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REGULAR ARTICLE

Changing edaphic conditions and exploitation of an expanded phenological niche allows for increased exotic (introduced) plant species dominance

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Abstract
Aims Global change agents are creating novel climatic and edaphic conditions that may favor introduced species. We attempted to identify mechanisms and impacts of *Bromus tectorum* invasion in the Colorado Front Range mixed-grass prairie under changing conditions.
Methods We conducted an in-situ experiment with three removal treatments (removal of *B. tectorum,* removal of *Pascopyrum smithii,* and no removal) and two nitrogen treatments (ambient and low N), and measured plant community response. We used isotopic analysis of δ^{18}O and δD in plants and soils to identify seasonal source water of *B. tectorum* and *P. smithii*.
Results We found that dominance of *B. tectorum* was greatest under high resource conditions (ambient N, wet winter) now common due to increased N deposition and climate change. However, its removal had little impact on native plant abundance or composition. Isotopic results show *B. tectorum* and *P. smithii* partitioning water use between shallow versus deeper soil layers during the dry summer season.
Conclusions Our results suggest that changing environmental conditions favor the introduced grass *Bromus tectorum* over native species, but probably not due to altered competitive relationships. Instead, *B. tectorum* appears to be opportunistically responding to expansion of a phenological niche and increased nutrient availability.

Keywords *Bromus tectorum* · Cheatgrass · Invasive species · Nitrogen deposition · Novel ecosystem · *Pascopyrum smithii*

Introduction

Agents of global change can affect plant communities both directly and indirectly. Range shifts of individual species are expected in response to changing climatic conditions based on their physiological tolerances and dispersal abilities (Ives 1995; Parmesan 2006). More difficult to predict are changes in species assemblage that may occur in response to altered strength or direction of interactions- including both plant-plant (Brooker 2006) and trophic interactions (Tylianakis et al. 2008), under novel climatic or edaphic conditions. Indeed, although there is evidence that biotic interactions can strongly determine direction of change and may even

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override the impacts of climate change on plant communities (Rysavy et al. 2016), there is a dearth of experimental research on this topic. An important question for plant scientists is how introduced species will interact with other agents of global environmental change (Bradley et al. 2010). Like all plant species, introduced species may experience range expansion or contraction due to direct effects of changing environmental conditions (e.g., Bradley and Wilcove 2009). They may also gain a competitive advantage over native species if, for example, they are better equipped to access limiting resources or to take advantage of resource pulses, tolerant of a broader range of environmental conditions or perturbations, or more resistant to disease or herbivory compared to native species (e.g., Smith et al. 2000; Littscheager et al. 2010; Dukes et al. 2011). Introduced plants that become invasive are often weedy, fast-growing species with high relative growth rates (Grotkopp et al. 2001; Pyšek and Richardson 2007), and some also benefit from differences in phenology compared to native co-occurring species (Wolkovich and Cleland 2011). These characteristics could facilitate their expansion under changing environmental conditions that influence the fitness of resident species.

Plant communities are already shifting in response to global change (Parmesan 2006), but the mechanisms are poorly understood. Where introduced species have become dominant, it may be assumed that these species themselves are the main drivers of biodiversity loss. However in the context of environmental change, it is difficult to differentiate systems where plant communities are shifting in response to directional changes in environmental conditions which favor introduced species over natives from those that are shifting due to the competitive displacement of natives by one or more introduced species. The outcome in both cases is a community with fewer native species. Investigations have pointed to the importance of distinguishing the role of introduced plants as drivers versus passengers of change (MacDougall and Turkington 2005). Passengers benefit indirectly from altered environmental conditions which negatively affect native species, while drivers are responsible for altered ecosystem properties which lead to declines in native species. We should note that attributing passenger or driver status to plant invaders is not always straightforward, as many species may fall somewhere in between (HillRisLanbers et al. 2010). However, the distinction remains important because the mechanism of invasion has implications for the success of management approaches. In the passenger model, ecosystem change is simultaneously driving the increased abundance of introduced species and the decline of native species, so removal of the environmental stressor would be needed to return to a native-dominant system. In contrast, by eradicating an invasive species that is acting as a driver, native species should directly benefit with increases in cover and/or richness (MacDougall and Turkington 2005).

Invasive species may be further characterized as having niche differences or fitness differences with native residents, or some combination of the two, to better understand their potential long-term impacts (MacDougall et al. 2009). Introduced species which are able to exploit an unoccupied niche may successfully establish without having great impacts on the native plant community. Ecological theory predicts that coexistence of introduced and native species is more likely to occur in such a scenario (MacDougall et al. 2009). In contrast, those that successfully establish through fitness differences with native species (e.g., superior competitor, resistance to herbivory) are more likely to lead to declines in biodiversity. Agents of global change may open up new opportunities for introduced species to invade via either mechanism. For example, increased resources via changing precipitation patterns could lead to expanded niche opportunities while some introduced species may become more competitive over natives under changing environmental conditions. Identifying which model (s) of invasion best characterizes an introduced species in a particular region, driver versus passenger and niche opportunist versus superior competitor, is important for understanding what its impacts are likely to be and what management approaches will work best to address those impacts. Here, we focus on a mixed-grass prairie ecosystem which has experienced recent increases in introduced species along with changes in climatic and edaphic conditions, making it an ideal system in which to explore these models.

In the Front Range of Colorado, the mixed-grass prairie plant community has been undergoing directional shifts in plant species composition over the last two decades (Beals et al. 2014), likely facilitated by ongoing global change drivers (Prevéy and Seastedt 2014; Concilio et al. 2016). The region has experienced increases in nitrogen (N) deposition and changing seasonality of precipitation (toward wetter winters), which both tend to favor the cool-season introduced species that are becoming dominant- particularly the annual grass *Bromus tectorum* (commonly known as cheatgrass; Concilio et al. 2016; Prevéy and Seastedt 2014). This suggests a passenger model of invasion. However, it is well-known that *B. tectorum* is driving loss of native species in other parts of its invaded range, most notably in shrub-steppe ecosystems of the Great Basin Desert, both by depleting soil resources early in the season when native species are still dormant (through priority effects; Wolkovich and Cleland 2011) and by altering fuel dynamics in a way which favors its own dominance (Brooks et al. 2004). These same changes to the fire regime have not been observed in invaded mixed-grass prairies of the Colorado Front Range, and would not be expected (Brooks et al. 2004). Instead, annual bromes tend to exhibit neutral or negative responses after fire in Great Plains grassland ecosystems (Porensky and Blumenthal 2016, Vermeire et al. 2011). However, priority effects associated with *B. tectorum*'s early phenology may be occurring in the Front Range. Prevéy and Seastedt (2014) found that *B. tectorum* cover in April was negatively correlated to species richness, diversity, and volumetric water content in June. One possible explanation for this pattern is that, like in the Great Basin, *B. tectorum* is depleting soil water and nutrient resources in the early spring that are then unavailable for native warm-season species, and thereby driving loss of biodiversity. This would make it a combination of niche opportunist with a fitness advantage, and a driver of change. Alternatively, *B. tectorum* could be benefiting from a niche difference without having much direct impact on the native plant community, which would make it a niche opportunist and passenger of change.

Here, we used an in-situ manipulative experiment to better understand mechanisms and impacts of *B. tectorum* invasion in this system, with a focus on competitive displacement of native species by *B. tectorum* under changing soil resource conditions. We used a removal experiment to compare the effects of *B. tectorum* on native plant diversity and productivity in both the spring and summer seasons to those of the only co-occurring native cool-season grass, *Pascopyrum smithii* (western wheatgrass), under current and simulated historic low nitrogen conditions in a mixed-grass prairie ecosystem of Colorado. The experiment was conducted over two years: one average and one exceptionally wet winter, allowing us to gain insight into effects of winter water availability on plant-plant and plant-soil interactions.

We tested hypotheses about overlapping resource use by *B. tectorum* and *P. smithii* by analyzing and comparing stable isotope ratios of xylem water from each species during different seasons over two years. Both species are C_3 grasses with maximum potential rooting depths in this system of ~150 cm, most of which are concentrated in the upper 30 cm for *B. tectorum* (Zouhar 2003) while the highly branched rhizomes of *P. smithii* begin about 5 cm below the soil surface (Coupland and Johnson 1965; Tirmenstein 1999). Although these species share considerable overlap in root distribution and density, grasses in prairie ecosystems may partition resource acquisition zones to reduce competition based on changes in soil moisture content in varying soil layers (Nippert & Knapp 2007; Asbjornsen et al. 2008). Indeed, stable coexistence of grassland species is theorized to occur, at least in part, due to niche differentiation (Weaver 1966, Nippert & Knapp 2007). Based on this same premise, those invasive species that gain dominance via niche differences with native species (with or without fitness differences) are likely to coexist in native plant communities with lesser impacts (MacDougall et al. 2009). We analyzed isotopic signatures in xylem water of *B. tectorum* and *P. smithii* for comparison with isotopic signatures in shallow vs deeper soil layers to determine where each species was acquiring water in the spring and summer seasons, and whether there was evidence for resource overlap or niche partitioning. Because isotopic fractionation does not occur during the uptake of water into root tissue, plant xylem water shares isotopic signatures of hydrogen and oxygen with its source water (Dawson et al. 2002), allowing these comparisons to be made.

If *B. tectorum* is displacing native species in the region due to its increased competitive advantage under changing resource conditions, then we would expect its removal to be associated with a relatively quick recovery in native plant abundance and diversity in high resource plots. Under historical resource conditions (low N availability), we expect *B. tectorum* to be a weaker competitor and thus would predict that its removal would cause relatively little change to the plant community. It is well known that *B. tectorum* has a rapid growth rate when resources are not limiting, which allows it to outcompete co-occurring native perennial species in the Great Basin (e.g., Concilio et al. 2015b). We hypothesize that it similarly acts as a superior competitor over the cool season perennial *P. smithii* when and where soil water and nitrogen are abundant. We expect to find evidence for this hypothesis through removal treatments: that is, removal of *B. tectorum* will result in increased *P. smithii*, whereas the removal of *P. smithii* will have little to no effect on *B. tectorum.* We would also expect the two grass species to use the same shallow soil layer source when water is available. If *B. tectorum* exhibits superior competitive ability for water uptake from shallow soil layers when water availability declines, we predict that *P. smithii* will rely on water from deeper sources. Our results will provide insight into whether and how *B. tectorum* is impacting the native plant community under changing environmental conditions, and thus provide important information for land management and invasion biology theory in an era of global change.

Materials and methods

We conducted an experiment in a mixed-grass meadow previously described in Prevéy and Seastedt (2014), 15 km northwest of Boulder, CO (40° 07′ N, 105° 18′ W; elev 1798 m). The site is on private land, has never been plowed, but was grazed by cattle until 2006 when livestock were removed from the land. Soils at the site are well-drained, colluvial, sandy loams (NRCS 2001), and vegetation is a mix of warm- and cool-season native grasses, native forbs, and (mostly cool-season) introduced forbs and grasses. See Concilio et al. (2015a) for a list of species. Temperature averages 10.9 °C and the site receives 525 mm of precipitation on average per year, ranging from a low of 277 mm in 1954 to a record high of 867 mm in 2013 (records are from 1893 to 2016; Western Regional Climate Center, http://www.wrcc.dri.edu/).

We established 60 experimental 1 × 1 m^2 plots in 2013 using a full factorial design to test the main and interactive effects of soil N (ambient and low N), precipitation (ambient and increased winter precipitation), and removal (*B. tectorum* removal, *P. smithii* removal, no removal). These plots were established within a larger global change experiment which included 96, 3 × 3 m^2 study plots to test effects of soil N availability and winter precipitation on plants and soils (and included an additional precipitation treatment: winter dry). For the current experiment, we used 15 plots each (60 plots total) of the following treatment combinations: ambient N- ambient precipitation; ambient N- increased winter precipitation; low N- ambient precipitation; low N- increased winter precipitation. Plots were placed in one corner of the 3 × 3 m^2 experimental plots, at least 0.5 m from the edge on both sides, where both focal species (*B. tectorum* and *P. smithii*) covered ≥10% of the plot. If this criteria was satisfied in the southeast corner of the larger plot, we chose that corner; if not, we looked to the southwest corner; and finally to the northeast.

The low N treatment was achieved by adding carbon (C) in the form of table sugar to half of the plots evenly throughout the growing season from March to October every 2–3 weeks at a rate of 500 g C m^{-2} $year^{-1}$ in 2013 and 2014. This treatment reduced N availability by an average of 63% in low N plots compared to ambient levels (Online Resource 1). Adding sugar affects soil microbial composition and particularly bacterial abundances by affecting C and N ratios of labile carbon substrates (e.g., Leff et al. 2015). While this method has additional, unmeasured effects on soil chemistry, this treatment has consistently reduced soil inorganic N availability in all studies we are aware of, and the outcome observed here was consistent with previous work (e.g., Cherwin et al. 2009).

We increased precipitation in winter wet plots by irrigating with water from a nearby creek in a series of simulated storm events delivered between January and May of 2013 and 2014. Winter wet plots received a total of +40 mm H_2O m^{-2} in 2013 and +34 mm of supplemental precipitation in 2014, representing a 20% increase over winter precipitation in 2013, but only 5% in 2014. This was due to an extreme precipitation event in September 2013 (with 430 mm delivered over a 6 day interval from Sept 10–15) and subsequent higher than normal soil moisture levels well into the next growing season. Consequently, there was no difference in volumetric soil moisture (or any of the response variables we measured) between winter wet and ambient precipitation plots in 2014 (Fig. 1), and we pooled data from the two precipitation treatments that year (within each season) for analysis**.** Another factor to note was that background soil moisture levels were on average higher in ambient than winter-wet treatment plots throughout the study period (Fig. 1). The winter wet treatment served to equalize, rather than increase, soil moisture levels compared to those of ambient precipitation plots. This probably further contributed to the lack of response we saw to precipitation treatments. Thus, we focused instead on the differences observed between 2013 (normal year) and 2014 (extreme precipitation event) to gain insight into how winter precipitation might influence plant-

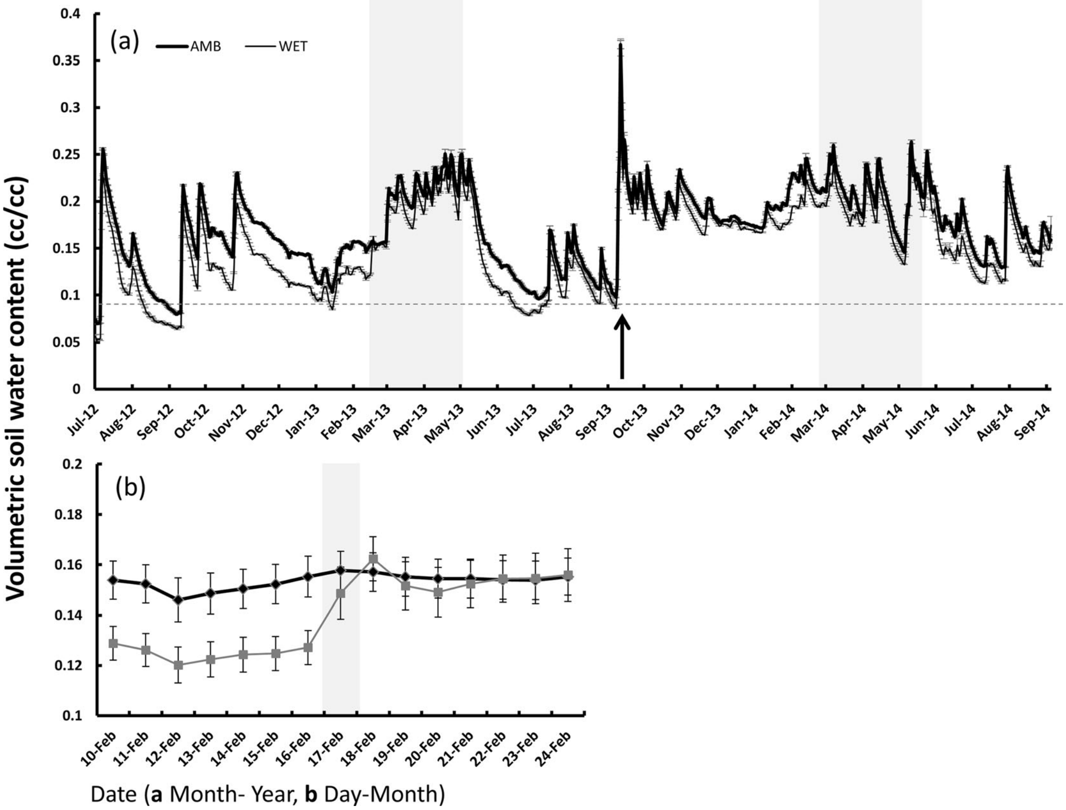

Fig. 1 Soil moisture collected hourly at 14 ambient precipitation (AMB) plots and 8 increased winter precipitation (WET) plots, and averaged by day **a** across two years from July 2012 to September 2014, and **b** from February 10–24, 2013, one week before and after a water addition was made to the WET plots. Grey bars represent the period of time when water additions were made in the WET plots in the top panel, and the date on which the addition was made (Feb 14) in the bottom panel. Winter-wet plots had background levels of soil moisture that were on average lower than ambient precipitation plots. Irrigation treatments elevated soil moisture so that there was no significant difference between AMB or WET plots, as illustrated in the bottom panel. The arrow in the top panel marks an extreme precipitation event, which occurred in Sept 2013. The dashed grey line helps illustrate how soil moisture in the driest period of 2014 remained well above that of previous years. Error bars represent the standard error of the mean daily soil moisture

plant interactions in this system. Spring and summer precipitation was similar in the two years, with 250 and 338 mm between April and August of 2013 and 2014, respectively.

To test the effect of each focal species on the rest of the plant community, we removed all individuals of *Bromus tectorum* from 20, 1 × 1 m^2 plots, all *Pascopyrum smithii* from 20 plots, and kept 20 plots as no removal controls. The removal treatments took place across equal numbers of each N-treatment and precipitation treatment, so that there were 5 plots each of all possible treatment combinations (5 replicates * 3 removal * 2 N * 2 precipitation treatments =60 plots). All individuals of *B. tectorum* and *P. smithii* were pulled from their respective removal plots shortly after seedlings germinated in 2013 (22 Mar - 6 Apr for *B. tectorum*, and 21–25 Apr for *P. smithii*). We removed the entire plant (roots plus shoots) for the annual *B. tectorum*, carefully so as to cause as little disturbance to the surrounding area as possible. For the rhizomatous native species *P. smithii*, we removed the entire aboveground portion along with several inches of root tissue using scissors to limit disturbance to the soil. In 2013, we found that a significant amount of *B. tectorum* germinated in most winter-wet treatment plots (but not ambient precipitation) after the removal treatments had been conducted, and some *P. smithii* resprouted in these same plots. The removal treatments in winter-wet plots were therefore ineffective in 2013, and we only used data collected in ambient precipitation plots in our analyses in 2013 ($n = 30$). In 2014, we removed both species from plots bi-weekly from 24 Mar to 16 Jul to ensure that treatments were effective. We dried and weighed all of the biomass that was removed from plots. The same plots were used both years for removal treatments, so weather effects were confounded with second-year removal effects in 2014.

To evaluate response to treatments, we measured species composition with the point-intercept method in the spring and summer of 2013 and 2014, and aboveground productivity in June 2013 and Sept 2014. We placed a 0.5 × 0.5 m^2 quadrat in the center of each plot and identified and tallied every plant that hit one of the 25 points in the grid. In order to capture information on rare species that would not have been accounted for otherwise, we assigned an arbitrary value of 1% cover (0.25 of a point out of 25 total points) to plants that were in the quadrat but not touching a grid point. At the time of sampling, all plant species that occurred within the full 1 m^2 plot were recorded and used for species richness comparisons. To calculate absolute cover, we divided the number of hits per species per plot by the total possible number of hits. For relative cover, we divided the number of times that each species hit a grid point by the total number of hits for all species in the plot. Measurements were made on June 19–20 and Aug 26–29, 2013 and on June 10 and Sept 3, 2014 to capture both cool-season and warm-season species responses.

We measured aboveground productivity by clipping all live plants within a 20 × 50 cm^2 quadrat placed in one corner of the 1 × 1 m^2 measurement area taking care not to clip in the same location where species composition was measured. Measurements were made in the late spring of 2013, from June 21–24. As we clipped, we separated plants into the following groups: *B. tectorum,* other introduced grasses, *P. smithii,* other native grasses, introduced forbs, and native forbs. Samples were dried for 48 h at 60 °C and weighed.

To test whether differences existed in the source of water used between *B. tectorum* and *P. smithii*, we compared stable isotope ratios of hydrogen and oxygen in plant xylem water from each species and soil water under different precipitation treatments and seasons. We collected non-photosynthetic crown tissue from *B. tectorum* (20–30 individuals $plot^{-1}$) and *P. smithii* (10–20 individuals $plot^{-1}$) twice per year in a subset of 16 plots, half of which received winter-wet and half received ambient precipitation treatments. To capture seasonal patterns, we sampled on April 21 and June 18, 2013 and April 29 and June 20, 2014. The spring sampling period in April occurred a few weeks after individuals of both species had started to germinate or become active. The summer sampling period was timed to occur before *B. tectorum* senescence, which takes place in late June- early July at the site. Samples were collected within the larger 3 × 3 m^2 plots so as to experience the precipitation and N treatments, but not the removal treatments. Because the sampling was destructive, we collected plant tissue only from areas of the 3 × 3 m^2 plot where no other sampling was taking place. For *B. tectorum* individuals, we pulled up the entire plant and cut off the roots and shoots, collecting only the crown tissue. Because *P. smithii* is a perennial plant, we scraped away the soil from the base of the plant and cut and collected the crown tissue leaving the roots in the ground and discarding the shoots. Surface soil samples (to a depth of 5 cm) were collected at each plot directly adjacent to locations of plant sampling (within the larger 3 × 3 m^2 plots, but not in an area that was being sampled for any other measurements) with a 5-cm-diameter corer. Mid-level soil samples (30 cm depth) were also collected at each point during spring 2013, but we reduced sampling effort in subsequent campaigns when it was clear that this layer was relatively homogenous across the site. To characterize deeper soil layers, we dug three soil pits (at high, mid, and low points in the meadow) and collected three samples per depth at 30, 50, and 70 cm. All plant tissue and soil samples were placed in sealed exetainer vials (Labco, UK) and immediately stored in a cooler on ice in the field. Samples were stored under refrigeration at 1-2 °C in the lab until analysis.

Water was cryogenically extracted from plant and soil samples under vacuum and then analyzed for the stable oxygen ($\delta^{18}O$) and hydrogen (δD) isotope ratios in the Stable Isotope Mass Spectrometry Laboratory at Kansas State University. Water samples were analyzed using a Picarro WS-CRDS isotopic water analyzer using ChemCorrect software to identify possible interference or sample contamination. The stable isotopic ratios of water samples ($\delta^{18}O$ and δD) are reported as deviations from international standards using δ- notation in parts per thousand (‰):

$$\delta = \left[\left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000\right] \tag{1}$$

where R is the absolute ratio of the rare to common isotope, respectively. In-house working standards were calibrated to the recommended primary water standards:

GISP, SLAP, V-SMOW. Variability of working standards within runs were <0.5‰ and 1.0‰ (for $\delta^{18}O$ and δD) and analysis of QA/QC standards were within 0.25‰ of calibrated value.

Data analysis

To test our first two hypotheses, we compared biomass, abundance of key functional groups, species composition, and biodiversity by removal and N treatments within each year. Both biomass and cover data were separated into the following groups: *B. tectorum*, *P. smithii*, total introduced grass (including *B. tectorum*), total native grass (including *P. smithii*), introduced forbs, native forbs, and introduced species dominance (calculated as abundance or biomass of all introduced species combined divided by total abundance or biomass). For each group, we analyzed differences by treatment with 2-way ANOVAs with fixed effects factors of N-treatment and removal treatment. We followed ANOVAs with post-hoc Tukey HSD tests when main effects were significantly different. To test differences in total cover and cover of each functional group between an average year (2013) and wet year (2014), we ran 3-way ANOVAs with fixed effects factors year, removal, and nitrogen treatment on a combined 2013 + 2014 dataset with ambient precipitation plot data only in spring and summer. Data from the winter wet plots were excluded from the analysis because removal treatments were not effective in those plots in 2013. Had they been included, effectiveness of removal treatments between the two years would be confounded with the environmental conditions that changed (which was what we were primarily interested in here). Variables that did not conform to the assumptions of ANOVA (because they were skewed) were normalized through log-transformation before analysis.

Species composition data were analyzed using Permutational Multivariate Analysis of Variance (PerMANOVA) after visualization with Nonmetric Multidimensional Scaling (NMDS) for each season (spring, summer) and year (2013, 2014) separately to test treatment effects on early versus late-season species after 1 and 2 years of removals. Analyses were performed on both raw (absolute abundance) and 4th root transformed data to test for differences in composition weighted for abundant versus rare species, respectively. We were most interested in the effects of *B. tectorum* and *P. smithii* on the rest of the plant community, so we analyzed each dataset both with and without these focal species included. We generated NMDS graphs with Bray–Curtis dissimilarities using random starts to find a stable solution (metaMDS, vegan package, R Statistical Software). To minimize stress, we used a 3-dimensional configuration. We analyzed differences in species composition by N-treatment, removal treatment, and their interaction using 2-way crossed PerMANOVAs with the adonis function in R (vegan package, again using Bray-Curtis dissimilarities). Prior to each analysis, homogeneity of multivariate dispersions within groups was tested using permutational analysis of multivariate dispersions (PERMDISP; using the permdisp function in R) to confirm that data conformed to the assumptions of the test.

We calculated and compared the following diversity indices for each plot using raw cover data: species richness, Shannon's diversity, Fisher's alpha, and Pielou's evenness (vegan package, R). Using 2-way ANOVAs by removal and N treatment (both fixed-effects factors), we analyzed differences in each of these indices with separate tests for each year and season.

Finally, we tested differences in stable isotopic ratios of $\delta^{18}O$ and δD in soil water and xylem water of *B. tectorum* and *P. smithii*. We used three-way ANOVAs to test whether $\delta^{18}O$ or δD differed by precipitation treatment (ambient precipitation, increased winter precipitation), season (spring vs summer), or water pool (surface soil, mid-deep soil, *B. tectorum, P. smithii*). Variables were tested for normality prior to running analyses to ensure that the assumptions of the test were met. When main effects were significant, we followed up ANOVAs with post-hoc Tukey HSDs. These analyses provide context of where plants were getting their water. For example, if plants are taking up water from a single source, we would expect isotopic signatures between plant xylem and that source to be identical. However, many plants take up water from multiple depths in the soil. To identify the degree to which each species was likely to be taking up water from various soil layers, we used the SIAR package in R to create isotopic mixing models (Parnell et al. 2010). These models describe the proportional contribution of multiple soil water sources: surface (5 cm depth) and mid-deep layers (collected at 30, 50, and 70 cm), using both $\delta^{18}O$ and δD data. We averaged results from the mid-deep soil layers because isotopic signatures of soil water between 30 and 70 cm depth were similar. Data were classified according to species, season, year, and precipitation treatment.

All statistical analyses were performed in R (v 3.1.1) and R Studio (V 0.98.994; The R Foundation for Statistical Computing), and we considered outcomes of analyses to be statistically significant using an α-level of 0.05. Variation of measurements is reported as standard error of the mean in all cases, unless stated otherwise. The datasets generated and analyzed during the current study are available in the Figshare repository (Concilio et al. 2016a).

Results

Effectiveness of removal treatments

In 2013, we removed 11.8 ± 3.8 g m^{-2} on average (±SEM) of *B. tectorum* and 8.4 ± 1.9 g m^{-2} of *P. smithii*. We estimated that *B. tectorum* density averaged 739 ± 248 individuals m^{-2} and *P. smithii* averaged 73 ± 16 tillers m^{-2} based on average mass per individual at the time of removal. In winter-wet plots, *B. tectorum* grew back to the same cover levels as no-removal plots, so we compared response to removal only in ambient precipitation plots in 2013. With these criteria, *B. tectorum* and *P. smithii* made up ≤36% cover in removal plots, and were reduced by 45 and 60%, respectively, compared to no-removal controls. Specifically, *B. tectorum* made up an average of 23.3 ± 8.9% cover in control no-removal plots compared to 12.8 ± 4.6% cover in the plots from which it was removed; *P. smithii* made up 27.6 ± 7.4 and 12.4 ± 3.8% cover in control versus removal plots, respectively.

In 2014, removal treatments were more effective as we weeded out the target species biweekly throughout the growing season. We removed a total of 22.2 ± 2.7 g m^{-2} of *B. tectorum* and 19.2 ± 2.9 g m^{-2} of *P. smithii* throughout the season, which corresponded to estimates of 1107 ± 149 individuals m^{-2} of *B. tectorum* and 288 ± 40 tillers m^{-2} of *P. smithii*. Cover of *B. tectorum* and *P. smithii* were on average 87 and 89% lower, respectively, in the plots from which they were removed compared to no removal controls in 2014.

Response of the plant community to removal treatments under ambient and low N conditions

With removal of both *B. tectorum* and *P. smithii,* we found that the main response was an increase in introduced species, whereas native species showed little response to removal treatments (Fig. 2). In the spring 2013 season, introduced forbs (primarily *Erodium cicutarium*) benefited from *B. tectorum* removal with increased productivity (though the difference was only marginally significant; $F_{2,24} = 2.82$, $p = 0.078$; Tukey HSD: $p = 0.08$) and *B. tectorum* increased in growth in plots where *P. smithii* was removed ($F_{2,24} = 4.96$, $p = 0.016$, Tukey HSD: $p = 0.01$). In contrast, total native productivity did not differ by removal, soil N, or their interaction, nor did native grasses (aside from *P. smithii*) or forbs ($p \geq 0.05$ in all cases). Further, although the low N treatment generally favored natives and reduced introduced species, functional group responses to removal treatments were not affected by N conditions (i.e., the interaction of N and removal treatment was not significant for any of the variables we tested). Total ANPP in spring 2013 was reduced under reduced N conditions ($F_{1,\ 24} = 4.6$, $p = 0.042$), but did not differ by removal treatment ($F_{2,\ 24} = 0.46$, $p = 0.64$).

Cover responses of plant functional groups in the spring of 2013 and 2014 roughly paralleled those of biomass (Table 1). Percent introduced cover was different by removal treatment in 2014, with higher levels in the *P. smithii* removal plots (61 ± 3.1%) compared to *B. tectorum* removal (45 ± 2.6%; $p = 0.0003$) and marginally so compared to controls (53 ± 3.0%; $p = 0.085$). Introduced forbs responded to removal treatments with greatest abundance in *B. tectorum*-removal plots (68 ± 9.7% cover) and least in controls (49 ± 4.9) in 2014 ($F_{2,54} = 5.7$, $p = 0.006$; differences were not significant in 2013). Native forbs were not different by removal treatment, and native grasses only differed in that they were lower in cover in *P. smithii*-removal plots (presumably due to the effectiveness of the removal treatment) in 2013 ($p = 0.05$) and 2014 ($p = 0.04$). The interaction of N and removal treatments was not significant for any of the variables we tested, although the low N treatment favored natives over introduced species. Introduced dominance was higher in ambient N than low N plots in spring of 2013 (48 ± 5.7% compared to 25 ± 4.3; $p = 0.004$) and 2014 (56 ± 3.3 compared to 46 ± 3.6%; $p = 0.005$). Introduced grasses showed the largest response to N treatments, with 240 and 103% higher cover in ambient N compared to low N plots in 2013 (50 ± 8.1 compared to 15 ± 4.4% cover; $p = 0.005$) and 2014 (69 ± 13 compared to 34 ± 8.3% cover; $p = 0.0003$), respectively. Introduced forb cover was also higher in ambient

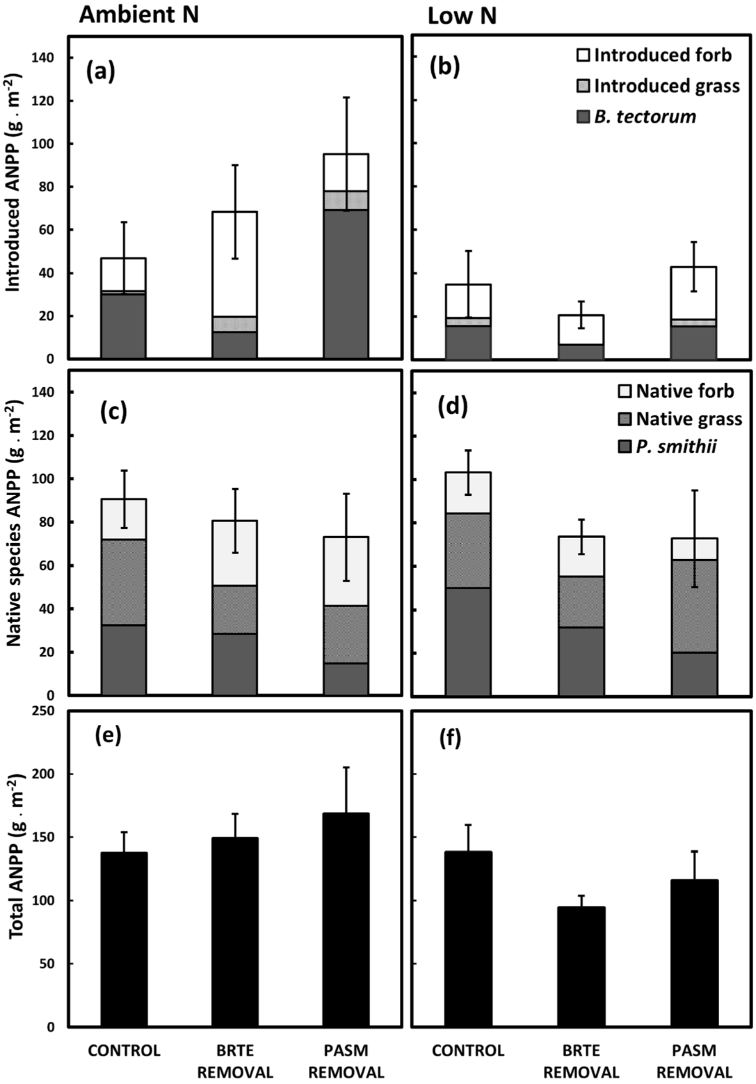

Fig. 2 Aboveground biomass of **a**, **b** introduced and **c**, **d** native grasses and forbs and **e**, **f** total ANPP in response to removal treatments under high (*left-panels*) and low (*right panels*) nitrogen conditions in spring 2013. Removal treatments included no-removal control, removal of introduced grass *Bromus tectorum* (BRTE), and removal of native grass *Pascopyrum smithii* (PASM). The introduced grass group includes all introduced grasses aside from *B. tectorum*, and the native grass group includes all native grasses aside from *P. smithii*. Samples were collected in ambient precipitation plots (n = 30). Bars represent the standard error of the mean of **a**, **b** total introduced ANPP, **c**, **d** total native ANPP, and **e**, **f** total ANPP, including both introduced and native grasses and forbs

compared to low N plots by 80 and 33% respectively in 2013 (from 40 ± 5.7 to 22 ± 5.2% cover; $p = 0.01$) and 2014 (from 67 ± 5.1 to 50 ± 6.5% cover; $p < 0.0001$). Native forbs were reduced in 2014 by 85% (from 39 ± 3.2 to 22 ± 4.3% cover; $p = 0.0006$) in response to the low N treatment. In contrast, native grasses had higher cover in low N compared to ambient N plots in 2013 (82.1 ± 6.2 and 53 ± 7.1% cover, respectively; $p = 0.003$).

We hypothesized that in plots where *B. tectorum* was removed, more resources would be available for warm-season species and we would therefore see increased productivity in those plots over control no-removal plots during the summer season. However, native warm-season species response to treatments was minimal (Fig. 3; Table 2). The removal treatments did affect native grass cover in both years, but not in the direction that we predicted. Post-hoc comparisons revealed that native grass cover remained significantly lower where *P. smithii* had been removed compared to control no removal plots in 2013 ($p = 0.0007$), but showed no difference in *B. tectorum* removal plots. In 2014, native grass cover was lower in *P. smithii* removal than *B. tectorum* removal plots ($p = 0.015$), but there was no difference between control no-removal plots and *B. tectorum* removal plots. There was no interaction of removal and N treatments, and no difference in native grass or forb cover by N treatment in either year, aside from a marginally significant decrease in native forb biomass in low N plots in 2014 ($p = 0.08$).

Table 1 Results from ANOVA tests on springtime cover of four functional groups (introduced forbs, introduced grasses, native forbs, and native grasses), dominance of introduced species (% cover Introduced species/ total cover) and four diversity metrics (species richness, Shannon's diversity, Fisher's alpha, and Pielou's evenness) in 2013 and 2104 by removal treatment (*B. tectorum* removal, *P. smithii* removal, no removal), nitrogen treatment (ambient N, low N), and their interaction. Data were collected in June of 2013 ($n = 30$, collected in ambient plots alone) and 2014 ($n = 60$, collected in both ambient and winter-wet treated plots). Significant results at or below the $\alpha = 0.05$ level are starred with an asterisk

	2013			2014		
	Removal d.f. = 2, 24	Nitrogen d.f. =1, 24	Removal * Nitrogen d.f. = 2, 24	Removal d.f. = 2, 54	Nitrogen d.f. = 1, 54	Removal * Nitrogen d.f. = 2, 54
Introduced forb	$F = 0.87, p = 0.43$	$F = 7.5, p = 0.01$ *	$F = 0.88 , p = 0.43$	$F = 5.7, p = 0.006$ *	$F = 18, p < 0.0001$ *	$F = 0.57, p = 0.57$
Introduced grass	$F = 0.25, p = 0.77$	$F = 9.5, p = 0.005$ *	$F = 0.75, p = 0.48$	$F = 42, p < 0.0001$ *	$F = 15, p = 0.0003$ *	$F = 0.62, p = 0.54$
Native forb	$F = 0.87, p = 0.43$	$F = 3.8, p = 0.06$	$F = 0.29, p = 0.75$	$F = 17, p = 0.84$	$F = 13.4,p = 0.0006$ *	$F = 0.27, p = 0.76$
Native grass	$F = 3.7 , p = 0.04$ *	$F = 10.73, p = 0.003$ *	$F = 0.33, p = 0.73$	$F = 4.39, p = 0.017$ *	$F = 0.45, p = 0.84$	$F = 0.27, p = 0.76$
% Introduced	$F = 1.9 , p = 0.18$	$F = 10.4, p = 0.004$ *	$F = 0.16, p = 0.85$	$F = 8.9, p = 0.0005$*	$F = 8.7, p = 0.005$ *	$F = 0.08, p = 0.92$
Total cover	$F = 0.03 , p = 0.97$	$F = 14.8, p = 0.0008$ *	$F = 0.06, p = 0.95$	$F = 4.0, p = 0.02$ *	$F = 47, p < 0.001$ *	$F = 0.37, p = 0.69$
Species richness	$F = 0.14 , p = 0.87$	$F = 0.13, p = 0.73$	$F = 0.63, p = 0.54$	$F = 1.6, p = 0.20$	$F = 3.31, p = 0.07$	$F = 0.24, p = 0.79$
Shannon	$F = 0.03, p = 0.97$	$F = 5.4, p = 0.29$	$F = 0.74, p = 0.49$	$F = 4.2, p = 0.19$	$F = 6.6, p = 0.01$ *	$F = 0.20 , p = 0.82$
Fisher's alpha	$F = 0.11, p = 0.89$	$F = 0.34, p = 0.57$	$F = 0.87, p = 0.43$	$F = 1.3, p = 0.27$	$F = 0.02, p = 0.89$	$F = 0.28, p = 0.75$
Evenness	$F = 0.06, p = 0.94$	$F = 15, p = 0.0008$ *	$F = 0.49, p = 0.62$	$F = 6.98, p = 0.002$ *	$F = 6.04, p = 0.02$ *	$F = 0.002 , p = 0.99$

We hypothesized that diversity would increase after *B. tectorum* removal. However, none of the biodiversity metrics that we tested differed by removal in spring or summer of 2013, and differences that were observed in 2014 were not in the direction that we expected (Tables 1 & 2). Pielou's evenness was lower after *P. smithii* removal compared to control plots in the spring ($p = 0.014$) and summer ($p = 0.01$) of 2014, and Shannon diversity was lower in *P. smithii* removal plots in spring 2014 ($p = 0.02$). *Bromus tectorum* removal had no effect on evenness or diversity. We also found that some diversity indices were different by N treatment: Shannon's diversity was higher in ambient than reduced N plots in spring 2014 and summer 2013, as was Pielou's evenness in spring of both years and summer of 2013. However, there was no interaction of N and removal treatment for any of the variables we tested.

Lastly, we tested differences in plant community composition in response to removal and N treatments (Fig. 4, Online Resources 2 & 3). Community analyses were done on two datasets: one with all plant species included, and the other with all species aside from our two focal grasses, *B. tectorum* and *P. smithii*. Although results from several of our PerMANOVA tests were significant, neither removal nor N treatment explained much of the variation in the samples (Online Resources 2 & 3). Significant results ($p < 0.05$) ranged from 0.03 to 0.25 in R^2 values- meaning that at the low end, only 3% of the variation in species composition could be explained by treatments. In general, more variation was explained by performing the analyses on raw data compared to square root transformed data- suggesting that differences in community structure were driven by common rather than rare species (Online Resource 2). Including *B. tectorum* and *P. smithii* helped explain more variation (Fig. 4a; Online Resource 2). When the two focal species were removed from the analysis, however, NMDS graphs showed very little differentiation among sites (Fig. 4b) and PerMANOVA tests showed corresponding low R^2 values (Online Resource 3). In general, results from 2014 (when removal treatments were more effective) showed stronger treatment effects than 2013.

Inter-annual differences in plant community response to removals

Total cover in spring 2014 was about 26% higher than that in 2013 ($F_{1,48} = 18.7, p < 0.0001$), but this increase did not vary by removal or N treatment. Most of the increase was due to higher introduced grass ($F_{1,48} = 4.6$, $p = 0.038$) and forb ($F_{1,48} = 23.9$, $p < 0.0001$) cover,

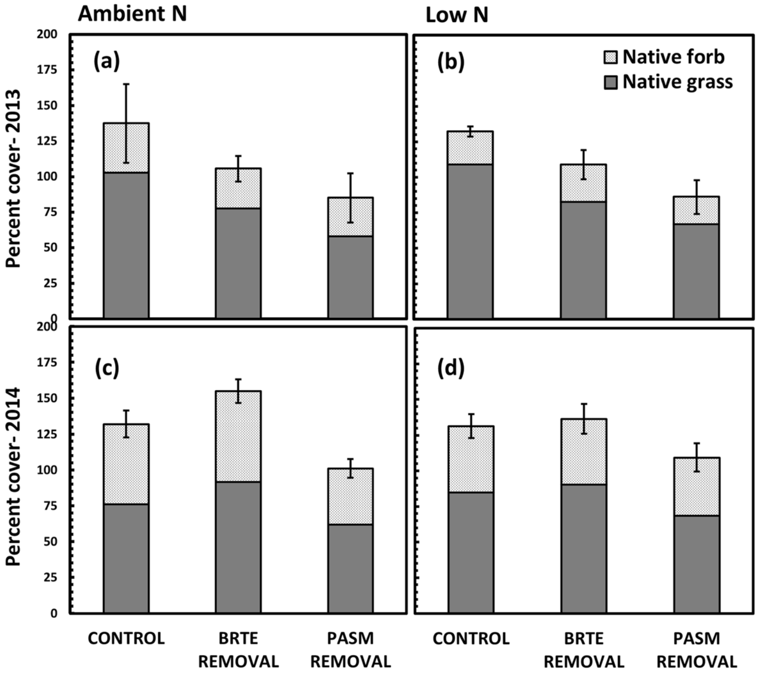

Fig. 3 Cover of native forbs and grasses during the summer growing season, collected in August 2013 in **a** ambient N and **b** low N plots and early Sept 2014 in **c** ambient N and **d** low N plots. Data were collected in ambient precipitation plots alone in 2013 (n = 30) and in both ambient precipitation and winter-wet treated plots in 2014 (n = 60). Most of the introduced grasses and forbs had senesced at this time, and are therefore not included. Bars represent the standard error of the mean native (forb + grass) cover

whereas neither native grass nor forb cover was different by year; consequently, introduced species dominance increased in 2014 ($F_{1,48}$ = 13.9, p = 0.0005; Online Resource 4). In the late summer sampling period, total cover was still increased in 2014 over 2013 ($F_{1,47}$ = 11.2, p = 0.002), but to a lesser degree (by an average of 14%). Native forb cover increased by about 40% in 2014 over 2013($F_{1,47}$ = 4.6, p = 0.037), while native grasses were not different by year (p > 0.05; Fig. 3).

Seasonal source water contributions to *B. tectorum* and *P. smithii*

We found significant differences in isotopic ratios of δ^{18}O and δD by pool (source or species) and season in 2013 and 2014, but not by precipitation treatment (Table 3). Notably, we found significant differences in isotopic signature between soil water from surface compared to deeper layers in summer (but not spring) of both years (Table 4). Surface

Table 2 Results from ANOVA tests on summer season cover of two functional groups (native forbs and native grasses), and four diversity metrics (species richness, Shannon's diversity, Fisher's alpha, and Pielou's evenness) in 2013 and 2104 by removal treatment (*B. tectorum* removal, *P. smithii* removal, no removal), nitrogen treatment (ambient N, low N), and their interaction. Data were collected in August of 2013 (n = 30, collected in ambient plots alone) and early September of 2014 (n = 60, collected in both ambient and winter-wet treated plots). Significant results at or below the α = 0.05 level are starred with an asterisk

	2013			2014		
	Removal d.f. = 2, 24	Nitrogen d.f. = 1, 24	Removal * Nitrogen d.f. = 2, 24	Removal d.f. = 2, 54	Nitrogen d.f. = 1, 54	Removal * Nitrogen d.f. = 2, 54
Native forb	$F = 0.03, p = 0.97$	$F = 0.31, p = 0.58$	$F = 0.16, p = 0.86$	$F = 1.1, p = 0.35$	$F = 3.2, p = 0.08$	$F = 0.66, p = 0.52$
Native grass	$F = 9.4, p = 0.0009$ *	$F = 0.67, p = 0.42$	$F = 0.02, p = 0.97$	$F = 4.2, p = 0.02$ *	$F = 0.41, p = 0.5$	$F = 0.18, p = 0.84$
Species richness	$F = 0.94, p = 0.41$	$F = 2.2, p = 0.15$	$F = 0.84, p = 0.44$	$F = 0.89, p = 0.42$	$F = 3.0, p = 0.09$	$F = 1.0, p = 0.37$
Shannon	$F = 0.44, p = 0.65$	$F = 6.5, p = 0.02$ *	$F = 2.5, p = 0.10$	$F = 2.7, p = 0.08$	$F = 1.3, p = 0.27$	$F = 1.6, p = 0.22$
Fisher's alpha	$F = 0.17, p = 0.84$	$F = 0.97, p = 0.34$	$F = 1.7, p = 0.20$	$F = 0.27, p = 0.76$	$F = 1.6, p = 0.21$	$F = 0.91, p = 0.41$
Evenness	$F = 0.04, p = 0.96$	$F = 4.4, p = 0.05$ *	$F = 2.1, p = 0.15$	$F = 5.1, p = 0.009$ *	$F = 0.22, p = 0.64$	$F = 0.63, p = 0.54$

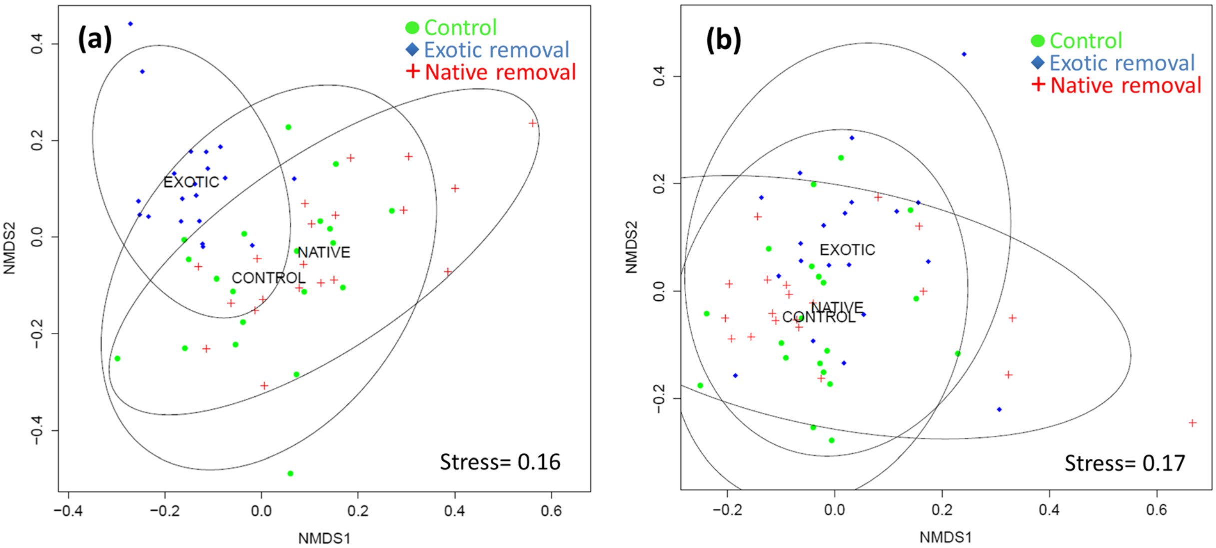

Fig. 4 NMDS graphs from June 2014 species composition data, using Bray-Curtis dissimilarities with **a** all data included, **b** all data aside from *B. tectorum* and *P. smithii* included. Ellipses represent 95% confidence intervals

soil water generally had higher values of δ^{18}O and δD than deeper soil layers, indicative of evaporative enrichment in surface soils. This effect was most pronounced in the summer season. Water extracted from xylem tissue of *B. tectorum* differed from that of *P. smithii* in the summer season of both years, indicating a difference in source water use among species for this time period.

To determine the most likely source contributions for each species, we used isotopic mixing models. We found that in the spring season of both years, both species were using water mainly from mid-deep soil water sources (Fig. 5). During this time, isotopic signatures did not show strong differentiation between surface and deeper sources possibly because soil moisture levels were relatively high in the surface layers (Fig. 1) and evaporative enrichment had not occurred yet. In the drier summer season, soil water from surface and deeper sources could be clearly differentiated, as could differences in xylem water isotopic signatures from the two focal grass species. During this dry season, *B. tectorum* was using an estimated 60% of its water from surface soils and 40% from deeper soil layers, whereas *P. smithii* showed the opposite pattern of resource use with about 65% of its water uptake coming from deeper layers. Results presented in Fig. 4 are from 2013 data alone, but patterns in 2014 were similar (Online Resource 5).

Table 3 Results from 3-way ANOVAs testing effects of season (spring vs. summer), water pool (*Bromus tectorum* xylem water, *Pascopyrum smithii* xylem water, surface soil water, mid-deep soil water) and precipitation treatment (ambient precipitation, increased winter precipitation) on isotopic ratios of δ^{18}O and δD in 2013 and 2014

		2013					2014			
		δD		δ^{18}O			δD		δ^{18}O	
Factor	d.f.	F	P	F	P	d.f.	F	P	F	P
Season	1, 97	98.11	< 0.0001	271.4	< 0.0001	1, 95	112.6	< 0.0001	144.6	< 0.0001
Pool	3, 97	18.56	< 0.0001	36.55	< 0.0001	3, 95	73.68	< 0.0001	71.85	< 0.0001
Precip	1, 97	0.037	0.848	0.029	0.864	1, 95	0.353	0.554	0.0021	0.964
Season * Pool	3, 97	13.27	< 0.0001	36.58	< 0.0001	3, 95	19.61	< 0.0001	13.15	< 0.0001
Season * Precip	1, 97	0.0001	0.991	0.0004	0.984	1, 95	0.017	0.896	0.354	0.553
Pool * Precip	3, 97	0.099	0.061	0.355	0.786	3, 95	0.144	0.865	0.275	0.761
Season * Pool * Precip	2, 97	0.126	0.882	0.459	0.633	2, 95	0.359	0.699	1.709	0.186

Table 4 Isotopic composition of plant xylem water in an invasive (*Bromus tectorum*) and native (*Pascopyrum smithii*) grass and soil water in the spring (April) and summer (June) of 2013 and 2014. Soils were collected at 5 cm (Surface soil) and 30-70 cm (Mid-Deep Soil). Data are means ± standard deviation of O^{18} ($\delta^{18}O$) and deuterium (δD) isotopic ratios. Samples sizes were different by year and season due to challenges extracting enough xylem water for analysis during the drier summer season and a reduced sampling effort with mid-deep soils after spring 2013. Lowercase letters indicate significantly different means within each season and year (α = 0.05)

	B. tectorum	*P. smithii*	Surface Soil	Mid-Deep Soil
Spring 2013, *n* = 64				
δD	-125.0 ± 11.1 ^{ab}	-128.4 ± 8.6 ^{a}	-114.8 ± 5.4 ^{c}	-119.5 ± 6.0 ^{bc}
$\delta^{18}O$	-14.3 ± 1.9 ^{b}	-15.9 ± 1.1 ^{a}	-14.8 ± 0.9 ^{ab}	-15.2 ± 1.2 ^{ab}
Summer 2013, *n* = 48				
δD	-98.0 ± 9.4 ^{b}	-108.8 ± 8.5 ^{ab}	-86.9 ± 17.1 ^{c}	-125.6 ± 10.9 ^{a}
$\delta^{18}O$	-8.2 ± 2.3 ^{b}	-11.7 ± 1.4 ^{b}	-4.6 ± 2.9 ^{c}	-15.4 ± 2.4 ^{a}
Spring 2014, *n* = 56				
δD	-120.8 ± 8.6 ^{a}	-120.2 ± 6.8 ^{a}	-98.7 ± 11.1 ^{b}	-103.6 ± 13.2 ^{b}
$\delta^{18}O$	-13.1 ± 1.8 ^{b}	-14.9 ± 1.0 ^{a}	-8.5 ± 2.3 ^{c}	-12.9 ± 1.6 ^{b}
Summer 2014, *n* = 53				
δD	-87.7 ± 8.2 ^{c}	-117.1 ± 6.7 ^{a}	-73.4 ± 5.4 ^{d}	-99.3 ± 9.5 ^{b}
$\delta^{18}O$	-7.1 ± 1.5 ^{b}	-10.9 ± 0.87 ^{a}	-6.4 ± 1.7 ^{b}	-12.4 ± 1.2 ^{a}

Discussion

Here, we found evidence to suggest that *Bromus tectorum*, which has increased in dominance throughout the Colorado Front Range mixed-grass prairie in recent decades, is behaving more like a passenger than driver of change in this system. As one would predict, removal of the environmental stressor (excess N deposition) alone led to a native-dominated system whereas removal of *B. tectorum* did little to affect plant community

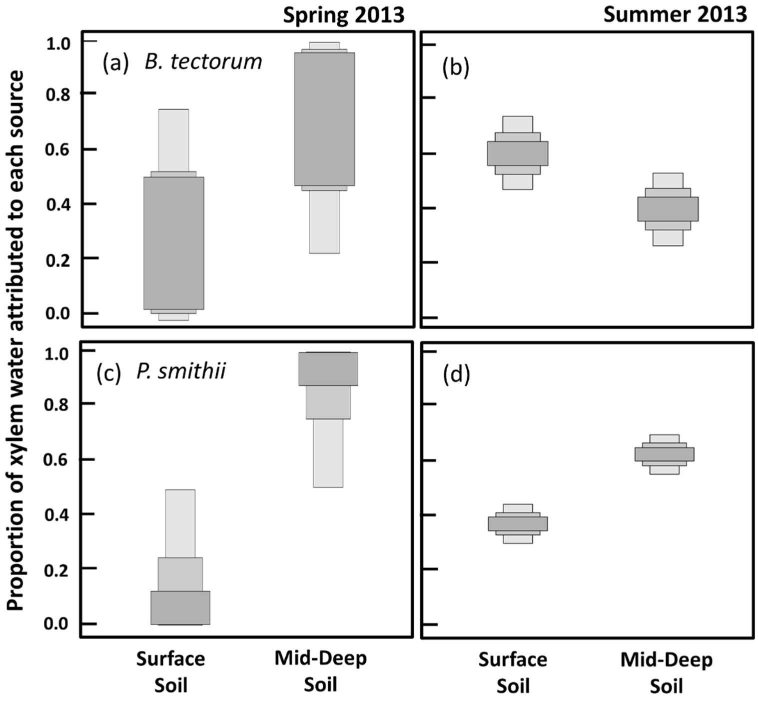

Fig. 5 Results from an isotopic mixing-model, showing the estimated proportion of water used by **a**, **b** *Bromus tectorum* and **c**, **d** *Pascopyrum smithii* from soil surface and deeper sources in the spring (**a**, **c**) and summer (**b**, **d**) seasons of 2013. Boxes represent the 50, 75, and 95% probability intervals for each source

composition. Instead, *B. tectorum* appears to be opportunistically responding to changing environmental conditions through a niche difference with resident species. Several lines of evidence from this research and other published studies support this finding: (1) Past work has shown that *B. tectorum* is favored over native species with increased winter precipitation and soil nitrogen availability (Concilio et al. 2015a; Concilio et al., 2016b; Prevéy and Seastedt 2014) and both these changes have been occurring in the Front Range (Baron et al. 2000; Prevéy 2014) concurrent with shifting plant community change. (2) We found here that removal of *B. tectorum* did not result in increased biodiversity or native species cover in the spring or summer season, suggesting that environmental conditions, not presence of *B. tectorum*, may be a more important determinant of native species cover and growth. (3) Finally, results from our isotopic analysis of xylem water indicate that *B. tectorum* and *P. smithii* are taking up water from different soil layers during the dry summer season, but not the spring. This suggests that niche differentiation may act as a means of stable coexistence for these two cool-season species in this system. There are several caveats to these findings and implications for management, discussed as follows.

Results from our multivariate analyses showed that less than 10% of the variation in community composition (of all species aside from *B. tectorum* and *P. smithii*) was due to removal treatment, indicating that other environmental variables (not presence of *B. tectorum* or *P. smithii*) were more important for driving differences in composition across plots. This suggests that *B. tectorum*'s direct impacts on the native plant community are likely to be minimal. However, recruitment or dispersal limitation could provide an explanation for the lack of response by the native plant community to removal treatments that we measured (e.g., Seabloom et al. 2003). For example, the seedbank may have been depleted of native species. We would expect this effect to be most pronounced for annual species, most of which are forbs at this site. We cannot rule out the possibility that *B. tectorum* has reduced annual forb diversity. However, most native grasses at the site are perennial and spread primarily via vegetative reproduction. We expected to measure increases in cover by these species in response to *B. tectorum* removal, particularly the fast-growing rhizomatous *P. smithii*. In contrast to our findings, other researchers have found that removal of annual brome species, including *Bromus tectorum* and the co-occurring *B. japonicus*, led to increases in native cool-season perennials in the northern mixed grass prairie, including *P. smithii* (Haferkamp et al. 1998; Haferkamp and Heitschmidt 1999). These researchers hypothesized that brome grasses can competitively displace *P. smithii*. If this were true at our site, we would have expected *P. smithii* to increase in growth after *B. tectorum* was removed. However, we found the opposite to be true: *B. tectorum* increased after *P. smithii* was removed. Our experiment was run over two years only, an average and above-average precipitation year. We may have found different results in a year when water was more limiting. Alternatively, there may be regional differences in the mechanisms and impacts of *B. tectorum* even within the same type of grassland, depending for example on soil chemical or biological characteristics.

A second caveat is that although removal of an invasive species that is acting as a driver should result in direct benefits for native species (MacDougall and Turkington 2005), those benefits may take time to emerge. Drivers of change can act via different mechanisms; depending on how they are affecting change, recovery of the native plant community may occur over a shorter or longer time-frame. We hypothesized that *B. tectorum* was driving change via competitive displacement in this system, and expected to measure a relatively quick response to its removal within the two years of our experiment. However, if *B. tectorum* was impacting ecosystem function, recovery of the native plant community would likely be delayed and not captured by our study. Therefore, we cannot rule out the possibility that *B. tectorum* is affecting soil chemical, physical, or biological characteristics or composition in this system in a way that inhibits growth or germination of native species even after its removal- i.e., through a legacy effect (Corbin and D'Antonio 2011). Other researchers have found that introduced species invasion can result in altered soil microbial community composition which favors invasive over native plant growth (e.g., Bozzolo and Lipson 2013). *Bromus tectorum* has been shown to alter soil biological communities of the Colorado Plateau (Belnap and Phillips 2001; Belnap et al. 2005), although it is uncertain whether these changes affect native species growth or germination. We find this scenario unlikely in our system given that sugar additions were so effective in increasing native plant growth and reducing invasive species growth at the site. Instead, our results coupled with past research suggest that the most likely way in which *B. tectorum* might be altering ecosystem properties at this site is by increasing N availability. Stark and Norton (2015) used a long-term experimental manipulation to demonstrate that *B. tectorum* created greater soil organic N pool sizes and stimulated N-cycling rates compared to similar-aged stands of sagebrush and native perennial grasses. Enhanced N can be found beneath *B. tectorum* at our site as well (O'Conner et al. 2015). Clearly, this N effect, in conjunction with winter precipitation, should benefit *B. tectorum* and other winter annuals whenever disturbance events diminish competition from native species. However, if this were the primary way in which *B. tectorum* was increasing in dominance at the site, we would expect to have measured an increase in native species diversity and/or growth in plots where both soil N and *B. tectorum* cover were reduced (i.e., sugar addition, *B. tectorum* removal plots), and we did not. There may be multiple mechanisms contributing to *B. tectorum* success in the region, and future research should focus on elucidating some of these gaps in our understanding.

Results from our isotopic analysis of xylem water suggest that *B. tectorum* and *P. smithii* were drawing water from the same soil layers early in the early spring season, and then diverged later in the season when surface soils were drier. *B. tectorum* drew a greater proportion of water from surface sources compared to *P. smithii* in the summer of both years, possibly because it has shallower roots and a greater proportion of shallow roots total. In contrast, *P. smithii* drew a greater proportion of its water from deeper sources regardless of season or year. For species like *P. smithii* that can access deeper water, coexistence with *B. tectorum* may be possible because of niche partitioning (Nippert & Knapp 2007). Although subsurface moisture is not inexhaustible, it remains high for much longer into the growing season because root density is lower and there is no evaporative loss. Many of the native warm season grasses and forbs at the site are deep rooted and may not have been impacted by *B. tectorum* for that reason. In years of very low water, we might expect to see impacts of *B. tectorum* on the community. The trend in this region over the last few decades has been toward a greater proportion of precipitation coming in the winter. If this trend continues, there may be plenty of soil moisture available for the coexistence of deep-rooted cool season species with *B. tectorum*, but dry summers are likely to impact warm season species (Prevéy and Seastedt 2014). Impacts on shallow rooted annual species are uncertain and may not have been captured in our experiment.

Despite the multiple caveats and uncertainties discussed above, our results highlight differences in invasion mechanisms and community-level impacts of the same species across different ecosystems. Although not historically considered problematic in the Great Plains (Karl et al. 1999), *B. tectorum* has had large impacts on ecosystems of the Intermountain West where it has come to dominate millions of hectares (Mack 1981). In this region, it is considered a transformer species for altering the fire cycle in a way which promotes its own spread and dominance (D'Antonio and Vitousek 1992), with cascading negative impacts on wildlife habitat, forage species, and biodiversity (Knapp 1996). Shrub-steppe ecosystems of the Great Basin may be particularly susceptible to *B. tectorum* invasion because (1) the climate is similar to that of *B. tectorum*'s native range, (2) native grasses of the region did not evolve with grazing pressure whereas *B. tectorum* did, and it is therefore more resilient to pressure by cattle, and (3) native grasses and shrubs do not resprout after fire and are slow-growing (Knapp 1996 and references therein). In contrast, resistance to annual grass dominance in the Great Plains has been attributed to the region's long history of association with herding ungulates and prevalence of rhizomatous grasses (Mack 1981; Karl et al. 1999). Thus, *B. tectorum* would not receive the same competitive advantage over native Great Plains grasses as it has over Great Basin grasses and shrubs with increases in grazing or fire. Further, the Great Plains historical climate pattern was more of a 'summer wet' system, a factor that allowed cool season grasses to exploit snowmelt moisture but also grow into the warmer months (Risser 1988). With changing climatic and edaphic conditions in the Colorado Front Range due to warming, shifts in seasonality of precipitation toward wetter winters, and increased nitrogen deposition, resource availability has increased at a time of year when many native mixed-grass prairie species are largely inactive. Our results support a scenario in which *B. tectorum* has been able to capitalize on these changing conditions and increase in dominance without outcompeting native species. This pattern is consistent with invasion theory, which predicts that species that gain a foothold in their invaded range due to niche differences (in our case, an earlier phenology), rather than fitness differences, are likely to become abundant without causing significant changes to the native community (MacDougall et al. 2009). Thus, this same introduced species, *B. tectorum*, has been increasing in abundance throughout large regions of the US West, but the reasons for its success are varied and so too are its impacts on the native plant community (ranging from almost complete displacement of some native plant communities in the Great Basin to coexistence in the Front Range). Accordingly, management perceptions and options should change as well.

In conclusion, our results suggest that the invasion of *B. tectorum* into Colorado Front Range mixed-grass prairie is being driven by changing environmental conditions. We cannot rule out the possibility that *B. tectorum* is impacting native plants in the region (e.g., during years of low soil moisture availability or via a legacy effect) and/or that it will not become problematic in the future. However, the removal of this invader alone will not result in restoration of the native plant community, at least in the short term. Management of this system for native dominance is likely to require (at the very least) shifting resource conditions over a landscape scale. This may be possible through high-level policy and/or regulatory changes that result in decreased anthropogenic N and greenhouse gas emissions. For individual land management and conservation agencies, however, landscape-level resource manipulation is an impractical goal. Instead, our results highlight the need for novel management techniques that aim to maintain or enhance ecosystem services in the mixed-grass prairie.

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