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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