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Soil conditions moderate the effects of herbivores, but not mycorrhizae, on a native bunchgrass

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ABSTRACT

Herbivores, microbial mutualists, and soil nutrients can affect plant survival, growth, and reproduction, demographic parameters that are essential to plant restoration. In this study we ask: 1) whether native plants that form early associations with mycorrhizal fungi are more tolerant of mammalian grazers, and 2) how early plant associations with mycorrhizal fungi influence mammalian grazing across gradients in soil nutrients. In eight grassland sites in California (USA), we transplanted seedlings of a native bunchgrass, *Stipa pulchra*, that were or were not pretreated with mycorrhizal fungi in exclosures designed to exclude different guilds of vertebrate grazers. Pretreated plants had greater establishment eight months after transplantation than untreated plants. Mycorrhizal inoculation resulted in twofold greater biomass and fourfold greater seed production when plants were protected from herbivores; inoculation with mycorrhizae resulted in twofold greater biomass and seed production when plants were accessible by all herbivores. Soil phosphate and potassium concentrations influenced herbivory: vertebrate grazing had less effect on transplant biomass and seed production at sites with high phosphate – low potassium soils, but the effects of grazing were more severe in low phosphate – high potassium soils. Pretreatment with mycorrhizal fungi can result in greater survival, growth, and reproduction of transplanted seedlings of native bunchgrass *S. pulchra*. Our results also illustrate that soil conditions may influence the extent to which the vertebrate herbivore community limits restoration of *S. pulchra*: the effects of some small mammalian herbivores (e.g., voles) was little affected by soil conditions, but grazing by larger herbivores had a greater effect on *S. pulchra* performance at sites with low phosphate – high potassium soils. In helping identify the contribution of soil nutrients, herbivores, and mycorrhizae to establishment and performance, our work has implications for the restoration of a species that is likely a fundamental component of pristine California grassland ecosystems.

1. Introduction

Vertebrate herbivores can have strong, deleterious effects on plant populations through direct or indirect effects on plant establishment and performance (Perevolotsky and Seligman, 1998; Howe and Brown, 1999), affecting important aspects of plant conservation (Augustine and McNaughton, 1998; Olff and Ritchie, 1998) and restoration (e.g., Howe and Lane, 2004; Howe et al., 2006; Orrock et al., 2009). The effect of herbivory on plant growth, survival, and reproduction may also depend on the status of important plant-microbe mutualisms (Gehring and Whitham, 2002; Bennett and Bever, 2007). For example, inoculation with arbuscular mycorrhizal (AM) fungi may confer tolerance or facilitate secondary defense for host plants against insect herbivores (Kempel et al., 2010; Jung et al., 2012). However, AM fungi-mediated resource acquisition also increases plant quantity and quality, both of which benefit the performance of certain insect herbivores (Gange et al., 1999; Koricheva et al., 2009), and the resulting attack on AM-infected seedlings may negate the competitive advantage often observed in plants inoculated with mycorrhizae (Fitter, 1977). Despite the importance of vertebrate herbivores and mycorrhizae in shaping plant populations, and evidence that mycorrhizae can alter the outcome of invertebrate herbivory, it remains unclear whether mycorrhizae alter the effect of diverse vertebrate herbivores.

There is also growing appreciation that the outcome of plant-

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herbivore interactions is often context-dependent (Maron and Crone, 2006; Hahn and Orrock, 2015), suggesting that the interplay of mycorrhizae and herbivores in affecting plants may vary depending upon local conditions. For example, gradients in soil fertility may alter the relationship between AM fungi and host plants (Johnson et al., 1997; Grogan and Chapin, 2000; Pietikäinen et al., 2005; Gerz et al., 2016) and indirectly generate spatial variation in herbivory (Olff and Ritchie, 1998). In nutrient-poor soils, infection by mycorrhizae may help limit herbivory by increasing the efficiency of nutrient uptake and permitting greater resource allocation to growth and herbivore defense; in these same systems, plants uninfected by AM fungi may be nutrient-limited and less capable of tolerating or defending against consumption (Jones and Last, 1991). Conversely, plants typically exhibit low mycorrhizal colonization in nutrient-enriched soils (e.g., Johnson et al., 2003) and may not require microbial mutualistic associations to acquire resources or mount defense against herbivore attack, suggesting infection by AM-fungi may have little direct effect on herbivory in systems with fertile soils. Although soil fertility gradients may be drivers of herbivore pressure (Olff and Ritchie, 1998; Hopcraft et al., 2010) and mycorrhizae may be critical elements of successful plant conservation and restoration (e.g., Requena et al., 2001; Korb et al., 2003), it is unclear how mycorrhizal status influences patterns of plant consumption by vertebrates under different soil conditions.

Degraded grasslands represent important restoration targets, but abiotic and biotic constraints can impede recovery of these systems (Bakker and Berendse, 1999; Halpern et al., 2016). In this study, we evaluate the interplay of mycorrhizal inoculation and vertebrate herbivores in affecting the establishment and growth of transplanted perennial bunchgrass seedlings into a degraded grassland habitat. We utilize factorial experiments conducted within different soil-fertility contexts and collect comprehensive data on plant establishment, growth, and reproduction. Our field experiments occur within the California grassland ecosystem, a once-diverse system that spanned 9.2 million hectares and that has been heavily degraded by invasive annual plants. The conservation and restoration of native plants is a priority within California grasslands (e.g., Stromberg and Griffin, 1996; Dyer and Rice, 1997; Corbin and D'Antonio, 2004; Orrock et al., 2008, 2009) and great effort is directed at promoting the recovery of the native perennial bunchgrasses, *Stipa pulchra* (Hitch.) (Purple needlegrass), a species that once likely dominated this grassland ecosystem (Stromberg and Griffin, 1996; Hamilton, 1997). The effect of herbivores and mycorrhizae on growth and reproduction may be particularly important for seedling survival and the demography of *S. pulchra* populations: both have been shown to independently affect *S. pulchra* plant establishment and reproductive potential (e.g., Nelson and Allen, 1993; Orrock et al., 2009), but little is known about the capacity for vertebrate herbivores and mycorrhizae to interact in their effects on this native grass species. In this study, we used field transplantations with seedling *S. pulchra* to determine whether inoculation with mycorrhizal fungi prior to transplantation confers growth and reproductive benefits to seedlings and to determine how pretreatment with AM-fungi influences consumption by different mammalian herbivores. Using eight different study sites and fencing to experimentally manipulate mammalian herbivore access we asked 1) how early inoculation with mycorrhizae influences the consumptive effects of different mammal groups on transplanted *S. pulchra* seedlings and 2) how gradients in environmental conditions (i.e., ambient soil fertility) alter the response of perennial grass seedlings pre-treated with mycorrhizal inoculum to herbivory by vertebrates.

2. Methods

2.1. Study area

Eight sites in open annual grassland were selected within Cheeseboro Canyon, part of the Santa Monica National Recreation Area (see Orrock et al., 2009 for site locations). Perennial bunchgrasses (e.g., *Stipa pulchra*) and sagebrush (e.g., *Artemisia californica*) likely dominated much of this region's historical grasslands (Stromberg and Griffin, 1996; Hamilton, 1997); the study area, however, is presently dominated by non-native annual grasses and forbs including *Bromus diandris*, *B. hordeaceous*, *Brassica nigra*, *Erodium cicutarium*, *Melilotus officianalis*, and *Medicago polymorpha* making it an ideal study location to evaluate the biotic factors (e.g., herbivory, soil mutualists) influencing the effectiveness of different restoration techniques. These southern California grasslands are characterized by a Mediterranean climate typified by hot, dry summers and mild winters with intermediate amounts of precipitation.

In order to ascertain soil type and quality at each site, four soil samples (0.47 L each) were taken from each plot on 15 July 2005, bulked and submitted for analysis (A and L Western Agricultural Laboratories, Modesto, California, USA). These analyses determined soil parameters known to influence plant performance, herbivore grazing, and plant-mutualist interactions: percent rock, percent organic content, soil pH, and the concentrations of phosphates ($NaHCO_3$–P), potassium, magnesium, calcium, and sodium.

2.2. Mycorrhizal treatment of seedlings

We use *Stipa pulchra* obtained from locally collected seeds, as the use of non-local seeds can artificially influence *S. pulchra* establishment likelihood and the severity of ungulate grazing (Hufford and Mazer, 2012). On 4 November 2004, seeds of *S. pulchra* were sown in nursery flats with either 1) potting soil that had been augmented with 4.92 g (1 teaspoon) MycoApply® EndoNet mycorrhizal granular inoculum (Rocky Mountain Bio Products, Denver, CO, U.S.A) or 2) untreated sterile potting soil. *Glomus intraradices* is the predominant active mycorrhizal fungus in EndoNet inoculum (~60,000 propagules/lb.). This fungal species is robust for restoration practices as it can occupy a broad variety of ecosystems (e.g., Renker et al., 2004; Tao and Zhiwei, 2005) and is effective at facilitating early phytoremediation in disturbed ecosystems (e.g., Requena et al., 2001; Caravaca et al., 2003; Oliveira et al., 2005). We applied the mycorrhizal treatment to seedlings (rather than inoculating plants directly in the field) because this approach allowed us to be certain that our treatments were imposed and effective. Moreover, from a pragmatic perspective, pre-transplant inoculation with commercially available mycorrhizae is a rapid approach that can be used by restoration ecologists to promote native plant recovery in degraded ecosystems (e.g., Richter and Stutz, 2002). Consequently, our use of pre-transplant mycorrhizal inoculation makes our findings of direct applied relevance and utility. Individual seedlings were transferred to conetainers (20.3 cm diameter) when seedlings were greater than 10 cm tall and had 2 or more true leaves; a seedling's soil treatment assignment in conetainers was identical to the seedling's soil treatment in the nursery flat (i.e. treated and untreated with mycorrhizal inoculum). Seedlings were maintained in the greenhouse for 3 months until transplanted into field plots.

2.3. Mycorrhizal treatments and mammalian herbivory

At each of the eight study sites we installed four experimental exclosures (2 × 5-m each) arranged in a rectangle with a 1-m buffer between exclosures (32 total exclosures; see Orrock et al., 2009 for diagram of exclosure layout). Briefly, each of these four exclosures received one of four treatments: (1) "No Consumer" (NC) exclosures prevented entry of all non-volant vertebrate consumers; (2) "Small" (S) exclosures permitted only small mammal consumers (i.e., voles and mice) but prohibited access by medium (i.e., rabbits) and large (i.e., deer) consumers; (3) "Small and Medium" (SM) exclosures permitted small and medium-sized mammals but prohibited access by deer; (4) "All" treatments (ALL) were unfenced thereby allowing consumers in all size classes access to the plot. All exclosures were trenched prior to exclosure installation and filled after exclosure installation to control for the disturbance created by fence installation. "NC" exclosures consisted of 1.22-m tall hardware cloth (0.8 × 0.8-cm mesh) imbedded 20 cm into soil. Fence tops were affixed with a 15–cm band of aluminum flashing to prevent rodents from climbing over the fence. "S" exclosures consisted of poultry wire (2.5 × 2.5 − cm holes) embedded in the soil with aluminum flashing affixed at the top. "SM" exclosures were identical to S exclosures but had 20 × 20-cm holes cut into the fence at uniform intervals along the poultry wire. Exclosure treatments did not control the entry of some potential consumers (i.e., pocket gophers, arthropods [e.g., ants, beetles, grasshoppers], birds); these un-excluded consumer groups, however, have equal access to all our experimental exclosures treatments, reducing opportunities for confounding the exclosure treatment effect. Additionally, although deer are capable of jumping over fencing surrounding NC, S, and SM exclosures, the small area of these exclosures (10-m^2) presented very little area within the exclosure for landing. Consequently, plot size most likely restricted deer entry and this estimation was corroborated by the observation that no deer tracks were seen within these exclosures during the entire course of the experiment. Small mammal trapping and observational data indicate that exclosures were most likely to be accessed by the expected guild of consumers (see Orrock et al., 2009).

Sites were tilled on 18–20 January 2005 and exclosures were installed 27–28 January 2005. In each exclosure three 1 × 1-m plots were positioned at least 0.5 m from each other and 0.5 m from the nearest exclosure fence. Two of the three plots were seed addition plots for a concurrent study described in Orrock et al. (2009); the third plot in each exclosure was used for the study described here. On 8 February 2005 sixteen *S. pulchra* seedlings were transplanted into each plot in a 4 × 4 grid pattern with approximately 20 cm spacing between individuals. Eight of these seedlings were grown in potting soil augmented with mycorrhizal fungi; the remaining eight were grown in untreated soil (*see above*). In order to restrict cross-contamination all seedlings grown in mycorrhizal inoculum-treated soils were planted in one randomly-assigned half of each plot, control seedlings were planted in the other half of the plot. We did not, however, control mycorrhizal inoculation of seedlings following transplantation into field plots; consequently seedlings raised in untreated soils likely acquired mycorrhizal mutualists at later life stages either through root contact with resident spores in the soil or through direct root contact with seedlings raised in inoculated soil. Factor level designations reflect the possibility of a late acquisition of mycorrhizae by untreated seedlings, we refer to greenhouse inoculated seedlings as "pretreated" seedlings and seedlings not pretreated with mycorrhizae as "untreated" seedlings.

2.4. Data collection and analysis

To quantify the effect of mycorrhizal inoculation and herbivore consumers on *S. pulchra* establishment, the status of each transplanted seedlings was monitored every 2–3 weeks from February to May 2005 (approximately once each month thereafter) and final assessments of establishment were made on 7 October 2005. From 8 to 13 June 2005, two plants in each exclosure (one pretreated plant, one untreated plant) were collected to measure biomass and provide estimates of reproductive output, consequently only seven individuals of each mycorrhizal treatment remained in each exclosure for viability assessment. Additionally, seedlings status check conducted on 7 October 2005 indicated that 27 transplanted seedlings in three exclosures at three different sites were burned or damaged by fire and two seedlings in two exclosures were buried by gopher activity. Buried *S. pulchra* seedlings were eliminated from analysis as their potential to establish is likely to be directly limited by gopher activity (Dyer and Rice, 1997), but burned *S. pulchra* seedlings were retained for plant diameter performance based analyses (*see below*) and analysis of establishment as burned *S. pulchra* typically remain viable post fire (Dyer and Rice, 1997).

Estimates of biomass and reproductive output were made from *S. pulchra* harvested in June 2005. Reproductive output (i.e., seed production per plant) was estimated by multiplying the average number of glumes found on three randomly selected reproductive tillers ($n = 3$) by the number of reproductive tillers on each harvested plant. In addition to June biomass sampling and estimates of seed production, the performance of transplanted seedlings was estimated by averaging two perpendicular measures of seedling diameter in June and October 2005. June plant diameter is a strong linear predictor of *S. pulchra* aboveground biomass and estimates of individual seed production (Fig. A.1, A.2). Although we did not destructively measure plant biomass and reproductive effort in October 2005, a strong correlation between June and October individual plant diameter (Fig. A.3) suggests that the effects we observed for June biomass and reproduction (see Results) are likely to persist into the start of the next growing season.

Soil characteristics were consolidated using a principal components analysis (PCA) to accommodate potential collinearity between variables and were used to describe major differences in soil composition across all eight sites. All soil variables measured were used in PCA. All values were transformed with a square root transformed and then centered prior to analysis. PCA analysis was conducted with a correlation matrix in order to account for differences in unit measures. We retained those principal components with eigenvalues greater than 1 (Everitt, 2005; Table A.1). One of the primary benefits associated with infection by mycorrhizal fungi is increased phosphate uptake for host plants (Javot et al., 2007; Parniske, 2008), consequently we focused our analysis on principal components that encompass variation in phosphate availability among sites as we expect the effect of mycorrhizal infection to be most influential along this gradient. The third principal component ("PC3" or "phosphorus PC" hereafter, 7.9% percent variance explained, Table A.1) satisfied both these requirements, being a contrast between soil phosphate and potassium concentration. Additionally, Supplementary Analyses suggest that PC1 and PC2 did not directly influence plant performance or alter the nature of model fixed effects (all P-values > 0.07); see below for fixed effects model specification.

We used generalized and general linear mixed models to evaluate seedling establishment, above ground biomass, seed production, and average plant diameter (June 2005) with exclosure type (NC vs. S vs. SM vs. ALL) and inoculation with mycorrhizae (Myco + vs. Myco-) as fixed factors. Our analyses used random-effects terms to explicitly incorporate the split-split-plot structure of our experimental design (i.e., variation occurred in three levels: all plants at a site had the same soil nutrients, exclosure treatment was applied within a site, and mycorrhizal treatment was applied to plants within an exclosure treatment). Seedling establishment analyses utilized a binomial error distribution, while plant size, biomass, and seed production were analyzed using a Gaussian error distribution. Seedling biomass and estimates of seed production were log(x+1) transformed prior to analyses. Denominator degrees of freedom were estimated using the Kenward-Rogers approximation (Littell et al., 2006). Recent work suggests a relationship between potassium and phosphorus uptake in mycorrhizal plants (Garcia and Zimmermann, 2014). Consequently, we retained the phosphorus PC in our analysis of plant performance and reproduction variables in order to test the influence of a soil phosphate-potassium continuum between pretreated and untreated plants and the effects of grazing across a diverse set of mammalian herbivores. We conducted contrasts to examine the effect of mycorrhizae treatment within each exclosure type at the 25% quartile, median, and 75% quartile of the phosphorus PC covariate; all analyses were conducted in SAS (SAS software 9.3, Cary, NC, USA).

3. Results

Inoculation with mycorrhizae led to significant increases in growth of *S. pulchra* prior to transplantation to the field: seedlings grown in mycorrhizae-treated soil were significantly taller (20.2 ± 0.7 cm vs. 12.5 ± 0.5 cm [mean ± SE]; t = 8.72, df = 57, P < 0.001; N = 32) and had a greater number of tillers (6.2 ± 0.3 tillers vs. 4.8 ± 0.2 cm [mean ± SE]; t = 4.17, df = 52, P < 0.001; N = 32) than seedlings raised in untreated soils, strongly suggesting mycorrhizal treatment resulted in early inoculation of pre-treated plants. Establishment of *S. pulchra* seedlings in the field was not influenced by exclosure type ($F_{3,\ 21} = 0.14$, $P = 0.935$), but was significantly affected by mycorrhizal inoculation ($F_{1,\ 28} = 4.97$, $P = 0.034$): establishment was greater for plants that had been inoculated with mycorrhizae prior to transplanting (95.2 ± 1.9% [least square means ± SE]) compared to untreated plants (91.7 ± 3.0%). There was no interaction between exclosure type and treatment with mycorrhizae ($F_{3,28} = 0.31$, $P = 0.822$).

Exclosure type had a significant effect on transplanted *S. pulchra* seedling biomass and reproductive output, but did not influence average plant diameter in June (Table 1, Fig. 1A–C). Pairwise contrasts between exclosure types indicate no difference in seedling biomass or seed production between complete exclosures (“NC”) and exclosures permitting small mammals (“S”) (NC vs. S: biomass, t = 0.90, df = 19.3, $P = 0.378$; seed production, t = 0.20, df = 19.1, $P = 0.843$, Fig. 1A–B) or between exclosures permitting both small- and medium-sized mammal herbivores (“SM”) and exclosures permitting all mammalian herbivores (“ALL”) (SM vs. ALL: biomass, t = −0.40, df = 18.4, $P = 0.694$; seed production, t = −0.28, df = 18.3, $P = 0.781$, Fig. 1A–B). *S. pulchra* seedling biomass was significantly lower in SM and ALL exclosure treatments than S exclosure treatments (SM vs. S, t = 2.85, df = 18.5, $P = 0.011$; ALL vs. S, t = 2.43, df = 19.0, $P = 0.025$, Fig. 1A), but differences in average *S. pulchra* seedling biomass in SM and ALL exclosure treatments versus NC exclosure treatments were not significantly different (SM

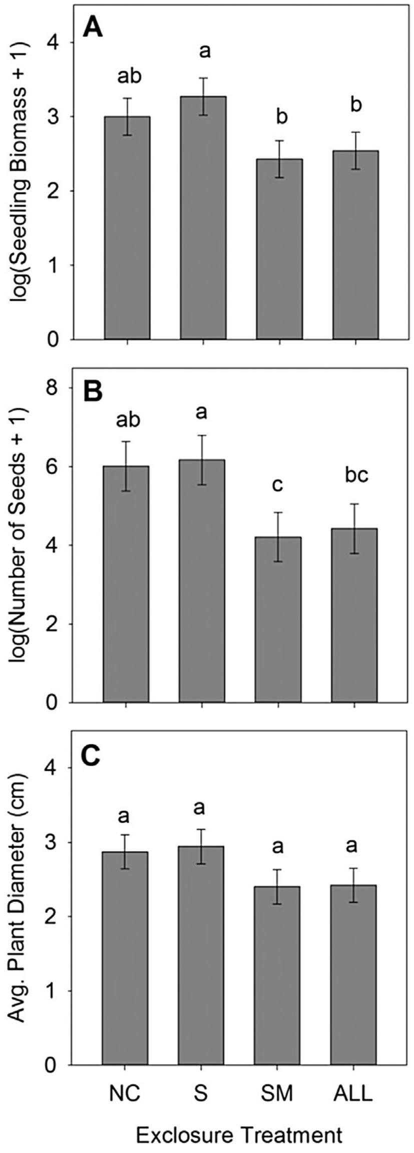

Fig. 1. Effect of mammal exclosure type, when averaged across mycorrhizae treatment and the phosphorus PC, on A) *S. pulchra* seedling biomass, B) average seed production per *S. pulchra*, and C) average *S. pulchra* diameter in June 2005. Exclosure type abbreviations indicate which mammalian herbivores had access to those treatment plots: “NC” – No consumers, “S” – Small mammals only, “SM” –Small and Medium mammals only, “ALL” – All herbivorous mammals. Bars are least square mean values ± SE. Seedling biomass and seed production are log(x+1) transformed. Different lower case letters above bars indicate significant differences at a type I error p = 0.05.

Table 1
Summary of type III test of fixed effects of exclosure type, mycorrhizae pretreatment, soil phosphate covariate (Phosphorus PC), and all possible interactions between factors on *S. pulchra* seedling biomass, average seed production per *S. pulchra* individual, and average *S. pulchra* diameter in June 2005. Biomass and seed production have been log(x +1) transformed. Values in bold indicate significant differences at Type I Error = 0.05.

Factor	log(Biomass + 1)			log(Number of seeds + 1)			Average diameter		
	F	df	P	F	df	P	F	df	P
Exclosure (EX)	3.28	3, 18.8	**0.044**	3.36	3, 18.7	**0.041**	1.61	3, 18	0.222
Mycorrhizae (MY)	13.91	1, 22.7	**0.001**	14.82	1, 22.5	**<0.001**	12.62	1, 24	**0.002**
Phosphorus PC (PC3)	2.62	1, 6.2	0.155	3.72	1, 6.3	0.100	2.96	1, 6	0.136
EX × MY	1.43	3, 22.7	0.260	1.20	3, 22.5	0.331	3.36	3, 24	**0.036**
EX × PC3	4.36	3, 20.0	**0.016**	3.45	3, 19.8	**0.036**	5.47	3, 18	**0.008**
MY × PC3	0.35	1, 23.6	0.563	1.06	1, 23.4	0.314	1.26	1, 24	0.272
EX × MY × PC3	0.40	3, 23.4	0.751	0.48	3, 23.2	0.701	0.49	3, 24	0.692

vs. NC, t = −1.92, df = 18.8, $P = 0.070$; ALL vs. NC, t = 1.51, df = 19.3, $P = 0.147$, Fig. 1A). Average *S. pulchra* seed production was significantly lower in SM exclosures than NC or S exclosures (SM vs. NC, t = −2.34, df = 18.6, $P = 0.030$; SM vs. S, t = 2.56, df = 18.4, $P = 0.020$) and significantly lower in ALL exclosures treatments that S exclosures (t = 2.23, df = 18.9, $P = 0.036$), but the difference in seed production between ALL exclosures and NC exclosures was only marginally significant (t = 2.05, df = 19.1, $P = 0.055$).

Inoculation with mycorrhizae resulted in *S. pulchra* seedlings with 53% greater biomass, 207% greater average seed production per plant, and 14% larger average diameters relative to the untreated seedlings in June 2005 (Table 1). Contrasts of mycorrhizae treatment by exclosure type indicates mycorrhizae pretreatment result in greater seedling biomass in NC and ALL exclosures (Figs. 2a and 2d). Similarly, pretreatment with mycorrhizae resulted in greater seed production in NC and ALL exclosures (Figs. 3a and 3d) relative to untreated plants within the same exclosure treatments. In all other exclosure treatment levels biomass accumulation and seed production by pretreated plants were generally quantitatively larger than, but did not statistically differ from, untreated plants.

In June 2005, seedling biomass, seed production, and average plant diameter were also influenced by the interaction between the phosphorus PC and exclosure type (Table 1). Examination by exclosure type suggests variation in phosphorus PC had no significant effect on seedling performance (biomass, spring average diameter) or seed production in NC or S exclosure treatments (Figs. 2a–b, 3a–b, 4a–b). However, in SM and ALL exclosure treatments both seedling performance measures and average seed production per plant were high in sites with high phosphate-low potassium soils and were significantly lower in sites with low phosphate-high potassium soils (Figs. 2c–d, 3c–d, 4c–d).

4. Discussion

Maintenance of the composition, productivity, and diversity of perennial grasslands is likely mediated by both mutualistic interactions (van der Heijden et al., 2008) as well as damage caused by herbivores (Augustine and McNaughton, 1998; Olff and Ritchie, 1998; Howe et al., 2006). Our work illustrates that local soil conditions may provide an important lens through which to view the interplay between mycorrhizal mutualists and herbivores in affecting plant growth, survival, and reproduction. Specifically, we show that (1) early mycorrhizal inoculation influences transplanted *S. pulchra* seedling establishment and that (2) pretreatment with mycorrhizae can result in greater seedling performance and reproductive potential when plants are subject to herbivory by the full complement of mammalian herbivores. Our results also indicate that (3) soil condition (i.e., phosphate/potassium

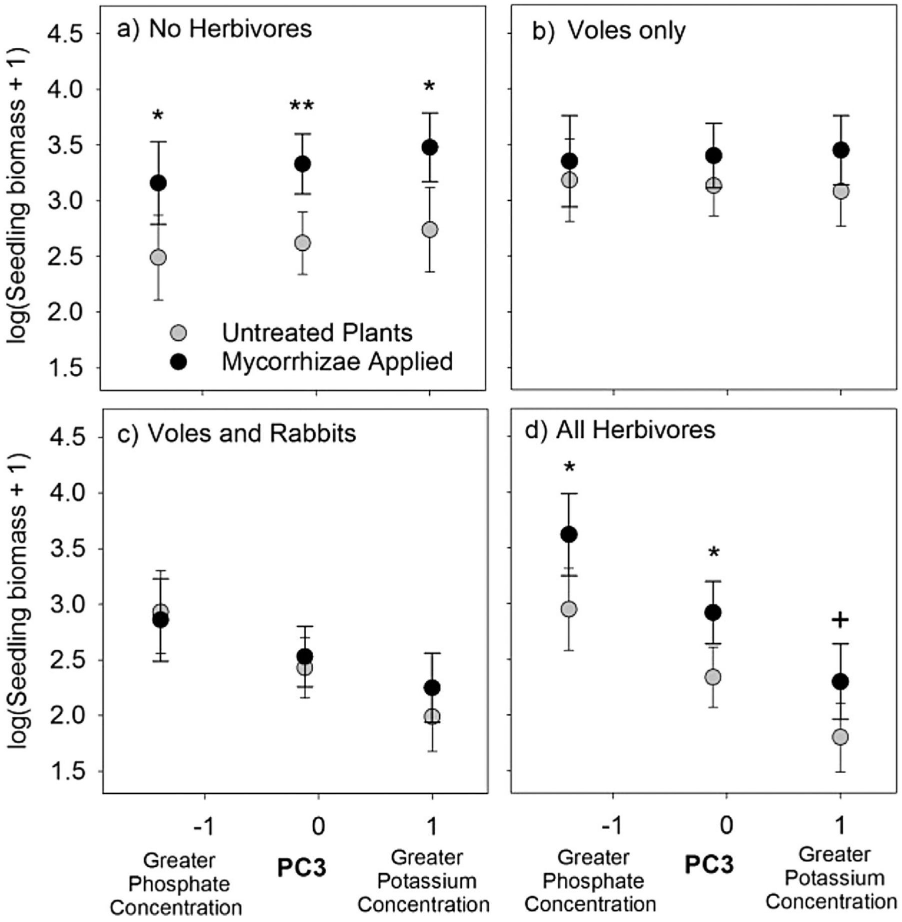

Fig. 2. Effect of pretreatment with mycorrhizae and the phosphorus PC on *S. pulchra* seedling biomass (June 2005) in exclosures permitting a) no mammalian herbivores ("No Herbivores", NC exclosures), b) only small mammalian herbivores ("Voles only", S exclosures), c) only small and medium mammalian herbivores ("Voles and Rabbits", SM exclosures), and d) all mammalian herbivores ("All Herbivores", ALL exclosures). Seedling biomass are log(x+1) transformed. Responses have been consolidated for analysis to least squares estimates at the 25% quartile (−1.39), the median (−0.12), and the 75% quartile (1.00) of the soil phosphate covariate within each exclosure type. Independent contrasts compare *S. pulchra* pretreated with mycorrhizae versus untreated plant at each quartile and the median. Error bars indicate one standard error for each least square mean value. Symbols above contrasts indicate p-value test results: no symbol = $p > 0.1$; "+" = $0.1 \geq p > 0.05$; "*" = $0.05 \geq p > 0.01$; "**" = $0.01 \geq p > 0.001$; "***" = $p \leq 0.001$.

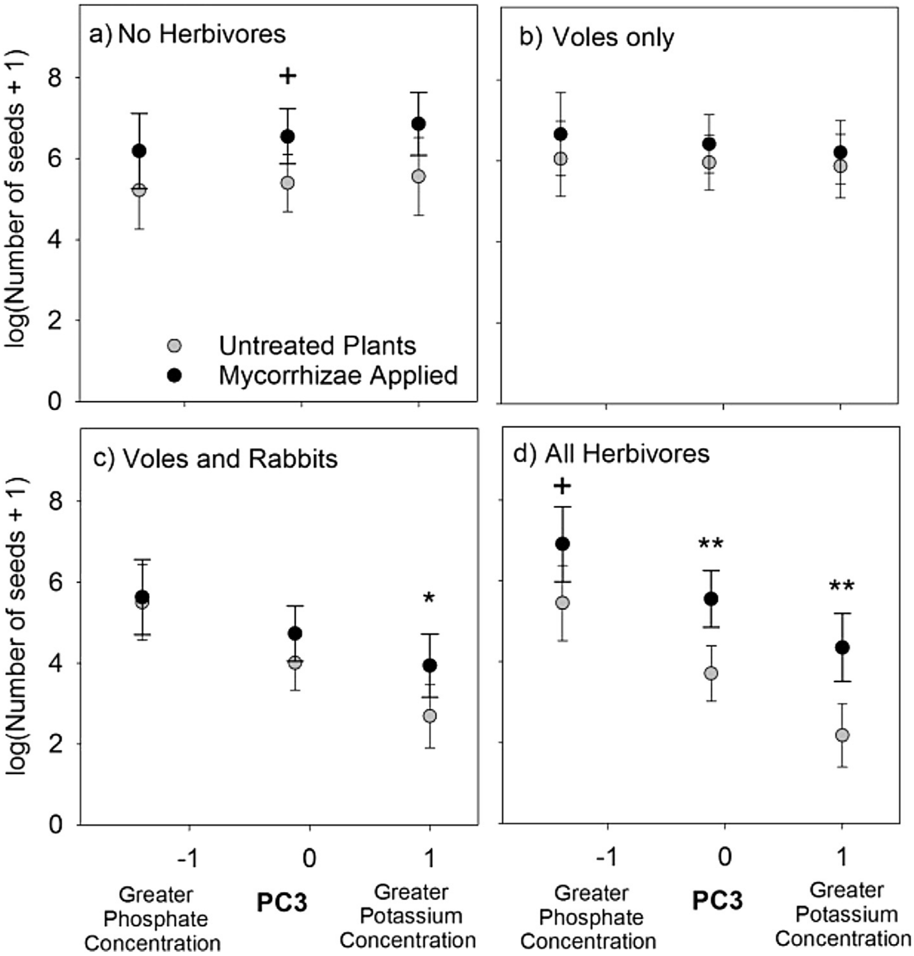

Fig. 3. Effect of pretreatment with mycorrhizae and the phosphorus PC on average *S. pulchra* seed production per plant (June 2005) in exclosures permitting a) no mammalian herbivores (“No Herbivores”, NC exclosures), b) only small mammalian herbivores (“Voles only”, S exclosures), c) only small and medium mammalian herbivores (“Voles and Rabbits”, SM exclosures), and d) all mammalian herbivores (“All Herbivores”, ALL exclosures). Estimates of seed production are log(x+1) transformed. Responses have been consolidated for analysis to least squares estimates at the 25% quartile (−1.39), the median (−0.12), and the 75% quartile (1.00) of the soil phosphate covariate within each exclosure type. Independent contrasts compare *S. pulchra* pretreated with mycorrhizae versus untreated plant at each quartile and the median. Error bars indicate one standard error for each least square mean value. Symbols above contrasts indicate p-value test results: no symbol = $p > 0.1$; “+” = $0.1 \geq p > 0.05$; “*” = $0.05 \geq p > 0.01$; “**” = $0.01 \geq p > 0.001$; “***” = $p \leq 0.001$.

concentrations) strongly influences the effect of herbivory on *S. pulchra* transplant performance and reproduction. Our work has several implications. First, our work provides a means to understand the context-specific nature of plant-herbivore and plant-fungi interactions, helping resolve the role of environmental heterogeneity in affecting the outcomes of restoration efforts (Valladares and Gianoli, 2007) and the prospects for long-term conservation. Second, although the outcomes of grazer access differed by herbivore guild, our work demonstrates strong, general benefits of mycorrhizal associations in promoting plant performance across a range of soil conditions. Third, our work demonstrates how the unique mammalian consumer guilds can have different effects on plant performance with specific consequences for grassland management strategies. We discuss each of these implications in greater depth below.

The importance of environmental gradients in shaping patterns of herbivory is becoming increasing apparent (Maron and Crone, 2006; Hahn and Orrock, 2015) and environmental heterogeneity likely plays a deterministic role in the survival and performance of plant communities in Mediterranean ecosystems (Valladares and Gianoli, 2007; Orrock et al., 2009; Coiffait-Gombault et al., 2012). In our study, herbivory had greater effects on *S. pulchra* performance and reproduction as site-level soil phosphate decreased and soil potassium increased. Systems with very fertile soils (e.g., those with high phosphate concentrations) can be more resistant to herbivore grazing (Proulx and Mazumder, 1998) and declines in soil phosphate may compromise plant resource availability for plant defense or regrowth. Nutrient limitation can lower plant tolerance to herbivory and allocation to secondary defense against generalist herbivores (Strauss and Agrawal, 1999; Stamp, 2003) and low soil phosphate concentrations may limit *S. pulchra* growth and herbivore tolerance. Our work suggests that local variation in phosphate-potassium soil condition can generate differential grazing pressure with greater herbivory occurring on *S. pulchra* seedlings in phosphate-poor, potassium-rich soils. Our work corroborates earlier work that indicates gradients in soil fertility influence herbivory (Harper, 1977; Louda et al., 1990; Eskelinen, 2008), and suggests that prior knowledge of the severity of herbivory on transplanted *S. pulchra* seedlings in southern California grasslands and knowledge of site-level soil fertility may help predict the effectiveness of *S. pulchra* seedling transplanting as a means of grassland restoration.

We also find evidence that early establishment of mycorrhizal mutualisms generally benefits plant survival, growth, and seed

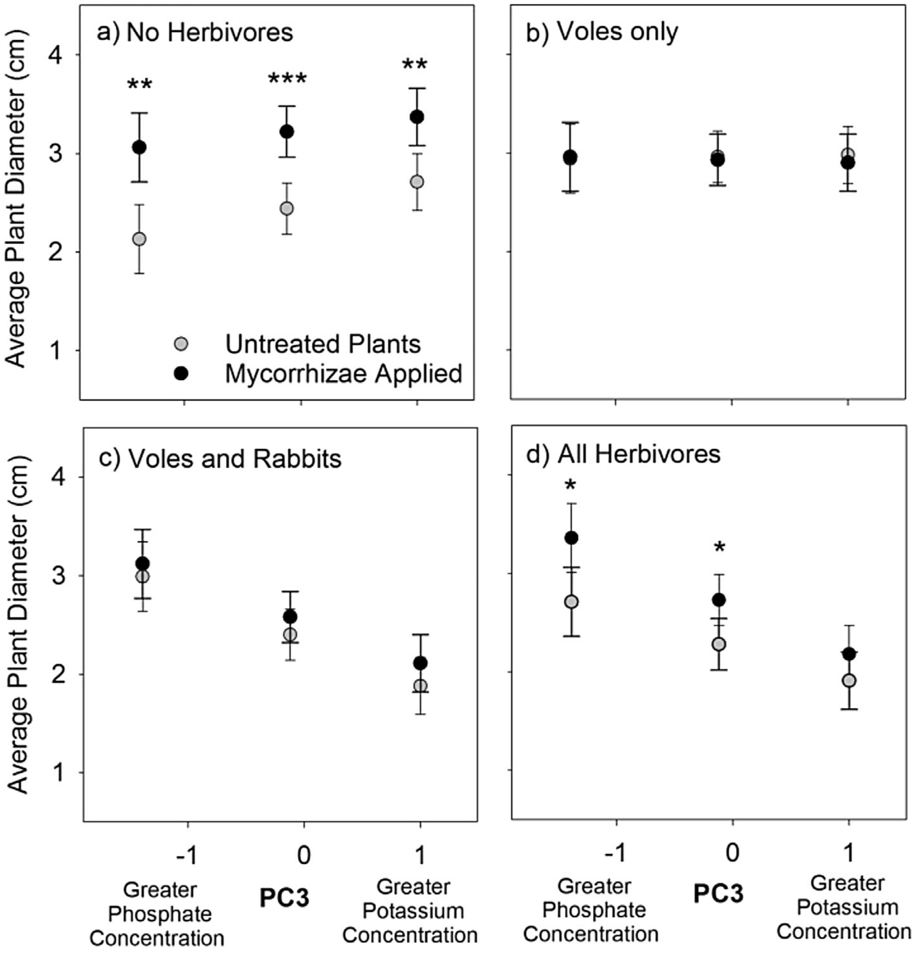

Fig. 4. Effect of pretreatment with mycorrhizae the phosphorus PC on average *S. pulchra* diameter (June 2005) in exclosures permitting a) no mammalian herbivores ("No Herbivores", NC exclosures), b) only small mammalian herbivores ("Voles only", S exclosures), c) only small and medium mammalian herbivores ("Voles and Rabbits", SM exclosures), and d) all mammalian herbivores ("All Herbivores", ALL exclosures). Responses have been consolidated for analysis to least squares estimates at the 25% quartile (−1.39), the median (−0.12), and the 75% quartile (1.00) of the soil phosphate covariate within each exclosure type. Independent contrasts compare *S. pulchra* pretreated with mycorrhizae versus untreated plant at each quartile and the median. Error bars indicate one standard error for each least square mean value. Symbols above contrasts indicate p-value test results: no symbol = $p > 0.1$; "+" = $0.1 \geq p > 0.05$; "*" = $0.05 \geq p > 0.01$; "**" = $0.01 \geq p > 0.001$; "***" = $p \leq 0.001$.

production. Notably, the effect of mycorrhizal inoculation on summer basal diameter was contingent upon herbivore access (Table 1), whereas there was no interaction between herbivore access and mycorrhizal inoculation for biomass and seed production. However, we note that mycorrhizae generally led to increased plant performance for all three metrics when the diversity of vertebrate herbivore was highest (Figs. 2d, 3d and 4d), suggesting that early inoculation with mycorrhizae may play an important role in mitigating the effect of herbivory. Colonization by AM fungi in some grasses results in a higher regrowth capacity following defoliation than for uncolonized conspecifics (Kula et al., 2005) suggesting that regrowth may be one strategy *S. pulchra* seedlings employ to tolerate herbivory in southern California grasslands. Mycorrhizae may also serve to generally increase the capacity for plants to mount induced defenses once attacked (Kempel et al., 2010; Jung et al., 2012). Moreover, recent work demonstrates that mycorrhizae-infected plants can communicate via mycelial networks (Song et al., 2010), informing neighbors of herbivory risk (Babikova et al., 2013). Plants receiving early inoculation may be able to more effectively transmit information regarding herbivory risk and induce defenses before an attack is underway. Although we did not measure plant defense during the course of our study, our work suggests that any allocation of resources to defense or tolerance did not adversely affect reproductive output. Our work also highlights how plant-mycorrhizal interactions may contribute to the potential for plant population persistence and spread: like many perennial plants of conservation concern (Turnbull et al., 2000; Seabloom et al., 2003), *S. pulchra* establishment is often limited by the number of viable seeds (Orrock et al., 2009). The increased rates of seed production we observed from inoculated plants suggest that mycorrhizal inoculation may be an essential method to employ to ensure future *S. pulchra* spread and population expansion, particularly in degraded habitats with a diverse community of vertebrate herbivores.

Mammalian herbivores limit *S. pulchra* recruitment through consumption at different plant life stages. Small mammals generate little reduction in *S. pulchra* seedling performance relative to complete exclosures, but strongly limit *S. pulchra* establishment via granivory (Orrock et al., 2009). Conversely, medium-sized mammalian herbivores, and to a lesser extent larger herbivores, predominantly limit *S. pulchra* performance and reproductive potential via grazing on vegetative and reproductive tillers (Orrock et al., 2009). Medium-sized herbivores (e.g., ground squirrels, rabbits), in particular, can significantly alter the composition and structure of grassland vegetation (e.g., Bartholomew, 1970) and reductions in *S. pulchra* reproductive capacity mediated by this group of herbivores (Fig. 1B) represent a direct obstacle to sustainability in restored populations. We found that little additional damage is attributable to large consumers (Figs. 1A–C). However, growth and reproductive benefits associated with mycorrhizal inoculation were most apparent when transplanted seedlings were exposed to the cumulative effects of all consumer guilds suggesting the ecological determinants of grazing severity associated with the full complement of vertebrate herbivores may permit transplanted seedlings to maximize growth and reproductive potential. While the effect of mycorrhizal inoculation was also apparent in exclosures that completely excluded mammalian herbivores, the effect of mycorrhizal inoculation was diminished in S and SM exclosures, suggesting that small and medium-sized consumers were foraging more intensively within these exclosures. We suggest two possible scenarios to explain these results: 1) competitive interactions with larger consumers limit the effects of small and medium-sized herbivores on transplanted seedlings or 2) S and SM exclosure structure provided a refuge from predation for small and medium-sized herbivores relative to ALL exclosure structure permitting more intense foraging within these exclosures (Orrock et al., 2009). Our work emphasizes the importance of considering how herbivore identity can influence the efficacy of different plant restoration or conservation strategies. For example, exclusion of small mammals (e.g., mice, voles) may be essential to ensure sustainable production following direct seeding (Howe and Brown, 2001; MacDougall and Wilson, 2007), whereas herbivore exclusion methods may be able to focus on only medium or large herbivores if sites are restored with seedling transplants.

5. Conclusions

Restoration of degraded grasslands is likely to hinge upon understanding the degree to which influential ecological interactions are context-specific (Bakker and Berendse, 1999). In finding that the performance and reproduction of a perennial native grass is contingent upon soil fertility, mycorrhizae, and herbivore guilds, our results inform effective management strategies, e.g., restoration of *S. pulchra* should consider how soil conditions interact with resident vertebrate herbivore guilds to identify locations best suited for seedling survival. Our results also highlight the need for more studies evaluating how environment context influences facultative or deleterious interactions within restored plant populations (e.g., Halpern et al., 2016). In particular, field studies assessing how anthropogenic disturbances (e.g., fire, plant invasions) or the techniques employed to assist plant reintroduction (e.g., tilling, weeding, pesticides) influence local soil conditions, AM fungal spore abundance, or both may directly inform the outcomes of herbivory on restored plant populations. Given that degraded grasslands can also have naturally depleted mycorrhizal potential (e.g., Requena et al., 1996, 2001), assessment of soil mycorrhizae spore abundance may be of particular importance in ensuring persistent growth and reproduction of transplanted seedlings when restoring grassland sites.

Author contributions

JLO, MSW conceived and designed research and performed experiments; BMC, JLO analyzed the data; BMC, JLO wrote the manuscript; BMC, JLO, MSW edited the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.actao.2016.09.005.

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