soil N and moisture alter interspecific relationships between juvenile *F. campestris* and *P. pratensis* plants, and (3) determine how defoliation, either alone or in combination with growing conditions, affect competitive dynamics between seedlings of these two species. We hypothesized that low concentrations of soil N, decreased soil moisture, and the absence of defoliation will each enhance the competitive ability of juvenile *F. campestris*, particularly when grown with neighboring *P. pratensis*. We also expected that older *F. campestris* seedlings grown with younger *P. pratensis* seedlings would be more resistant to competition compared with *F. campestris* seedlings grown with equal-aged competitors.

Methods

Experimental Design

Testing direct competitive effects is most effective in "mesocosms" (Drake et al. 1996), which allows the examination of individual and multiple factors simultaneously while generating results comparable with field studies. Two 6-month-long greenhouse experiments using either equal-aged (experiment 1) or unequal-aged (experiment 2) competitors of *Poa pratensis* grown with *Festuca campestris* were conducted at the University of Alberta, the first from October 2007 to April 2008, and the second from May 2008 to November 2008. Plants were placed in a greenhouse at 21°C, and exposed to 16 hour of light and 8 hour of dark each day. Treatments included species composition (*F. campestris* alone, hereafter "monoculture"; or *F. campestris* with neighboring *P. pratensis*, hereafter "mixture"), two N levels (ambient or reduced), two watering (W) levels (low or high), and defoliation (D; absence or presence, consisting of cutting to 1 cm). Each experiment had nine replicates of all possible treatment combinations in a completely randomized design.

Seed for both species was obtained from wild populations currently found within fescue grasslands of SW Alberta. *Festuca campestris* and *P. pratensis* seed was initially planted in 8 cm deep by 3 cm wide root trainers and grown in a greenhouse prior to use. Seedlings were watered regularly to near-field capacity but not provided supplemental nutrition. In experiment 1, seedlings of both species were grown for 3 months prior to planting at equal ages. In experiment 2, *F. campestris* and *P. pratensis* were grown for 4 and 2 months, respectively, to evaluate the use of older *F. campestris* seedlings (e.g. as transplants) to aid re-establishment under competition.

Seedlings were transplanted as soil plugs into 24 cm diameter by 13 cm deep pots containing Orthic Black Chernozemic soil (U.S.A. Udic Boroll). Soil was collected from a fallow field near Cremona, Alberta, in the Foothills Fescue region, and had 15.9% organic matter, pH 7.0, and a clay-loam texture (34% sand, 24% silt, 42% clay), with a cation exchange capacity of 24.5 meq/100 g. Soil was weighed into each pot, with weights later used in the watering treatments. Five grass plugs were planted in each pot using one of two configurations. In the first, a single *F. campestris* focal plant was surrounded by four conspecifics (monoculture), and in the second, a single *F. campestris* plant was surrounded by four *P. pratensis* plants (mixture). These configurations facilitated comparison of intra- and interspecific competition on *F. campestris* focal plants, with all other treatments (N, W, and D) added to these configurations in all possible combinations.

Nitrogen reduction was achieved using sucrose additions (hereafter called "N reduction") to the soil (0.012 g C m^{-3} pot^{-1}) just prior to planting. This level of C addition represents 1,500 g C m^{-2} addition in the field, similar to that used to immobilize plant available N in previous studies (Corbin & D'Antonio 2004), and was intended to reduce N for the 6-month duration of each experiment. To evaluate the response of available N following sucrose addition, we quantified soil N monthly in additional (unplanted) pots containing soil with and without C addition, in combination with low and high W treatments. Soil samples were assessed for available NO_3^-–N and NH_4^+–N using spectral absorption after extraction with a 1:5 mixture of soil to 2 M KCl (Maynard & Kalre 1993). In experiment 1 (using 3-month-old *F. campestris* plants and equal-aged *P. pratensis*), the desired effect was achieved of reducing available soil N throughout the 6-month experiment (Fig. 1A). Despite similar results initially in experiment 2 (using 4-month-old *F. campestris* and younger *P. pratensis*), no difference in soil N was detected after 3 months (Fig. 1B). As a result, only N manipulation data from the first 3 months of the second 6-month-long experiment are presented.

Water addition treatments were initially calibrated based on the water-holding capacity of soil. Random soil samples were dried for 3 days at 40°C and weighed to determine dry mass, then saturated with water for 24 hour, and weighed again to determine field capacity. During each experiment, half the pots were watered twice weekly to field capacity (high treatment), while the other half (low treatment) were watered to 50% of field capacity. The low W treatment was intended to create intermittent water stress for plants.

Defoliation of all plants (*Festuca* and *Poa*) to 1 cm height occurred in half the pots 3 months after initial planting (i.e. at the mid-point of the monitoring period). This intensity was intended to mimic a single severe, mid-summer grazing event that is known to alter the abundance of *P. pratensis* and *F. campestris* in fescue grasslands (Willms et al. 1985).

Vegetation Measurements

Sampling of focal *F. campestris* plant response variables included tiller numbers and biomass. An initial tiller number was taken at the time of planting for use as a covariate. Tiller numbers of focal plants were subsequently counted after 3 months of experiment 1, and at 3 and 6 (end-of-trial) months of experiment 2. In addition to quantifying the aboveground biomass that was removed during defoliation after 3 months into each experiment, both aboveground and belowground biomass of each focal plant were measured at the end of each experiment. Shoot biomass in defoliated plants was quantified as the combination of biomass removed at the initial defoliation combined with the final remaining shoot biomass 3 months later. Roots were harvested by carefully washing soil away and gently separating roots of the focal plant from neighbors. All biomass samples were dried at 40°C for 36 hour and weighed, and root and shoot data were used to quantify the root:shoot (R:S) ratio for each focal plant.

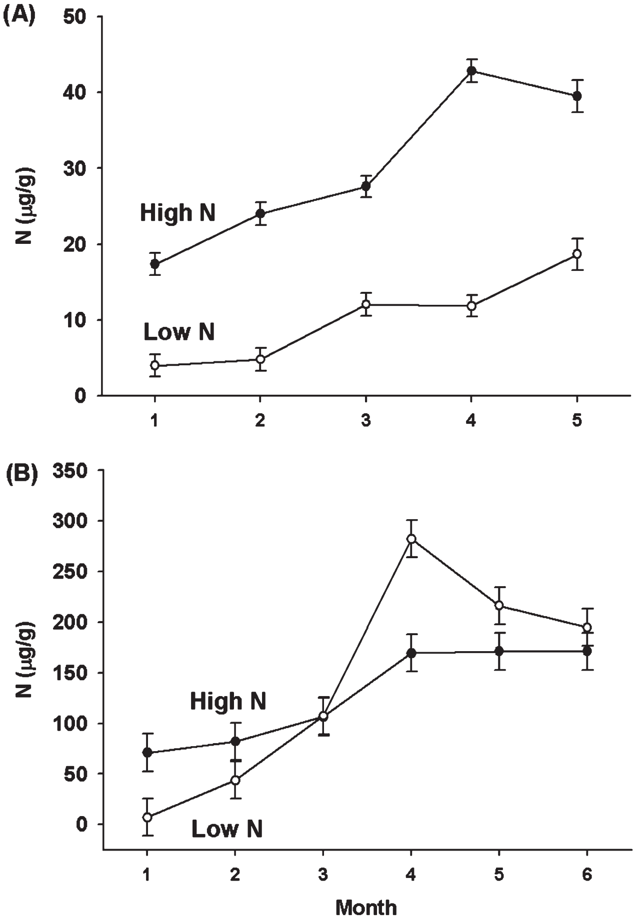

Figure 1. Concentrations of available N (µg/g) in soil exposed to carbon addition (low N) and without carbon addition (ambient N) over a 5-month period in each of (A) the experiment using young, equal-aged *Festuca campestris* and *Poa pratensis* seedlings, and (B) the experiment using older *F. campestris* seedlings grown with younger *P. pratensis* seedlings. Values shown are means with ± SE.

Statistical Analysis

Results of each experiment are presented in the context of *F. campestris* plants grown with either equal-aged *P. pratensis* competitors (i.e. experiment 1; $n = 144$ plants) or younger and unequal-aged *P. pratensis* competitors (i.e. experiment 2; $n = 144$ plants). Treatment effects on *F. campestris* tiller dynamics were analyzed by taking the difference between the initial number of tillers and the last tiller count. Biomass was also analyzed using initial tiller counts as a covariate to adjust for differences in the size of initial seedlings at planting. Statistical analysis was completed using Proc Mixed in Statistical Analysis Software, version 9.2 (SAS Institute, Inc., Cary, NC, U.S.A.), with defoliation (D, presence or absence), neighbor identity (focal plant monoculture or mixture), moisture level (W, high or low) and N (ambient or reduced) treatments as fixed factors, and replicate as random. Significance for main effects and interactions was set at $\alpha = 0.10$ to minimize the risk of a type 2 error (i.e. overlooking a biologically relevant effect), while post hoc mean separation was conducted using least significant difference (LSD) tests and $\alpha = 0.05$. Prior to analysis, data were transformed where needed to meet assumptions of normality and equality of variances. A square root transform was performed on R:S ratios in experiment 1, and on the change in tiller densities [0–3 months, and 0–6 months] and root biomass in experiment 2. Shoot biomass in experiment 2 was natural log transformed. To facilitate interpretation, only non-transformed data are presented.

Results

Response of Young *F. campestris* Seedlings Grown With Equal-Aged *P. pratensis*

Summary analysis of variance (ANOVA) results from experiment 1 are provided in Table 1. Shoot and root biomass were affected by the interaction between neighbor identity and water addition. Shoot and root biomass were greatest in *Festuca campestris* plants grown with conspecifics and high water conditions (Table 2). In contrast, both the presence of *Poa pratensis* and a reduction in water availability reduced above- and belowground biomass of *F. campestris*.

Defoliation interacted with neighbor identity to alter the biomass of focal plant shoots and roots (Table 1). Shoot and root biomass of *F. campestris* seedlings were both lower when these plants were defoliated and simultaneously exposed to competition from similar-aged *P. pratensis* (Table 2). While *F. campestris* shoot and root biomass tended to be lower under exposure to *P. pratensis* in the absence of defoliation, these differences remained non-significant. Similarly, when grown with conspecifics, one-time severe defoliation did not affect root or shoot biomass of *F. campestris* seedlings ($p > 0.05$).

Reduction in soil N led to several significant biomass responses in young *F. campestris* seedlings. While final root biomass increased ($p = 0.01$) under reduced N (from 2.17 ± 0.26 to 3.14 ± 0.25 g/plant), shoot biomass was further affected by the interaction of defoliation and N reduction (Table 1). Shoot biomass decreased to similar levels under exposure to reduced N, defoliation, or the combination of these two treatments (Table 3).

Resulting R:S ratios of young *F. campestris* seedlings were affected by the interaction of moisture and defoliation (Table 1), with low moisture in the absence of defoliation leading to the greatest R:S ratio (Table 3). In general, R:S ratios were low under defoliation regardless of moisture treatment. Additional responses in the R:S ratio of young *F. campestris* plants were observed in relation to the interaction of N treatment and defoliation (Table 1), such that non-defoliated *F. campestris* plants experiencing low N had the greatest R:S ratios (Table 3). Root:shoot ratios of young *F. campestris* were also affected by the interaction of N reduction and watering, which further depended on neighbor identity (Table 1). Regardless of neighbor identity, high moisture and ambient N conditions combined to decrease R:S ratios of *F. campestris* compared with the reduced N treatments with both low and high moisture (Fig. 2). Relative divergence in R:S ratios among N and moisture treatments was also greater in mixed cultures, and exposure to *P. pratensis* generally increased R:S ratios of *F. campestris* compared with that to seedlings grown under the same soil conditions but in monoculture (Fig. 2).

Finally, changes in tiller counts among young *F. campestris* plants during the first 3 months were affected by the interaction between moisture and N (Table 1). Both low moisture and reduced N decreased tiller counts compared with the more

Table 1. ANOVA results for the effect of water, nitrogen levels, neighbor identity, defoliation, and all interactions thereof, on 3-month tiller changes, final shoot biomass, root biomass, and R:S ratios, of young focal *Festuca campestris* seedlings grown with equal-aged competitors. Bolded *p*-values indicate those $p < 0.10$. Initial tiller counts were used as a covariate for all biomass data.

	Change in Tillers		*Shoot Biomass*		*Root Biomass*		*R:S Ratio*	
Source	F *Value*	p > F	F *Value*	p > F	F *Value*	p > F	F *Value*	p > F
Water (W)	5.45	**0.02**	10.12	**0.002**	1.01	0.32	3.62	**0.059**
Nitrogen (N)	9.5	**0.003**	2.82	**0.096**	6.54	**0.011**	23.07	**<0.0001**
W × N	3.39	**0.068**	2.73	0.10	1.28	0.26	4.97	**0.03**
Neighbor (P)	0.18	0.67	20.5	**<0.0001**	4.59	**0.03**	8.9	**0.003**
W × P	0.5	0.48	3.97	**0.049**	3.36	**0.069**	0.01	0.93
N × P	0.59	0.45	0.21	0.65	0.51	0.48	0.17	0.68
W × N × P	< 0.1	0.98	1.6	0.21	0.28	0.60	4.15	**0.04**
Defoliation (D)	—	—	1.81	0.18	2.27	0.13	4.27	**0.04**
W × D	—	—	1.72	0.19	0.75	0.39	6.44	**0.01**
N × D	—	—	10.7	**0.001**	0.67	0.41	6.06	**0.02**
W × N × D	—	—	2.67	0.11	0.63	0.43	0.31	0.58
D × P	—	—	8.01	**0.005**	5.3	**0.02**	0.34	0.56
W × D × P	—	—	0.49	0.48	0.01	0.93	0.85	0.36
N × D × P	—	—	0.02	0.90	0.17	0.68	0.34	0.56
W × N × D × P	—	—	0.02	0.88	0.55	0.46	0.87	0.35
Covariate	—	—	67.0	**<0.0001**	41.95	**<0.0001**	1.7	0.20

Table 2. Plant neighbor interactions with water addition and with defoliation on young *Festuca campestris* seedling shoot and root biomass (g/plant) when grown with either conspecifics or similar-aged *Poa pratensis*. Values are means ± SE. $N = 36$ per observation. Different letters in a column and grouping indicate different means ($p \leq 0.05$).

Neighbor	*Treatment*	*Shoot Biomass*	*Root Biomass*
F. campestris	Low water	3.26 (±0.32)b	2.70 (±0.35)b
F. campestris	High water	5.38 (±.33)a	3.53 (±0.36)a
P. pratensis	Low water	2.51 (±0.33)b	2.35 (±0.35)b
P. pratensis	High water	3.04 (±0.33)b	2.03 (±0.36)b
F. campestris	Non-defoliated	4.33 (±0.19)ab	3.20 (±0.35)a
F. campestris	Defoliated	4.52 (±0.19)a	3.03 (±0.37)a
P. pratensis	Non-defoliated	3.72 (±0.18)b	2.53 (±0.35)a
P. pratensis	Defoliated	2.99 (±0.18)c	1.86 (±0.35)b

Table 3. Interactions of defoliation with either nitrogen level or watering regime on young *Festuca campestris* seedling shoot biomass (g/plant) and R:S ratios. Values are means ± SE. $N = 36$ per observation. Within a column and treatment grouping (N or W), means with different letters differ ($p \leq 0.05$). NS indicates not significant ($p > 0.10$).

Treatment	*Defoliation*	*Shoot Biomass*	*R:S Ratio*
Ambient N	Defoliated	2.90 (±0.32)b	0.70 (±0.08)b
	Non-defoliated	4.88 (±0.34)a	0.74 (±0.08)b
Reduced N	Defoliated	3.43 (±0.33)b	0.88 (±0.08)b
	Non-defoliated	2.99 (±0.32)b	1.28 (±0.08)a
High water	Defoliated		0.82 (±0.08)b
	Non-defoliated	NS	0.85 (±0.08)b
Low water	Defoliated		0.76 (±0.08)b
	Non-defoliated		1.17 (±0.08)a

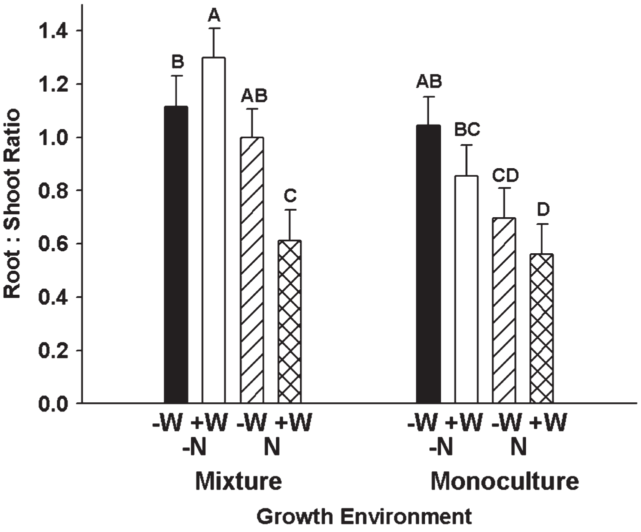

Figure 2. Changes in the root:shoot ratio of young *Festuca campestris* seedlings grown with conspecifics or equal-aged *Poa pratensis* competitors when exposed to varying nitrogen, water, and neighbor treatments ($n = 18$ per treatment). Values shown are means + SE. Means with different letters differ ($p < 0.05$). N, ambient nitrogen; −N, reduced nitrogen; +W, high water; −W, low water; mixture, *Festuca* focal plants with *Poa* neighbors; monoculture, *Festuca* focal and exterior plants.

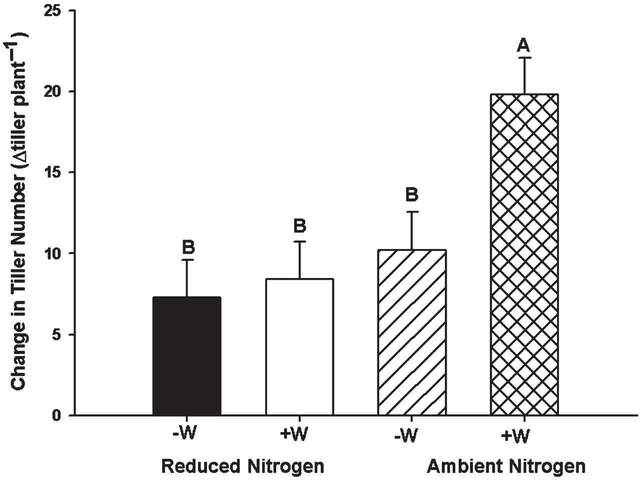

Figure 3. The interaction of watering (W) regimen and nitrogen (N) status on the change in tiller numbers (Δtiller/plant) of young *Festuca campestris* seedlings ($n = 36$) during the first 3 months of growth. Values shown are means + SE. Different letters indicate statistically different means ($p \leq 0.05$).

optimal growing conditions of high moisture and ambient N (Fig. 3).

Response of Older *F. campestris* Seedlings Grown With Younger *P. pratensis*

Summary ANOVA results from experiment 2 are shown in Table 4. Root biomass of older *F. campestris* plants was affected by the interaction of water and neighbor identity (Table 4). Under high moisture conditions, monocultures of *F. campestris* led to greater ($p < 0.05$) root biomass (1.50 ± 0.13 g/plant) than did mixtures (0.64 ± 0.13 g/plant). In contrast, under low moisture conditions, interspecific competition did not alter root biomass (monocultures, −W: 1.175 ± 0.13 g/plant; mixtures, −W: 0.65 ± 0.13 g/plant) significantly ($p > 0.05$) despite a tendency to follow the same trend as shoot mass. Effects of nitrogen reduction were not tested on final biomass of older *F. campestris* plants because of the loss of N immobilization after 3 months.

Plant neighbor identity and defoliation interacted to alter final shoot biomass and root biomass of older *F. campestris* seedlings (Table 4). Observed responses were similar to those in experiment 1 for younger *F. campestris* (i.e. biomass and tillering declined from defoliation and exposure to *P. pratensis*), though larger and more consistent decreases in shoot biomass were attributed to *P. pratensis* in comparison to defoliation, while reductions in root biomass were similarly affected by *P. pratensis* and defoliation (Table 5).

Root:shoot ratios of older *F. campestris* seedlings responded to the main effects of neighbor and defoliation, but no interactions were significant (Table 4). Presence of neighboring *P. pratensis* led to a large reduction (−77%) in *F. campestris* shoot biomass (4.2 ± 0.21 vs. 0.94 ± 0.20 g/plant) and a moderate decline (−51%) in root biomass (1.34 ± 0.09 vs.

Table 4. ANOVA F-values and significance tests for the effects of water level, neighbor identity, and defoliation, together with all interactions thereof, on 6-month tiller changes, shoot biomass, root biomass, and R:S ratios, of older focal *Festuca campestris* seedlings in the experiment using unequal-aged competitors. Bolded p-values indicate those $p < 0.10$. Initial tiller counts were used as a covariate for all biomass data.

	Change in Tillers		*Shoot Biomass*		*Root Biomass*		*R:S Ratio*	
Source	F *Value*	p > F	F *Value*	p > F	F *Value*	p > F	F *Value*	p > F
Water (W)	0.90	0.34	0.5	0.48	0.4	0.54	2.0	0.16
Neighbor (P)	169.4	**<0.0001**	206.7	**<0.0001**	33.6	**<0.0001**	27.1	**<0.0001**
W × P	2.6	0.11	0.5	0.50	4.1	**0.04**	0.1	0.74
Defoliation (D)	15.1	**0.0002**	1.3	0.26	12.1	**0.0007**	4.6	**0.03**
W × D	0.5	0.46	0.1	0.72	0.4	0.55	0.0	1.00
D × P	23.9	**<0.0001**	14.5	**0.0002**	13.0	**0.0004**	0.2	0.70
W × D × P	0.2	0.65	0.9	0.35	0.0	0.95	0.1	0.79
Covariate	—	—	14.2	**0.0002**	5.5	**0.02**	0.7	0.41

Table 5. Effects of plant neighbor identity and defoliation on older *Festuca campestris* seedling total shoot and root biomass (g/plant), and the final change in tillers (tillers/plant), when grown with unequal-aged *Poa pratensis*. Values are means ± SE. $N = 36$ per observation. Within a column, different letters indicate different means ($p \leq 0.05$).

Neighbor	*Treatment*	*Shoot Biomass*	*Root Biomass*	*Change in Tillers*
F. campestris	Non-defoliated	5.15 (±0.30)a	1.86 (±0.13)a	61.11 (±3.62)a
F. campestris	Defoliated	3.28 (±0.30)b	0.82 (±0.13)b	29.39 (±3.67)b
P. pratensis	Non-defoliated	0.74 (±0.30)c	0.65 (±0.13)b	−3.64 (±3.62)c
P. pratensis	Defoliated	1.15 (±0.30)c	0.64 (±0.13)b	−0.02 (±3.57)c

0.65 ± 0.09 g/plant), which together increased ($p < 0.0001$) R:S ratios (0.32 ± 0.06 in monoculture, vs. 0.75 ± 0.06 in mixture). In contrast, defoliation decreased R:S ratios of older *F. campestris* plants (from 0.62 ± 0.06 to 0.45 ± 0.06), a pattern that coincided with reductions in root biomass (1.26 ± 0.09–0.73 ± 0.09 g/plant) rather than shoot biomass (2.07 ± 0.29 vs. 1.57 ± 0.29 g/plant).

Tillers of older *F. campestris* seedlings also had a strong response to the presence of *P. pratensis* (Table 4), where exposure to *P. pratensis* led to little or no increase in tiller numbers regardless of defoliation (Table 5). In contrast, *F. campestris* plants grown with conspecifics markedly increased tiller numbers, with defoliation moderating this increase. While water treatment had no effect on final tiller dynamics of older *F. campestris* seedlings (Table 4), tiller counts 3 months into the trial indicated that initial tiller increases were lower ($F = 6.40$, $p = 0.01$) under high moisture (22.6 ± 2.5 tillers/plant) than under low moisture (30.7 ± 2.5 tillers/plant).

Although N reduction could not be tested on final plant responses in older *F. campestris* seedlings of experiment 2, initial tiller responses at 3 months did indicate an N by neighbor interaction ($F = 6.40$, $p = 0.01$). Tiller counts at that time responded favorably to ambient N compared with those responded to reduced N, but only when grown with conspecifics, while *P. pratensis* presence suppressed tiller numbers regardless of soil N status (Fig. 4).

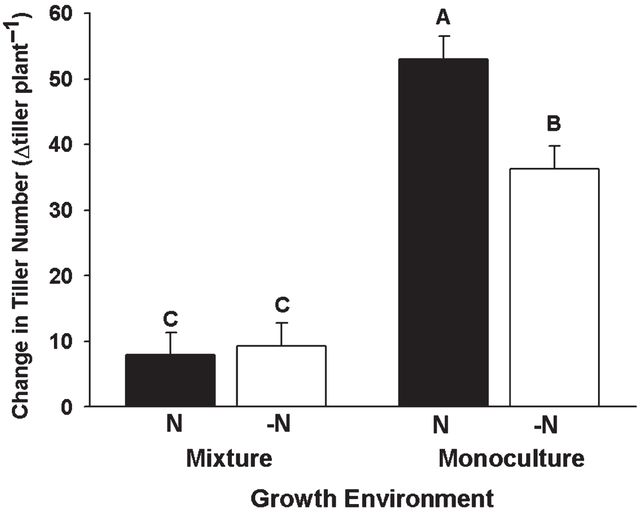

Figure 4. Change in tiller counts (Δtiller/plant) of older *Festuca campestris* seedlings ($n = 36$) grown with younger *Poa pratensis* competitors after 3 months of growth with reduced nitrogen (−N) or ambient nitrogen (N). Mixture, *Festuca* focal plant with younger *Poa* neighbors; monoculture, *Festuca* focal plant and conspecific neighbors. Values shown are means + SE. Means with different letters differ ($p < 0.05$).

Discussion

Exposure to *Poa pratensis* instead of conspecifics reduced *Festuca campestris* biomass in both younger and older seedlings grown with *P. pratensis*. However, unlike older plants, reductions in the biomass of young *F. campestris* seedlings induced by *P. pratensis* did not occur unless coinciding with high moisture or severe defoliation. Similar to trends in biomass, decreases in tiller number occurred with exposure to *P. pratensis*, though only in older *F. campestris*. Negative impacts of *P. pratensis* were also disproportionately greater on older *F. campestris* as evident by amplified differences in R:S ratios when grown in mixtures. The reduced frequency of negative responses among young *F. campestris* plants suggests potential for resistance to interspecific competition.

The greater sensitivity of older *F. campestris* seedlings to *P. pratensis* was unexpected and fails to support the notion that older seedlings may be more resistant to interspecific competition. Our finding could reflect a greater demand for soil resources among older *F. campestris* seedlings, which in turn would have increased their susceptibility to competition, particularly under greenhouse conditions. While older *F. cam*pestris plants were visually observed to have more root biomass than younger plants at harvest, and this could have reduced opportunities for sustained root growth in a potting study, no treatments were considered “root-bound” at final harvest (personal observation). Moreover, older *F. campestris* seedlings demonstrated favorable biomass and tiller responses when grown in monoculture, where all plants (focal + neighbors) were comprised of equal-aged plants. As the replacement of older conspecifics with younger *P. pratensis* as neighbors in experiment 2 led to more negative effects on the bunchgrass, this clearly suggests neighbor identity rather than age was the key factor regulating *F. campestris* growth. Although we did not find a benefit to using older *F. campestris* plants to resist competitive effects from *P. pratensis*, further study directly comparing the effects of plant age under field conditions would be required to fully understand how seedling age impacts the competitive ability of juvenile *F. campestris*.

Older *F. campestris* plants experienced reduced soil N levels for less than 3 months, in part due to much greater initial soil N levels. As the soil used in experiment 2 was collected in May (spring) rather than October (autumn), N mineralization over the dormant season may have increased initial N levels, which in turn would have required much greater carbon addition to achieve sustained N immobilization. Nevertheless, low N suppressed *F. campestris* tillering during the first 3 months with exposure to intraspecific competition, though no additive effect of N was evident on *F. campestris* already experiencing interspecific competition. With young *F. campestris* plants in the first experiment, extended N reduction over 6 months may account for the differential growth responses to modified N compared with the second experiment using older plants. Among young *F. campestris* plants, low N suppressed tiller development and promoted allocation to root biomass (i.e. increased R:S ratios), presumable as a strategy to increase access to soil N. While this plastic response of *F. campestris* could represent an important competitive advantage over species such as *P. pratensis* in the field (Callaway et al. 2003), we found no direct evidence that the competitive effects from *P. pratensis* varied directly with N level in the greenhouse.

Young *F. campestris* seedlings were negatively affected by low moisture and reduced N as represented by reduced biomass and tillering. Moreover, presence of *P. pratensis* eliminated the benefit of high moisture, universally decreasing the biomass of young *F. campestris* seedlings. While the known adaptation of *P. pratensis* to high moisture (Aronson et al. 1987; Dong et al. 2011) was expected to give this species an advantage over *F. campestris*, which our results support, we found that this result unexpectedly extended to low moisture conditions as well. Consequently, in contrast to our initial hypotheses, both low moisture and reduced N failed to shift the competitive balance away from *P. pratensis* and toward young *F. campestris* seedlings grown in mixtures. Despite the fact that *P. pratensis* is known to benefit from high N and associated N mineralization (Wedin & Tilman 1990) and can even displace tallgrasses under N addition (Tilman 1987), our findings cast doubt on whether the reverse is true. That is, in this study soil conditions were not modified to the point of enhancing the establishment of *F. campestris* seedlings simultaneously exposed to *P. pratensi*s.

Defoliation during the growing season is known to be detrimental to *F. campestris* (Willms & Fraser 1992; Willms et al. 1998; Bogen et al. 2003). Our observations suggest these negative effects further depend on seedling age and competition from neighboring plants. While older *F. campestris* seedlings grown in monoculture clearly responded negatively to defoliation (biomass and tiller counts), younger *F. campestris* seedlings in monoculture appeared to be more resistant to defoliation, as evidenced by fewer and more modest decreases in shoot biomass, and only under ambient N. Smaller (i.e. younger) *F. campestris* plants may have been more plastic in growth response and recovered more readily from defoliation, and plant morpho-physiology could have played a role in these results whereby younger plants may have suffered less biomass removal during defoliation, a pattern observed previously in field studies using *F. campestris* (Bogen et al. 2003). Defoliation-induced decreases in the R:S ratio of young *F. campestris* seedlings grown under low N or low moisture also suggest that defoliation can reduce the ability of *F. campestris* to access belowground resources, further decreasing its competitiveness against neighbors, particularly those adapted to favorable soil resource conditions. Such changes eventually reduce the competitive ability of slow-growing plants such as *F. campestris* (Willms & Fraser 1992), and could account for the marked increases in *P. pratensis* observed within grasslands undergoing progressively heavier stocking from cattle (Willms et al. 1985).

While *F. campestris* seedling biomass decreased due to defoliation in both younger and older bunchgrass plants, an even larger decline in biomass due to defoliation occurred in young *F. campestris* seedlings simultaneously exposed to *P. pratensis* as neighbors. This reinforces the detrimental impact that *P. pratensis* has on *F. campestris* during early establishment, and highlights the importance of minimizing interspecific competition when attempting to re-establish this bunchgrass. While young *F. campestris* plants may be moderately tolerant of defoliation alone, the added presence of competition from *P. pratensis* will sharply reduce establishment of juvenile *F. campestris*, an effect observed in other grass species as well (Ang et al. 1994). As the defoliation treatments in this study were imposed on both *F. campestris* and *P. pratensis* when growing in mixtures, our test of defoliation on competitive dynamics between these species assumes similar levels of defoliation. Previous work in mature grasslands indicates that *F. campestris* is initially selected by cattle over *P. pratensis* during the critical spring green-up period at low stocking rates, a preference that disappears at moderate stocking (Moisey et al. 2005). However, the opposite was found during autumn where *P. pratensis* was selected over *F. campestris* (Moisey et al. 2005). These results highlight that selection by herbivores for either *F. campestris* or *P. pratensis* may further alter competitive pressure on *F. campestris* seedlings, depending on whether the native bunchgrass or *P. pratensis* is defoliated, and to what extent.

In this greenhouse study, while *F. campestris* seedling growth was reduced by low moisture, reduced N, and defoliation, the most consistent factor determining the above- and belowground biomass as well as tiller production of seedlings was whether or not *P. pratensis* was a neighbor during early growth. Negative effects of *P. pratensis* were also more consistently apparent in older *F. campestris* seedlings, while being limited in younger seedlings to conditions coinciding with high moisture or severe defoliation. Overall, these results suggest that *P. pratensis* is capable of acting as a strong driver of early successional dynamics during grassland restoration, a result that has been observed in parallel field trials exploring the effect of this species on community dynamics within old-growth grasslands of the region (White et al. 2013). Establishment of *F. campestris* may be increased with the reduction of *P. pratensis* from affected areas, potentially through the use of an extended mechanical or chemical fallow period prior to revegetation. However, given the large seed bank of *P. pratensis* within these grasslands (Willms & Quinton 1995), complete removal of this invasive remains unlikely. Where *P. pratensis* cannot be eliminated, reductions in defoliation may also be used to aid bunchgrass recovery. Ultimately, reducing the effect of *P. pratensis* on establishing *F. campestris* seedlings appears to be a critical step toward re-establishing and conserving these native grasslands.

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