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Facilitation of an Exotic Grass Through Nitrogen Enrichment by an Exotic Legume☆

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ABSTRACT

Invasive species control requires understanding the mechanisms behind their establishment and their interactions with other species. One potential ecosystem alteration influencing the establishment and spread of invasive species is anthropogenic nitrogen enrichment, from sources like introduced or invasive nitrogen (N)-fixing legumes, which can alter competition between native, non-native, and invasive plants. Kentucky bluegrass *(Poa pratensis)* and N-fixing yellow sweet clover *(Melilotus officinalis)* are exotic to the Great Plains and are currently invading and degrading native rangelands by altering ecosystem processes and displacing native plants. Therefore, we investigated how N enrichment from yellow sweet clover affects the aboveground biomass production of Kentucky bluegrass and western wheatgrass *(Pascopyrum smithii)*, a native cool-season grass, the ranges of which overlap in the northern Great Plains. In a controlled greenhouse environment, we conditioned experimental pots by growing yellow sweet clover and terminating each plant after 8 wk. Conditioned soils contained ≈340% more plant-available N than untreated soils 2 wk after yellow sweet clover death. We then grew Kentucky bluegrass and western wheatgrass transplant seedlings in interspecific and intraspecific pairs in pots conditioned either with or without yellow sweet clover for 12 wk. Aboveground biomass production of both Kentucky bluegrass and western wheatgrass grown in interspecific and intraspecific pairs increased in conditioned soils. However, when grown together in conditioned pots, the increase in Kentucky bluegrass biomass relative to untreated pots (520%) was double that of the increase in western wheatgrass biomass (260%). Our results reveal that Kentucky bluegrass can use increased soil N to produce proportionally more aboveground biomass than western wheatgrass, a native grass competitor. Thus, our results suggest yellow sweet clover and other sources of N enrichment may facilitate the invasion of Kentucky bluegrass.

Introduction

Biological invasions are recognized as one of the leading anthropogenic threats to biodiversity (Wilcove et al., 1998). Exotic invasive species compete directly with native species, but they can also indirectly change various aspects of their native ecosystems including alterations in nutrient cycling, hydrology, litter accumulations, and other attributes that can further alter disturbance regimes (Vitousek et al., 1997b; Brooks et al., 2004). Environmental damage, economic losses, and control costs associated with invasive species total ≈$120 billion/yr in the United States (Pimentel et al., 2005). The overall number of exotic species invasions, however, is continually increasing and associated costs are likely increasing as well (Pimentel et al., 2005). Research on the mechanisms driving exotic species establishment and invasions will advance control strategies and help reduce control costs by improving their efficiency.

Nitrogen (N) availability is a major determinant of primary productivity in terrestrial ecosystems, especially in N-limited grasslands (Tilman, 1987; Wedin and Tilman, 1990). Consequently, increases in N availability where it is typically limited can increase plant productivity (Chapin et al., 1986). Most plant species have different N requirements, uptake rates, and use efficiencies, which can result in different growth responses in environments with increased N availability (Chapin et al., 1986). Species that produce the highest rates of biomass in high N environments have a competitive advantage and can outcompete native species for area and light (Chapin et al., 1986; Wedin and Tilman, 1990). Consequently, increases in N availability can influence the invasibility of ecosystems and may shift plant community composition (Tilman, 1987; Wedin and Tilman, 1990). Research on the biomass production of invasive species compared with native species under high N conditions may reveal if enhanced N availability acts as a positive feedback mechanism to promote invasion.

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Despite the awareness that N enrichment in terrestrial systems is linked with numerous human activities, little is understood regarding the impacts of N-fixing legume introductions and invasions on rangeland ecosystems and plant interactions (Vitousek et al., 1997a). Yellow sweet clover *(Melilotus officinalis)* is a biennial N-fixing legume native to Eurasia that has the potential to become invasive in the northern Great Plains (Lesica and DeLuca, 2000). Yellow sweet clover plants fix atmospheric N into plant-available N in root nodules that develop when plants are naturally inoculated with N-fixing *Rhizobium* bacteria (Franche et al., 2009). Mineralization of dead yellow sweet clover root nodules can release substantially higher rates of inorganic N than native legumes into the mineral soil (Lesica and DeLuca, 2000; Van Riper et al., 2010). Consequently, invasion by yellow sweet clover has the potential to shift the competitive balance in favor of plants that thrive under high N conditions because it enriches soil N more than native legumes and can be highly pervasive.

Kentucky bluegrass *(Poa pratensis)* has invaded native grasslands of the northern Great Plains and is altering plant community composition and ecosystem functions with consequences that remain largely unknown (Murphy and Grant, 2005; Toledo et al., 2014). Kentucky bluegrass is a cool-season, perennial grass native to Europe and parts of North America outside of the northern Great Plains that is displacing the perennial, cool-season grasses that typically dominate the region including western wheatgrass *(Pascopyrum smithii)* and green needlegrass *(Nassella viridula)*. Although its abundance has increased substantially over the past few decades, its invasion has only recently received attention and knowledge gaps exist regarding the consequences of, and mechanisms behind, the invasion (Toledo et al., 2014). Kentucky bluegrass has a relatively high N uptake rate compared with its N use efficiency (Jiang et al., 2000), which may promote its dominance in high N systems if it correlates with enhanced biomass production. There is a lack of empirical evidence, however, to suggest whether the potential interaction between soil N enrichment, among other factors, and Kentucky bluegrass invasion exists.

Although exotic species invasions are increasingly recognized for their far-reaching ecological impacts (Murphy and Grant, 2005; Toledo et al., 2014), the consequences of those impacts remain largely unknown and there is a lack of research revealing the mechanisms behind them. As increased N deposition from various sources may influence ecosystem invasibility (Tilman, 1987; Wedin and Tilman, 1990), research on how invasive and native species interact under enhanced N conditions is necessary to understand how to repair invaded ecosystems successfully. Therefore, we studied the interaction of Kentucky bluegrass and native western wheatgrass *(Pascopyrum smithii)* in control and N-enriched soils as a model. Specifically, our objectives were to quantify the aboveground biomass production of Kentucky bluegrass as compared with western wheatgrass in control soils and soils conditioned with decaying yellow sweet clover roots as the ranges of these three species overlap in the northern Great Plains. Given that 1) soil N content can differentially influence plant growth, 2) that invasive yellow sweet clover can elevate soil N more than native legumes (Nichols and Johnson, 1969; Lesica and DeLuca, 2000), and 3) invasive Kentucky bluegrass may have a higher N uptake efficiency under high N conditions, we hypothesize that yellow sweet clover invasion may facilitate Kentucky bluegrass by enhancing its biomass production.

Methods

We conducted this study at the North Dakota State University Agricultural Experiment Station Research Greenhouse Complex in Fargo, North Dakota, United States. We germinated yellow sweet clover seeds in vermiculite and transplanted two seedlings into polystyrene pots (11.2 L) containing peat moss/sand growth media (1:3) one wk following germination. Additional pots were filled with growth media and did not receive seedlings. Plants received 15 hr of daylight supplemented with artificial lights and day and night temperatures of 27°C and 21°C, respectively, to simulate growing-season conditions. Pots received water daily to maintain soil moisture at field capacity. We clipped yellow sweet clover plants at the soil surface to top-kill each plant following 8 wk of growth.

Conditioned and control soils were tested in the laboratory to determine soil pH, nitrate-N, and ammonium-N 2 wk following termination of yellow sweet clover plants. We transplanted Kentucky bluegrass and western wheatgrass seedlings into experimental and control pots when soil samples were collected under interspecific (different species) and intraspecific (same species) competition pairings after 1 wk of growth to establish an even-age class. We arranged plants in one of three species combinations in pots either conditioned with yellow sweet clover (treatment) or not (control): 1) Kentucky bluegrass + western wheatgrass, 2) Kentucky bluegrass + Kentucky bluegrass, and 3) western wheatgrass + western wheatgrass. Soil and species combination treatments were randomly arranged into four blocks (a bench being a block) with 10 replications within each block for a total of 40 observations (pots) per treatment to account for light and temperature differences. We harvested aboveground biomass when grasses reached boot stage (approximately 12 wk post germination for both species). Biomass was oven dried at 60°C to a constant weight and recorded. Soil N, pH, and aboveground biomass treatment means were compared using analysis of variance (ANOVA) procedures in SPSS 23 using Tukey's B means separation (IBM-SPSS, 2016). To determine relative biomass increase, we calculated the percent increase between control and conditioned pots for each species within each replication for all species-treatment combinations. We then compared mean relative biomass increase for each replication across each species-treatment combination ($n = 4$) using ANOVA procedures in SPSS 23 using Tukey's B means separation (IBM-SPSS, 2016). Plant nomenclature and distribution followed the National Plant Database (USDA-NRCS, 2018).

Results

Analysis revealed a nonsignificant block effect for all variables. Therefore, respective treatments were pooled across blocks. Laboratory analyses revealed plant-available N increased significantly following yellow sweet clover conditioning ($P \le 0.05$). Relative to untreated control soils, plant-available soil N increased by approximately 340% following yellow sweet clover treatment (Fig. 1). Soil pH averaged 6.8 and was not significantly different among soil treatments ($P > 0.05$).

Aboveground biomass of Kentucky bluegrass and western wheatgrass in control pots was generally low (< 200 mg). In control pots, western wheatgrass biomass was greater with interspecific planting than any other species-treatment combination and all others were similar (Fig. 2). In conditioned pots, western wheatgrass biomass was

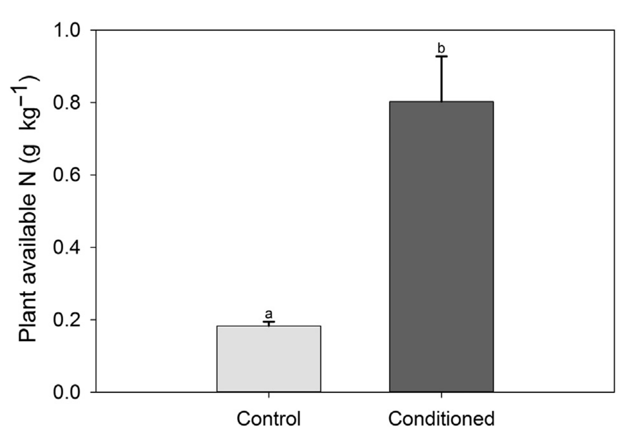

Figure 1. Mass concentration of total plant available nitrogen (N) in control pots and pots conditioned with yellow sweet clover at the planting time of interspecific and intraspecific Kentucky bluegrass and western wheatgrass transplant seedlings. Bars represent treatment means ± standard error and were significantly different at $P \le 0.05$.

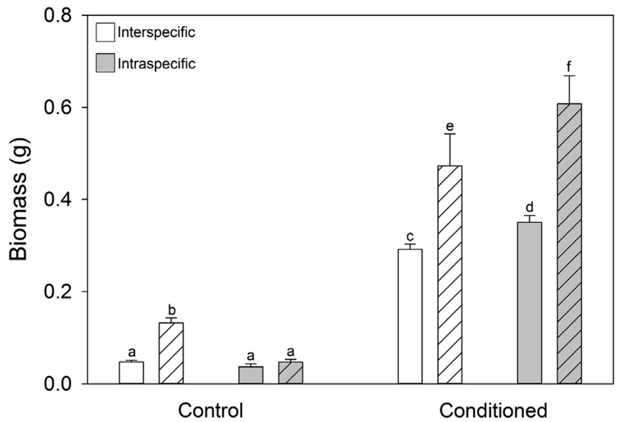

Figure 2. Aboveground biomass of western wheatgrass and Kentucky bluegrass harvested after 12 wk of growth in interspecific and intraspecific combinations in both control and conditioned pots. Hashed bars indicate western wheatgrass treatment means ± standard error. Solid bars indicate Kentucky bluegrass treatment means ± standard error. Treatment bars with different letters are significantly different at $P \leq 0.05$.

always higher than Kentucky bluegrass and intraspecific planting produced more biomass than interspecific planting for both species (see Fig. 2). Although aboveground biomass increased for both species in conditioned pots relative to untreated control pots, there was a significant difference between the magnitude of their relative increases in interspecific and intraspecific planting (Fig. 3). Relative to untreated controls, the increase in Kentucky bluegrass and western wheatgrass in conditioned pots with intraspecific planting was not significantly different (see Fig. 3). The increase in Kentucky bluegrass biomass (520%), however, was double that of western wheatgrass (260%) with interspecific planting (see Fig. 3). Therefore, Kentucky bluegrass produced aboveground biomass at a rate higher than that of western wheatgrass when grown together with N enrichment from yellow sweet clover.

Discussion

Changes in resource availability and nutrient cycling can influence plant community composition by altering competitive dominance (Wedin and Pastor, 1993; Levine et al., 2003). Our results suggest that Kentucky bluegrass increases aboveground biomass production with enriched soil N at a faster rate than western wheatgrass, a potential native grass competitor in the northern Great Plains. Kentucky bluegrass invasion can alter resource dynamics and reduce their availability or available niches for other species because it regenerates quickly through tillering, which occupies lateral space across the landscape (Toledo et al., 2014). Consequently, our results imply the need for further research to investigate how enhanced aboveground biomass production of Kentucky bluegrass in the field affects resource availability. Turf grass research has revealed that increased soil N is associated with increased species homogeneity in Kentucky bluegrass turf environments (Heckman et al., 2000). Several field-based N-addition studies also attribute declines in species richness to competition from the increased dominance of fewer species, which exploit enhanced N more rapidly than their neighboring species (Tilman, 1987; Wedin and Tilman, 1990; Tang et al., 2017). Therefore, we suggest that increased Kentucky bluegrass dominance in the northern Great Plains may be associated, in part, with its exploitation of enhanced N levels.

The N enrichment by yellow sweet clover in our study significantly affected the competitive balance between non-native Kentucky bluegrass and native western wheatgrass. Although N-fixing plants are recognized for their impacts on the competitive balance between native and invasive species in terrestrial ecosystems, few studies have revealed the impacts of yellow sweet clover on the invasibility of rangelands (Tilman, 1987; Maron and Connors, 1996; Lesica and DeLuca, 2000). Death of established yellow sweet clover plants can result in N mineralization and N accumulations roughly two to three orders of magnitude greater than native range uninvaded by yellow sweet clover, which may increase rangeland productivity and agrees with our soil testing results (Nichols and Johnson, 1969; Lesica and DeLuca, 2000). In the field, it is conceivable that dead, rather than live, yellow sweet clover stands may facilitate Kentucky bluegrass invasion in following years, as our results show that Kentucky bluegrass is more productive than native grasses at high levels of N availability and warrants further investigation that considers all associated variables like water dynamics and belowground biomass production.

Our results reveal that yellow sweet clover may have an indirect facilitative effect on Kentucky bluegrass invasion as enhanced N from decaying yellow sweet clover roots allows Kentucky bluegrass to produce high rates of biomass. Facilitation is increasingly recognized for not only its importance in structuring plant communities but also the potential role it may have in promoting exotic plant invasions (Callaway, 1995; Maron and Connors, 1996; Holmgren et al., 1997). Similar to our findings, Carino and Daehler (2002) observed that N-fixing partridge pea *(Chamaecrista nictitans)* indirectly facilitated the growth of fountain grass *(Pennisetum setaceum)* only after it had died. Yellow sweet clover is a biennial species, which implies that its enrichment of soil N and potential facilitation of Kentucky bluegrass invasion may be seasonal, or episodic if plant death is required. Additional research is required to determine the legacy or lag effects of yellow sweet clover soil fertility on exotic plant invasions over various periods of time.

Management Implications

Although the short duration, greenhouse conditions, and study of only one competing native grassland species limit the application of our findings to field conditions, our research provides empirical evidence to suggest elevated soil N may facilitate Kentucky bluegrass by enhancing its aboveground biomass production. Consequently, additional research on this interaction in the field is needed as it could be a factor affecting species richness declines in the northern Great Plains with Kentucky bluegrass invasion. The enhanced aboveground growth response of Kentucky bluegrass to N addition from decaying yellow sweet clover roots implies that reductions in or removal of N inputs in invaded northern Great Plains grasslands may have the potential to limit or slow the aboveground biomass production of Kentucky bluegrass and promote the conservation of native species richness and ecological function. Belowground biomass production, however, was not investigated in this study and represents an additional research avenue as biomass production dynamics and competition could differ

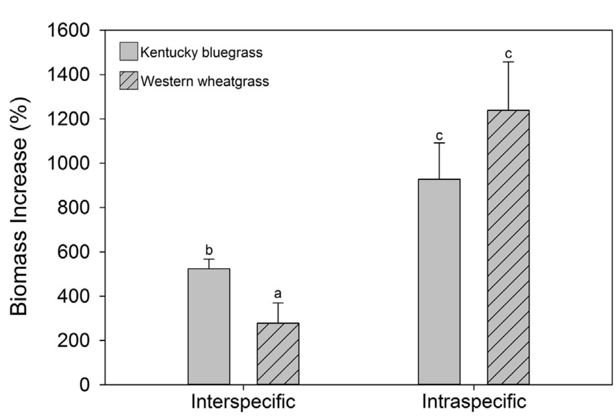

Figure 3. Relative increase in aboveground biomass production of western wheatgrass and Kentucky bluegrass harvested after 12 wk of growth in interspecific and intraspecific combinations in conditioned pots as compared with controls. Bars represent treatment means ± standard error. Treatment bars with different letters are significantly different at $P \leq 0.05$.

belowground. In agreement with related studies on sweet clover invasion, our results support findings that yellow sweet clover may facilitate invasion or enhance the invasibility of native grasslands by exotic species that exploit associated increases in N supply (Lesica and DeLuca, 2000; Van Riper et al., 2010). N-fixing plants like yellow sweet clover can and have been used to improve rangeland forage production (Nichols and Johnson, 1969). However, our research shows they can also shift the competitive balance in favor of exotics that thrive in N-enriched soils and may facilitate further rangeland plant invasions or plant community shifts (Maron and Connors, 1996; Lesica and DeLuca, 2000; Van Riper et al., 2010).

Interactions among invasive species, other invasive species, and native species should be taken into account when managing imperiled and invaded ecosystems. In sum, our study provides valuable insights into the potential relationships between Kentucky bluegrass, N enrichment, and yellow sweet clover in imperiled grassland ecosystems. Our findings suggest that yellow sweet clover and Kentucky bluegrass management in the northern Great Plains may require management for both exotic invaders where they coexist as yellow sweet clover may facilitate Kentucky bluegrass production. Research on components of anthropogenic change, such as invasive species and resource availability interactions, helps inform management that aims to conserve biodiversity and maintain the ecosystem services society depends on in the face of increasing global change. Land managers may not be able to control other anthropogenic sources of N deposition but should be aware of its potential effects on plant interactions, ecosystem functions, vegetation structure, invasion potential, and biodiversity.

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