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Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming

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Abstract. High-elevation ecosystems are expected to be particularly sensitive to climate warming because cold temperatures constrain biological processes. Deeper understanding of the consequences of climate change will come from studies that consider not only the direct effects of temperature on individual species, but also the indirect effects of altered species interactions. Here we show that 20 years of experimental warming has changed the species composition of graminoid (grass and sedge) assemblages in a subalpine meadow of the Rocky Mountains, USA, by increasing the frequency of sedges and reducing the frequency of grasses. Because sedges typically have weak interactions with mycorrhizal fungi relative to grasses, lowered abundances of arbuscular mycorrhizal (AM) fungi or other root-inhabiting fungi could underlie warming-induced shifts in plant species composition. However, warming increased root colonization by AM fungi for two grass species, possibly because AM fungi can enhance plant water uptake when soils are dried by experimental warming. Warming had no effect on AM fungal colonization of three other graminoids. Increased AM fungal colonization of the dominant shrub *Artemisia tridentata* provided further grounds for rejecting the hypothesis that reduced AM fungi caused the shift from grasses to sedges. Non-AM fungi (including dark septate endophytes) also showed general increases with warming. Our results demonstrate that lumping grasses and sedges when characterizing plant community responses can mask significant shifts in the responses of primary producers, and their symbiotic fungi, to climate change.

Key words: Achnatherum; Carex; *climate change; dark septate endophyte; elevation; grass; infrared heating; mycorrhizal fungi;* Poa; *sedge; subalpine meadow, Rocky Mountains, Colorado, USA; temperature.*

Introduction

Understanding how communities of primary producers respond to warming is essential for predicting the effects of climate change on ecosystems and for identifying positive feedbacks between vegetation and climate (Luo 2007, Zhou et al. 2012). Past research has shown that warming can increase plant biomass (Rustad et al. 2001, Wu et al. 2011*b*), shift plant species composition and phenology (Walker et al. 2006, Wolkovich et al. 2012), and differ in its effects on particular plant functional groups (Lin et al. 2010, Elmendorf et al. 2012). For example, 12 years of experimental warming in Swedish tussock tundra doubled the biomass of evergreen shrubs relative to control plots, but reduced the biomass of the dominant sedge (Molau 2010). Identifying the species and functional groups most sensitive to warming can refine predictions on the community and ecosystem impacts of climate change.

High-elevation ecosystems are expected to be particularly sensitive to warming (e.g., Pauli et al. 2012) because of the temperature constraints on biological processes. Low temperatures can affect plant productivity by slowing growth and respiration (Scott and Billings 1964, Arft et al. 1999) and by limiting decomposition and nutrient cycling (e.g., Wu et al. 2011*a*). Climate warming can relax these constraints, but also can reduce water availability, with negative effects on plants (e.g., Harte et al. 1995, Huelber et al. 2011).

Warming may affect plants directly by changing physiology, phenology, or demographic rates (e.g., Price and Waser 1998, Dunne et al. 2003, Inouye 2008) or indirectly by altering interspecific interactions (Tylianakis et al. 2008, van der Putten 2012). Of particular interest are plant–microbe interactions, which are nearly ubiquitous, and can have large effects on plant performance (Rodriguez et al. 2009, Johnson et al. 2013). Given their rapid generation times, microbial populations may respond more quickly to environmen-

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tal conditions than plants, contributing indirectly to the primary-production response (Lau and Lennon 2012), yet these interactions are not well studied in high-elevation ecosystems.

Our recent meta-analysis showed that both aboveground and belowground fungal symbionts can buffer plants under drought and warming (Kivlin et al. 2013). Arbuscular mycorrhizal (AM) fungi and other root-associated fungi (Porras-Alfaro and Bayman 2011) may also be important in soil carbon pools by forming soil aggregates and producing recalcitrant fungal biomass (Clemmensen et al. 2013). Most studies in a recent synthesis showed positive effects of warming on AM fungal colonization and hyphal length, but the impacts of temperature varied among study systems (Compant et al. 2010). Drought increased the levels of root colonization in ~50% of studies (Auge 2001), but generally reduced extraradical hyphae, in part by reducing plant biomass (Staddon et al. 2003, Hawkes et al. 2011). AM fungi comprise the majority of plant–fungal symbioses in most ecosystems, but dark septate endophytes (DSEs) may be more beneficial than AM fungi at high latitudes/elevations (Alberton et al. 2010), where organic nutrient uptake is favored (Newsham 2011). DSEs occur in >600 plant species across a range of habitats (Read and Haselwandter 1981). However DSE responses to warming are not well resolved. In soils, extraradical septate hyphal biomass can decline with warming and drought (Allison and Treseder 2008, Hawkes et al. 2011), but the generality of this pattern remains uncertain. In some plants, DSEs confer drought or thermal tolerance (Olsrud et al. 2010); in others, DSE are detrimental (Reininger and Sieber 2012) or neutral (Olsrud et al. 2010).

We investigated the effects of experimental warming on the composition of graminoids and their fungal associates in a high-elevation ecosystem. A focus on graminoids is justified because of their global diversity (~10 000 grass species and ~5500 sedge species; Barker et al. 2011) and ecological importance (Shantz 1954). Early results from this same experiment showed no overall response in total graminoid biomass or cover, which comprised ~38% of aboveground plant biomass in the system (Harte and Shaw 1995, Price and Waser 2000). However, individual taxa may respond differentially to warming, with potential ecosystem-level consequences (e.g., Pendall et al. 2011). We examined species-specific responses following 20 years of warming in a subalpine meadow to ask: (1) Does experimental warming shift the relative frequency of grasses vs. sedges? (2) Does warming alter the species composition or diversity of graminoids? (3) Do subalpine plants have altered associations with soil fungi (mycorrhizal and non-mycorrrhizal) under warming?

Methods

Study site

The experiment was conducted in a subalpine meadow at the Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado, USA (38°57′ N, 106°59′ W; elevation, 2920 m). The study area spanned east–west gradients in elevation and moisture across a small glacial moraine (see illustrations in Harte et al. 1995, Price and Waser 2000), from a dry ridgetop dominated by sagebrush (*Artemisia tridentata*), rabbitbrush (*Ericameria parryi*), and bunchgrasses (*Festuca thurberi*, *Achnatherum* spp.) to a moist swale dominated by willow (*Salix* spp.), shrubby cinquefoil (*Dasiphora fruticosa* spp. *floribunda*), and monkshood (*Aconitum columbianum*) (elevation range, 1–2 m). The plant community also included ~60 herbaceous perennial forb species and a few annuals (Price and Waser 2000, de Valpine and Harte 2001).

Warming experimental design

Treatments were applied to 10 plots (10 × 3 m), each oriented with the long axis running from ridgetop to swale and alternating between warmed and control treatments ($n = 5$ plots/treatment). Beginning in fall of 1990 two electric heaters (15 W/m^2 infrared radiation) were suspended 1.5–2.5 m above the long axis of warmed plots. Heating began 6 January 1991. A third heater was added between the two existing heaters in May 1993, increasing total infrared flux to 22 W/m^2. From this point onwards, the treatment simulated soil surface heating expected under a doubling of atmospheric CO_2 (Harte and Shaw 1995). Heaters shaded ~2% of plot surface area in heated plots; no structures were hung over control plots to mimic this minimal shading.

Each plot was divided into three blocks: an upper dry ridgetop, a middle rocky slope, and a lower moist swale. In each block, soil temperature and moisture were logged every 2 h at 5, 12, and 25 cm depth. Experimental heating has warmed the top 15 cm of soil by ~2°C, dried it by 10–20% (gravimetric basis) during the growing season, and prolonged the snow-free season at each end by an average of ~12 d (Harte and Shaw 1995, Saleska et al. 2002). Effects of warming on abiotic conditions were strongest in the upper blocks (Harte and Shaw 1995, Harte et al. 1995). Responses of non-graminoids and ecosystem processes are reported elsewhere (Harte and Shaw 1995, Harte et al. 1995, Price and Waser 1998, 2000, Saleska et al. 1999, de Valpine and Harte 2001, Shaw and Harte 2001, Perfors et al. 2003, Lambrecht et al. 2007).

Graminoid censuses: belt transects

On 4–7 June 1990 (prior to warming), 15–16 June 1994 (26 d post-snowmelt, 4 yr of warming) and 5 July 2010 (54 d post-snowmelt, 20 yr of warming), we censused all graminoid species in 32 adjacent 0.25 × 0.25 m quadrats along a belt transect (0.25 × 8 m) parallel to the long axis of each plot. The belt transect was located 0.75 m north of the southern edge of each plot and covered ~6.7% of total plot area. Quadrat size was chosen to reflect the scale at which plant species are

likely to compete vegetatively. For every graminoid species, we scored presence or absence per quadrat based on presence of living tissue. We first tested for an effect of warming on the change in frequency of grasses (scored as present/quadrat if any grass species was present) and sedges between 1990 and 2010 by calculating the percentage of occupied quadrats/plot in 2010 minus percentage occupied in 1990. We applied ANOVA with the warming treatment as a fixed effect (Proc MIXED, SAS version 9.1; SAS Institute 2008). Plot was the unit of replication. Residuals from the model met assumptions of homogeneity of variances and normality. For visualization, we show both the absolute change from 1990 to 2010 and the mean percentage of quadrats occupied on each census.

We next examined individual species responses along the belt transect. Prior to this analysis, we conservatively combined two species pairs (*Carex obtusata* + *C. geyeri*, *Bromus porteri* + *Ceratochloa carinata*). Observers differed between early vs. late censuses, and data suggested a failure to distinguish between these species in some censuses. Our repeated-measures model used presence/absence of the species per quadrat as a binomial response and included the fixed effect of warming, repeated effect of year, the warming × year interaction, the continuous effect of quadrat location (1–32) along the transect, and the random effect of plot (nested within treatment) (Proc GLIMMIX, SAS version 9.2; SAS Institute 2009). For rare species, this approach was more powerful than taking average occupancy per plot because it accounted for spatial distributions. For significant warming × year interactions, we examined a priori contrasts for warming within each year. We report percentages of quadrats occupied per plot.

Frame censuses

The belt transect did not sample a large enough area in any one year to permit a robust species-level compositional analysis. Thus, we collected more spatially extensive data following 21 yr of warming on 29–30 June 2011 (22 d post-snowmelt) and 17 August 2011 (71 d post-snowmelt). We used a 1.4 × 1.4 m frame divided into 49 quadrats (0.2 × 0.2 m) that sampled 20% of each block (147 quadrats/plot). Frames were centered in each block and did not overlap the belt transect, which was positioned closer to the southern edge of each plot.

We examined the frequency of grasses, sedges, and each graminoid species using the proportion of quadrats occupied/block (logit-transformed; Warton and Hui 2011). We also tested mean species richness/quadrat and the evenness index (J'), using the proportion of quadrats occupied/block. Mixed general linear models had fixed effects of block, warming, date, all interactions, and the random effect of plot (nested in warming) (Proc Mixed, SAS version 9.2). For significant warming × block effects, we tested a priori contrasts within blocks.

Graminoid species composition

We analyzed the effect of warming on graminoid composition in 2011 using the percentage of the 147 quadrats/plot occupied by each species. We applied nonmetric multidimensional scaling analysis (NMS) with a Bray-Curtis distance metric and 500 restarts. The distance matrix was 10 × 10 with $n = 5$ plots per treatment (June and August tested separately). PerMANOVA tested for an effect of warming (unrestricted permutation of raw data, 9999 iterations, Primer v. 6.1.10; Clarke and Gorley 2009). Indicator species analysis (SIMPER, Primer) showed which species contributed most to dissimilarity.

Root and soil fungi

In the upper and lower blocks, we collected roots and rhizospheric soil (~2 g) from dominant grasses: *Achnatherum lettermanii*, *Festuca thurberi*, *Poa pratensis*; sedges: *Carex geyeri*, *C. obtusata*; and the dominant shrub *Artemisia tridentata* (25–26 July 2011, 48 d post-snowmelt). We limited sampling to dominants to ensure sufficient replication. For each species, we sampled three individuals nearest to the center of each block. Samples were collected into sealed, sterile plastic bags kept on ice until processing, which occurred within 48 h.

Intraradical fungal colonization

Roots were cleared in 10% KOH and stained with acid fuchsin (McGonigle et al. 1990). Proportion of root length colonized by hyphae, arbuscules, and vesicles was quantified by the grid-line intersection method at 200× (McGonigle et al. 1990). Aseptate and septate hyphae were counted separately as indicators of AM fungi and non-AM fungi, respectively. To summarize fungal colonization at the scale of each block, we calculated *extrapolated root colonization* $= \sum r_i \times p_i$, where r_i was percentage root length colonization and p_i the average frequency of the host plant (proportion of quadrats occupied, 2011) for each graminoid sampled (i). Data were averaged across three replicates per block and analyzed with mixed-model ANOVA including warming, block, warming × block, and the random effect of plot nested in warming. We logit-transformed extrapolated fungal root colonization. We also tested for rank correlations between graminoid richness/evenness and fungal responses.

Extraradical fungal network

Within each block, we mixed equal amounts of soil from each root sample for soil hyphal extraction, producing a single replicate per block ($N = 20$ samples). Hyphae were extracted from 5 g of soil vortexed in 5% sodium hexametaphosphate for 5 min., filtered through a 0.22-μm nylon filter, and stained with acid fuchsin (Brundrett et al. 1994). Hyphal length was quantified via

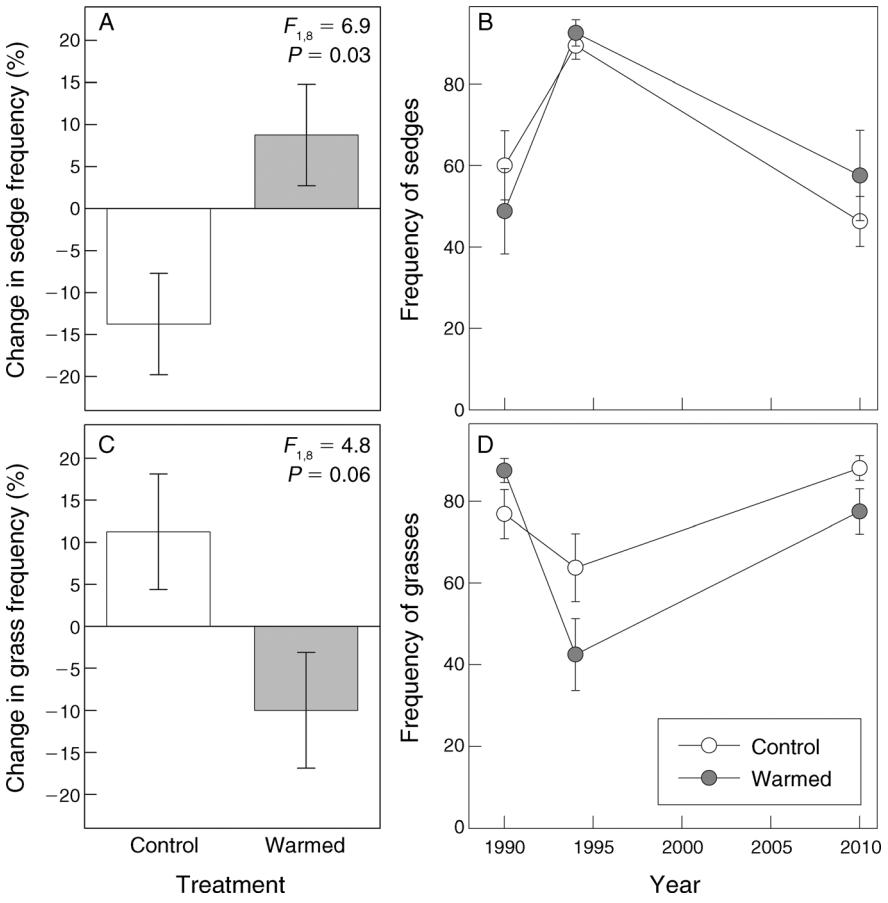

FIG. 1. Responses of (A) sedges and (C) grasses and to 20 years of warming. For the belt transects, the change in frequency (mean ± SE) = (percentage occupied quadrats/plot in 2010) minus (percentage occupied in 1990). (B, D) For sedges and grasses frequency is the percentage of occupied quadrats/plot (mean ± SE) by sampling year and treatment.

the grid-line intersect method (100 grids per sample, 200×; Brundrett et al. 1994) and log-transformed. Mixed-model ANOVA included the fixed effects of block and warming, and the random effect of plot nested in warming.

RESULTS

We observed a total of 16 graminoid species in the warming meadow (Appendix A). All species were native to the Colorado Rocky Mountains (USA) with the possible exception of *Poa pratensis*, which may be either circumpolar or European in origin (Shaw 2008). We next discuss our results in terms of our original questions.

(1) Does experimental warming shift the relative frequency of grasses vs. sedges?

Belt transects.—After 20 years of warming, sedge frequency had increased in warmed plots and declined in controls (Fig. 1A, B). Grasses showed the opposite trend (Fig. 1C, D; Appendix B). Occurrence of graminoid species along the longitudinal belt transects significantly varied among plots prior to the warming treatment, but in the opposite direction of post-warming patterns (Appendix B). In 1990, prior to warming, the grass *Poa pratensis* was 33% more frequent in warmed plots than in controls; however, by 2010 *P. pratensis* showed a 23% lower frequency in warmed plots (warming × year, $F_{1,24} = 5.2$, $P = 0.014$; Appendix B). The grass *Achnatherum lettermanii* responded early to warming with a 44% lower frequency in warmed plots than in controls after three years of warming (1994) and a marginally significant (33%) decline by 2010 (warming × year, $F_{1,24} = 3.7$, $P = 0.041$; Appendix B). Within warmed plots, *A. lettermanii* declined by 26% from 1990 to 2010. We could not track temporal changes in the sedge *Carex obtusata* alone, because unambiguous pretreatment data were not available for this species, which can be difficult to identify. However, early years showed lower combined *C. obtusata*/*geyeri* frequencies under warming, but this pattern was reversed by 2010 (Appendix B). A few species lacked sufficient belt-transect coverage to permit temporal analysis.

Frame censuses.—The long-term shift evident from belt transects was confirmed by the more spatially extensive frame census conducted after 21 years of

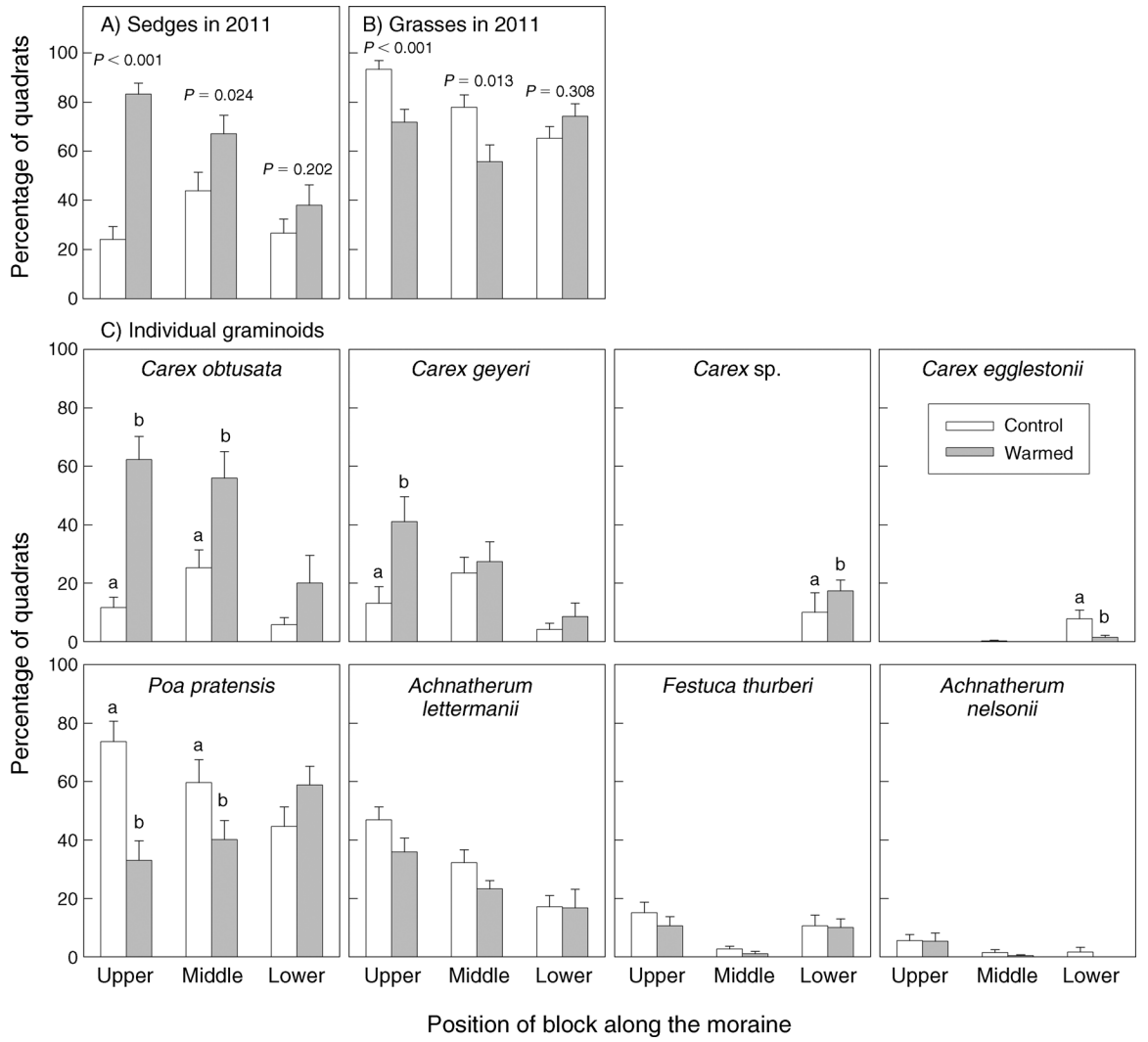

FIG. 2. Frequency (mean + SE) of (A) sedges and (B) grasses, and (C) the eight most responsive individual graminoids estimated by frame censuses in 2011 after 21 years of warming. Frequency is the percentage of occupied quadrats/block. Upper (dry ridge), Middle (rocky slope), and Lower (moist swale) refer to elevation positions of blocks along the moraine. *P* values show a priori contrasts within each block. Different lowercase letters indicate significant differences ($P < 0.05$) within a block (upper, driest; lower, wettest) ($n = 5$ plots/treatment). Data are averages for June and August (interactions with census date were not significant; Appendix C).

warming. Averaged across blocks, warmed plots had 100% higher sedge frequency than controls: 63% of the 147 quadrats in warmed plots were occupied by sedges, whereas just 32% of control quadrats had sedges (Fig. 2A, $F_{1,8} = 30.6$, $P < 0.001$). In contrast, warmed plots had 15% lower grass frequency than controls (79% of control quadrats, 67% of warmed, Fig. 2B, $F_{1,8} = 7.5$, $P = 0.026$; Appendix C).

Graminoid responses to warming were strongest on the upper, dry ridgetop of the moraine. Collectively, sedges responded most positively to warming in the upper block (250% increase), significantly in the middle transition zone (50% increase), and nonsignificantly in the lower, most mesic block (Fig. 2A, warming × block, $F_{1,40} = 7.0$, $P = 0.002$, Appendix C). Grass responses also varied spatially, with 25% lower frequency under warming in both upper and middle blocks but no significant difference in the lower block (Fig. 2B, warming × block, $F_{1,40} = 6.5$, $P = 0.015$). Despite this spatial variability, graminoid responses were temporally consistent across the 2011 growing season (warming × date, all $P > 0.4$; Appendix C).

(2) Does warming alter the species composition or diversity of graminoids?

Frame censuses.—Warming altered overall graminoid species composition (Fig. 3). Effects were consistent between early (June) and late (August) sampling dates (compare Fig. 3A with 3B), confirming that the difference was not caused by shifts in plant phenology.

Carex obtusata contributed most to warming-induced changes in community composition on both dates (SIMPER % contribution to treatment dissimilarity: June, 27%; August, 34%), and was 220% more frequent in warmed than in control plots (440% in the upper block, Fig. 2C; Appendix C). *Carex geyeri* showed a similar, although less dramatic, pattern with 90% higher frequency in warmed plots than in controls (Fig. 2c, SIMPER: June, 17%; August, 15% contribution to dissimilarity). Among the grasses, *Poa pratensis* showed the strongest warming response (Fig. 2C, SIMPER: June, 19%; August, 15%), and *Achnatherum lettermanii* ranked fourth among graminoids in its contribution to community dissimilarity (SIMPER: June, 11%; August, 11%), although warming was not significant in individual analysis (Appendix C).

One species responded against the general trends. The sedge *Carex egglestonii* had 80% lower frequency in warmed plots relative to controls (Fig. 2C; Appendix C). Unlike *C. geyeri* and *C. obtusata*, which are common in warm, dry habitats (A. A. Reznicek, *personal communication*), *C. egglestonii* prefers mesic conditions and may be more sensitive to soil drying under warming.

Warming affected local graminoid species richness, with opposing effects on sedges vs. grasses. Sedge richness per quadrat increased by 400% with warming in the upper, dry ridgetop (warmed, 1.0 ± 0.1 [mean ± SE]; control, 0.2 ± 0.1; $P < 0.001$) and by 60% in the middle transition zone (warmed, 0.8 ± 0.1; control, 0.5 ± 0.1; $P = 0.012$; Appendix C). In contrast, grass richness was significantly reduced under warming (by 40%), but only in the upper ridgetop (warmed, 0.9 ± 0.1; control, 1.5 ± 0.1; $P < 0.001$). Patterns reflect the positive relationship between abundance and species richness: when conditions favored a higher cover of sedges, it was more likely that additional sedge species were observed.

(3) Do subalpine plants have altered associations with soil fungi under warming?

Intraradical fungal colonization by AM fungi.—Across warming treatments, grasses had higher levels of root colonization by AM fungi than did sedges (grasses, 13% ± 1% [mean ± SE]; sedges, 4% ± 1%, $F_{1,24} = 56.4$, $P < 0.0001$), although overall colonization was generally low (average: 9.3% ± 0.6%), which is consistent with other reports from the Rockies (Schmidt et al. 2008). Warming increased AM fungal colonization relative to control plots in some cases, but effects varied among plant species and location. For *Achnatherum lettermanii*, warming increased total AM fungal root colonization (hyphae, arbuscules, and vesicles combined) in the upper blocks, but reduced colonization in the lower blocks (Fig. 4A), suggesting that warming interacts with the soil moisture gradient present along the moraine. In *Festuca thurberi*, arbuscules, the sites of plant–fungal nutrient exchange, increased by 280% under warming, but only in the lower blocks (warmed, 0.65% ± 0.18% [mean ± SE]; control, 0.17% ± 0.11%, $P = 0.05$); there were no significant effects of warming on total colonization (Fig. 4B) similar to results for *P. pratensis* (Fig. 4C). Neither sedge species showed significant AM colonization responses to warming (Fig. 4D–E). The dominant shrub (*Artemisia tridentata*, absent from lower blocks) showed 60% higher colonization under warming (Fig. 4F), similar to the pattern for *A. lettermanii*. There was no warming-induced shift in extrapolated root colonization by AM fungi at the plot scale ($F_{1,8} = 0.03$, $P = 0.88$). Also, plot-level correlations between graminoid diversity and AM fungal root colonization were nonsignificant ($P > 0.80$).

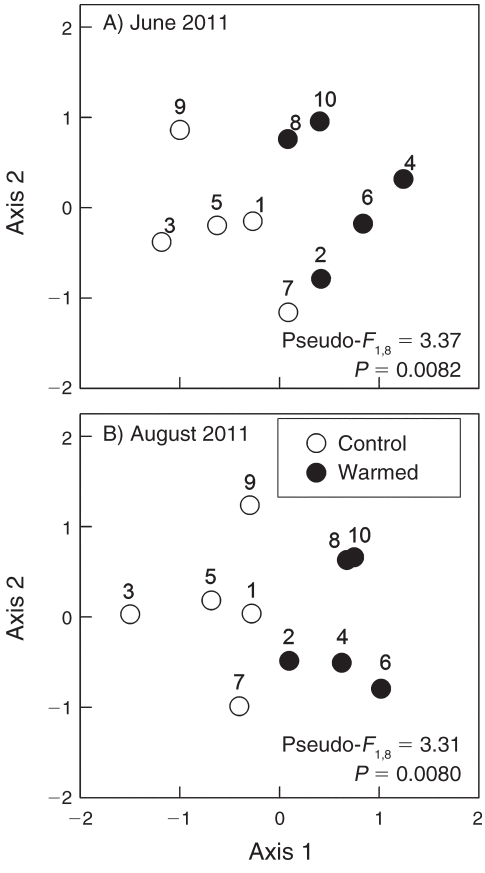

FIG. 3. Graminoid species composition estimated by nondestructive frame sampling (three 1.4 ×1.4m frames per plot, 0.2 × 0.2 m quadrats) in 2011 after 21 years of warming. Nonmetric multidimensional scaling (NMS) plots show composition for (A) June 2011, NMS two-dimensional stress = 0.070 (low risk for drawing false inferences; McCune and Grace 2002) and (B) August 2011, NMS two-dimensional stress = 0.099. Each symbol is a plot. The numbers show the relative positions of plots along the north–south axis of the moraine. Axes are NMS values, with statistics from perMANOVA.

Intraradical fungal colonization by non-AM fungi.—Although grasses had higher AM fungal colonization than did sedges, the two groups of graminoids did not differ in root colonization by non-AM fungi (grasses,

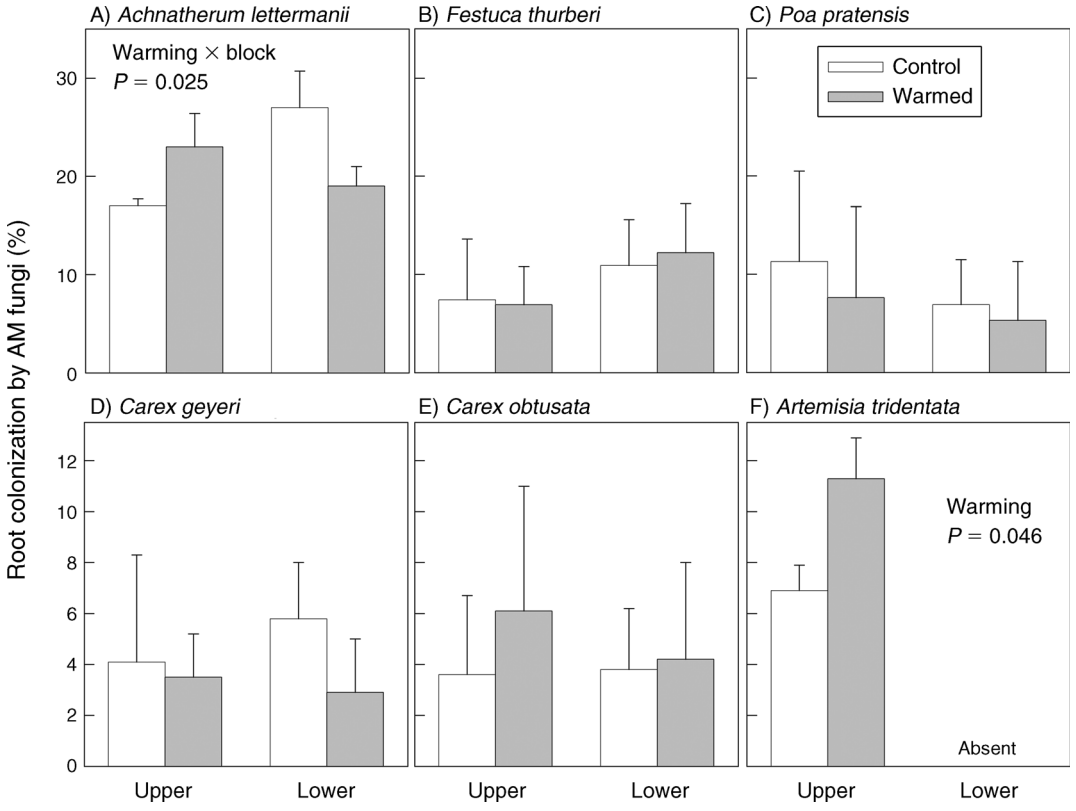

FIG. 4. Arbuscular mycorrhizal (AM) fungi percentage (mean + SE) of root length colonized (hyphae, arbuscules, vesicles) in response to warming and block (upper, dry; lower, mesic).

22% ± 1%; sedges, 23% ± 1%; $F_{1,24} = 0.4$, $P = 0.55$). Warming did not affect root colonization by non-AM fungi in analyses of individual plant species (all $P > 0.26$). However, small differences in colonization rates combined with shifts in graminoid species composition resulted in nearly 50% higher extrapolated non-AM fungal root colonization in warmed plots than in controls (Fig. 5A). Spatial patterns were consistent with, but stronger than, warming effects. Dry, upper blocks had 100% more non-AM fungal colonization than did lower swales (Fig. 5B). Correlations with graminoid diversity were nonsignificant ($P > 0.30$).

Extraradical fungi.—Non-AM fungi dominated the extraradical fungal assemblage. Warmed plots had fewer septate extraradical hyphae than did controls, but this trend was not statistically significant (Fig. 5C). Spatial patterns were stronger than warming effects, with 40% fewer extraradical hyphae in the dry ridgetop than in the low swale (Fig. 5D). At the plot scale extraradical hyphal length was negatively associated with extrapolated root colonization by AM fungi (Spearman $r = -0.45$, $P = 0.048$, $n = 20$ subplots) and non-AM fungi (Spearman $r = -0.40$, $P = 0.081$, $n = 20$ subplots). Graminoid diversity was not correlated with extraradical hyphal length ($P > 0.2$).

DISCUSSION

Subalpine graminoid species composition has responded strongly to 20 years of experimental warming, despite the lack of change in total graminoid biomass or cover (Harte and Shaw 1995, Price and Waser 2000). Several prior warming experiments have lumped grasses and sedges, often detecting no significant graminoid response (e.g., Grime et al. 2008, Jagerbrand et al. 2009, Hudson and Henry 2010). Our results show that ignoring species-specific responses within the graminoids can conceal important shifts in plant composition. Grasses and sedges are separated by 55–59 million years of evolutionary history (Wikstrom et al. 2001) and can differ in their effects on ecosystem processes, such as rates of soil respiration or nutrient leaching (e.g., Johnson et al. 2008, Phoenix et al. 2008). If grasses and sedges make unique contributions to decomposition, carbon storage, nutrient cycling, or water dynamics, either directly or through microbial interactions, shifts in graminoid composition will alter ecosystem process rates.

Mechanisms driving warming-induced shifts in graminoid composition

The positive response of sedges was unexpected, given that warming reduced soil moisture in this subalpine

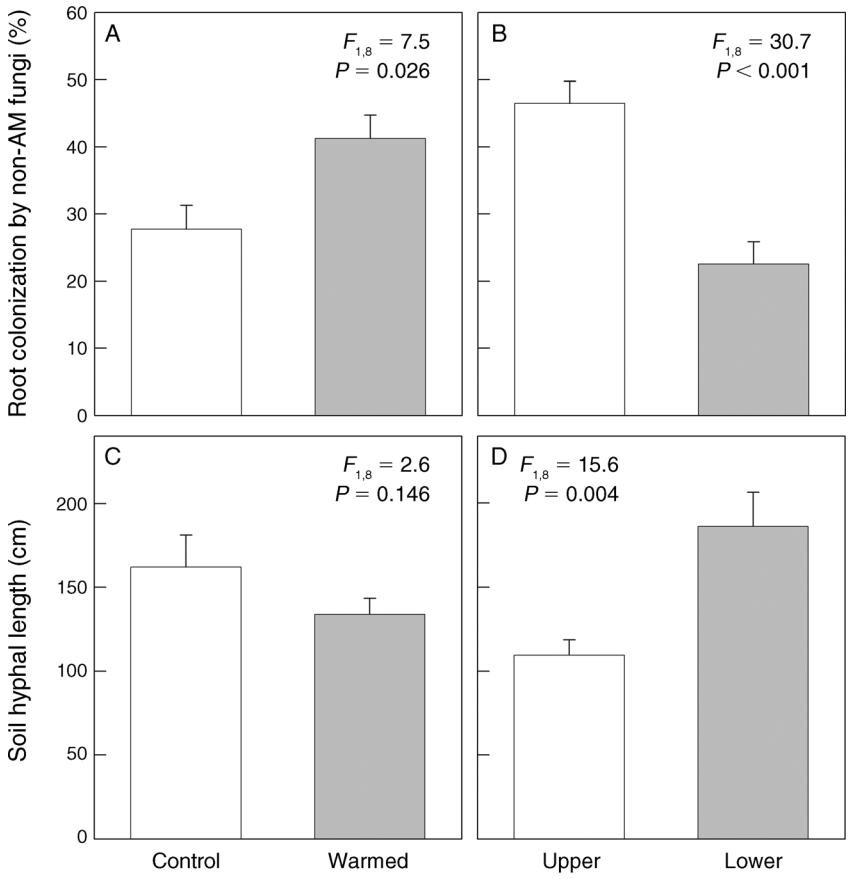

FIG. 5. Non-AM soil fungi responses to experimental warming and location within the meadow. Data are means + SE. (A) Extrapolated root-length colonization by non-AM fungi in response to warming and (B) block within the meadow (upper, dry; lower, mesic); there was no significant interaction (warming × block, $F_{1,8} = 2.7$, $P = 0.14$) ($n = 5$ replicates per treatment). Soil hyphal lengths in response to (C) warming and (D) block (treatment × block, $F_{1,8} = 1.44$, $P = 0.26$).

meadow (Harte and Shaw 1995), and sedges are often associated with mesic soils (Ball et al. 2003). Direct effects of higher temperatures, as well as correlated changes in soil moisture (e.g., earlier snowmelt, reduced summer soil moisture) may underlie the differential responses of sedges and grasses. The two dominant sedges (*Carex geyeri*, *C. obtusata*) thrive in warm, dry habitats (A. A. Reznicek, *personal communication*). In addition, prior research showed that *C. geyeri* cover was negatively correlated with snow duration (Knight et al. 1977), suggesting a direct benefit of a longer growing season. More complex, indirect mechanisms may also play a role. For example, if the dominant shrub (*Artemisia tridentata*) becomes a better competitor under warming (either directly or via increased benefits of AM fungi; Stahl et al. 1998), then it may preferentially outcompete grasses over sedges. Other biotic mechanisms, such as warming-altered herbivory, could also indirectly drive shifts toward sedge dominance.

It is not surprising that the effects of warming often depended on spatial location along the moraine. While earlier snowmelt caused by warming may negatively affect plants growing in dry meadows and ridgetops due to reduced water availability, it may positively affect wet meadows by extending the growing season (Knight et al. 1979). We observed the largest shifts in grasses and sedges in the driest (upper) blocks, including changes in species' frequency (up to 250%) and species richness (up to 400%), with little effect in the lower, mesic blocks where plants may be less affected by the soil-drying effects of warming. The reduction in grass species richness resembled that of a warming experiment in northern China (Yang et al. 2011). Although a review of 61 experiments in tundra found the strongest positive response of grasses to warming in dry sites and of sedges in wet sites (Elmendorf et al. 2012), tundra sites are generally wetter than subalpine meadows, which may explain the opposite pattern that we observed here.

Over 20 years in the control plots, grass frequency increased whereas sedge frequency declined, in contrast to responses observed under experimental warming. By isolating the effect of warming, the experimental treatment may have different effects on graminoids than actual (multifactor) climatic trends, particularly if altered precipitation and/or CO_2 trump the effects of warming (see also Wolkovich et al. 2012). Alternatively,

responses to experimental heaters may not mimic species responses to more gradual temperature changes (~0.5–1°C increase per decade for the Rocky Mountains; Rangwala and Miller 2012). Interannual variation in climate is an additional correlate of temporal changes in plant composition, and was considerable between 1990 and 1995 (Price and Waser 2000). Some of that variation relates to winter precipitation: 1990 was relatively dry, 1994 and 2010 were similarly average, and 2011 was wet. An effect of warming should be to dampen year-to-year variation in precipitation effects—so altered variance in abiotic conditions may explain the greater temporal variability in controls relative to warmed plots. Price and Waser (2000) showed that warming reduced year-to-year variation in several variables, including plant species richness.

Fungal responses to warming

Shifts in plant species composition were accompanied by changes in soil fungi. Non-AM fungi dominated the soil matrix associated with graminoids. Fungal investment shifted from higher soil colonization in wetter soils to higher root colonization in drier soils. Changes in septate fungal biomass of either saprotrophs, pathogens, or dark septate endophytes (DSEs) can occur through a variety of mechanisms. First, fungal biomass may respond directly to warmer temperatures or declines in soil moisture. Extraradical saprotrophic hyphal biomass often decreases with drought (e.g., Hawkes et al. 2011), consistent with the patterns observed here. Extracellular enzyme activity can also decline with soil drying, reducing nutrient uptake and carbon allocation to fungal biomass (Henry 2012). DSEs, which often do not produce extensive extraradical networks (Porras-Alfaro and Bayman 2011), have been proposed to function in nutrient acquisition (like AM fungi) in high-elevation sedges (Haselwandter and Read 1982) and may be favored by roots experiencing warm, dry conditions. Second, belowground fungal species composition can vary with soil moisture (Hawkes et al. 2011), affecting how fungal biomass is allocated between root colonization vs. extraradical hyphae. Third, plant–fungal interactions may contribute to the shifts we observed. For example, under drought, plants may invest more in fungi with less extensive extraradical networks, such as some DSEs (e.g., Henry et al. 2007). It is not yet possible to disentangle alternative hypotheses because we lack data on fungal species composition. Nevertheless, because soil fungal biomass can alter carbon storage belowground (Yuste et al. 2011, Clemmensen et al. 2013), fungal shifts associated with temperature, moisture, and plant composition have the potential to affect ecosystem processes.

Despite the dominance of non-AM fungi in graminoid rhizospheres, AM fungal colonization of roots increased with warming for some plant species. For example, root colonization rates increased ~10–15% under experimental warming. A seminal paper by Johnson (1993) showed that even smaller changes in root colonization (~3–5% under nutrient addition) had large repercussions for plant fitness. Therefore, root colonization per se cannot be taken as a direct indicator of the magnitude of benefit that plants get from arbuscular mycorrhizal fungi. While manipulations of AM fungi would be needed to assess benefits, any benefit associated with increased colonization of grass roots by AM fungi was insufficient to compensate for the decline of grasses under warming (Fig. 2). Last, not only were the responses of soil fungi specific to plant species, but spatial patterns also suggest that fungal responses depend upon water availability and vary among fungal clades.

Future directions and caveats

Several aspects of the system deserve further attention. First, understanding differences between grasses and sedges in carbon recalcitrance, decomposition rates, and contributions to carbon stocks would shed light on the potential for shifts in ecosystem process rates. Second, it would be useful to explore whether grasses have stronger responses to warming belowground than aboveground, as a recent meta-analysis suggested (Lin et al. 2010). Third, teasing apart the mechanisms underlying the heating effect would be informative. While we observed effects of warming on plants and microbes, we do not yet know if these were direct effects of temperature, indirect effects of altered snowmelt and soil drying (Harte et al. 1995), indirect effects of altered species interactions, or some combination of these pathways. Finally, a look at fungal symbionts on forbs and other shrubs in this ecosystem would provide a more holistic perspective on the responses of the symbiotic fungal assemblage.

In conclusion, our long-term study showed that graminoid composition can be substantially altered by experimental warming, with concurrent shifts in root-associated fungi. Effects of warming on both graminoids and their fungi were strongest under the driest soil conditions. Our results demonstrate that lumping grasses and sedges can mask important responses of primary producers, and their fungal symbionts, to climate change.

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Supplemental Material

Appendix A

List of the graminoid species in the experimental warming meadow, Rocky Mountain Biological Laboratory, Gunnison County, Colorado, USA (*Ecological Archives* E095-169-A1).

Appendix B

Belt transects: means and statistical results showing the effects of the warming treatment and year on individual graminoid species frequencies for 1990, 1994, and 2010 (*Ecological Archives* E095-169-A2).

Appendix C

Frame censuses: statistical results for the effects of experimental warming on graminoids in 2011 (*Ecological Archives* E095-169-A3).