Communication

Priority Treatment Leaves Grassland Restoration Vulnerable to Invasion

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Abstract: Priority effects can be used to promote target species during restoration. Early planting can provide an advantage over later-arriving species, increasing abundance of these early-arrivers in restored communities. However, we have limited knowledge of the indirect impacts of priority effects in restoration. In particular, we do not understand how priority effects impact non-target species. Of particular conservation concern is how these priority effects influence establishment by non-native species. We use a field-based mesocosm experiment to explore the impacts of priority effects on both target and non-target species in California grasslands. Specifically, we seeded native grasses and forbs, manipulating order of arrival by planting them at the same time, planting forbs one year before grasses, planting grasses one year before forbs, or planting each functional group alone. While our study plots were tilled and weeded for the first year, the regional species pool was heavily invaded. We found that, while early-arrival of native grasses did not promote establishment of non-native species, giving priority to native forbs ultimately left our restoration mesocosms vulnerable to invasion by non-native species. This suggests that, in some cases, establishment of non-native species may be an unintended consequence of using priority treatments as a restoration tool.

1. Introduction

Community assembly, the process by which species come together to form a community, is an important driver of community structure and function [1]. Often, early-arriving individuals tend to exhibit an advantage, performing better than individuals that arrive later—a principal known as priority effect [2]. Priority effects can be driven by several forces, including size-asymmetric competition [3,4] and soil legacy effects [5,6]. While these dynamics play a role in driving the composition of incipient communities, they can also have long-lasting effects, potentially impacting community trajectories well into the future [7,8].

Restoration practitioners have begun harnessing the concepts of community assembly to facilitate successful restoration outcomes. In particular, practitioners can use priority effects to enhance desirable but difficult-to-establish species by planting them early [9]. In fact, as little as two-weeks of a head start has been shown to significantly enhance a species representation in a community in some cases [10,11]. However, there has been no exploration of how the use of priority effects in restoration practices may influence non-target species in the community. In particular, it is possible that priority effects could have unintended consequences if the treatment reduces biotic resistance, leaving these areas more vulnerable to invasion.

Here, we harness the power of a field-based priority mesocosm experiment to explore the relationship between priority effects and vulnerability to invasion. In particular, we assembled communities of native grasses and forbs, manipulating the order of arrival of these two functional

groups. In these assembled native California grass and forb communities, we found that a one-year delay in seeding dominant grasses promoted invasion by non-native plant species.

2. Materials and Methods

2.1. Mesocosm Study

We assembled communities of native forbs and/or native grasses, altering order of functional group arrival to explore how priority impacts community composition. Mesocosms were assembled at three sites in north-central California: the UC Davis Agricultural Experiment Station in Davis, the McLaughlin Natural Reserve in Lower Lake, and the Hopland Research and Extension Center in Hopland. All sites were on level ground recovering from past agricultural use within a Mediterranean grassland habitat and had high levels of invasion in the regional species pool. Soil at all sites was a relatively fertile clay loam. For more specifics on sites, see Young et al. [12].

Prior to planting, sites were tilled to control weeds just before the first germinating rain of the growing season, and then one to two weeks following this rain. Plots were then seeded in November of 2011. We broadcast seeds into square plots, 1.25 m on a side and spaced 1 m from adjacent plots. Alley-ways between plots were managed with either mowing or herbicide spraying to limit weed cover, though weeds were present and varied to some extent across sites. Plots were lightly raked immediately before planting and seeds were sown by hand in one of five planting treatments: 1) forbs sown alone (F), 2) grasses sown alone (G), 3) grasses and forbs sown at the same time (GF), 4) forbs sown one year before grasses (FtG), 5) grasses sown one year before forbs (GtF). Each treatment was replicated five times at each of the three sites. Seeding rates were based on common regional restoration practices (Table 1). For more details on planting, see Young et al. [12]. Non-seeded species were hand-weeded from plots for the first growing season after planting.

Table 1. Seeding rates (live seed/m2) for seeded grasses and forbs.

Grasses		Forbs	
Species	**Rate**	**Species**	**Rate**
Bromus carinatus	100	*Achillea millefolium*	175
Elymus glaucus	100	*Asclepias fascicularis*	50
Hordeum brachyantherum	100	*Croton setigerus*	50
Stipa pulchra	100	*Eschscholzia californica*	125

We surveyed cover of each species in each mesocosm in May of 2015 (after four growing seasons) by visually assessing percent cover of each species. We then summed cover for the following species groupings: 1) seeded grasses, 2) seeded forbs, 3) non-native species.

2.2. Analysis

Invasive cover was log-transformed to achieve normality. To assess the impact of priority planting treatment on cover of 1) seeded grasses, 2) seeded forbs, 3) non-native species, we used ANOVA, blocked by site, with priority treatment as the explanatory variable. A Tukey's HSD test was used to determine differences between priority treatments. Analyses were conducted in R version 3.6.1.

3. Results

Priority treatments influenced native grass cover in our study plots (site: $F_{2,68} = 4.98$, $p = 0.01$; treatment: $F_{4,68} = 18.06$, $p < 0.0001$; Figure 1A). Specifically, seeded grasses were significantly more abundant when they arrived in the first year of planting, regardless of whether they were seeded alone (G), seeded along with forbs (GF), or seeded a year before forbs (GtF), while they were significantly less abundant when seeded a year after forbs (GtF v FtG: $p < 0.0001$; G v FtG: $p < 0.0001$; GF v FtG: $p = 0.0005$).
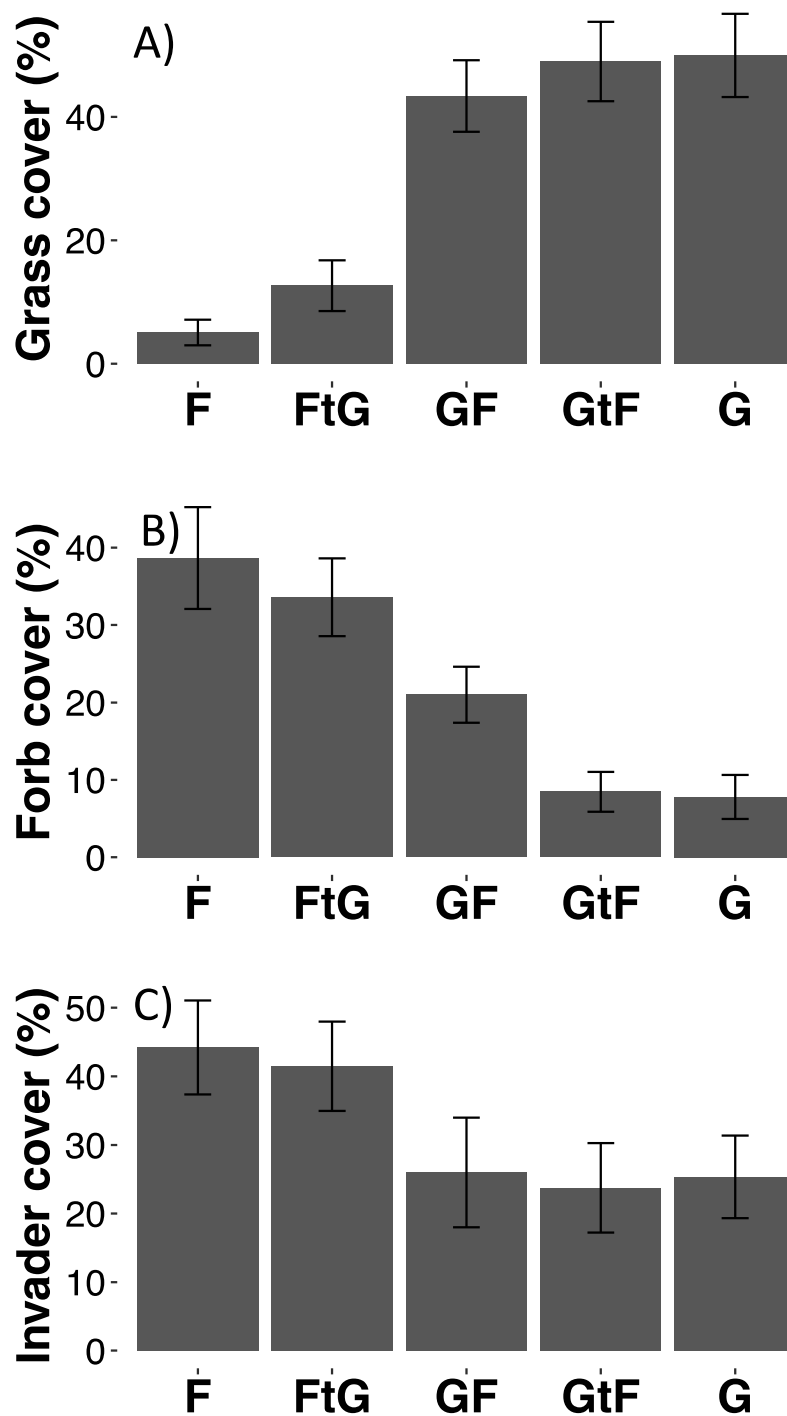
Figure 1. Mean cover (± standard error) of (**A**) native grasses, (**B**) native forbs, (**C**) exotic plants across priority planting treatments including forbs seeded alone (F), forbs seeded a year before grass (FtG), grasses and forbs seeded at the same time (GF), grasses seeded a year before forbs (GtF), and grasses seeded alone (G).

Priority treatments also influenced forb cover in the plots (site: $F_{2,68} = 10.59$, $p < 0.0001$; treatment: $F_{4,68} = 13.17$, $p < 0.0001$; Figure 1B). Specifically, forb cover did not differ between plots in which forbs were given one-year priority (FtG) and plots in which forbs were seeded alone (F) ($p = 0.89$). Forb cover in forb priority plots (FtG) was significantly higher than plots in which grasses were given priority (GtF) ($p = 0.0002$), and also (but not significantly) than plots in which the functional groups arrived at the same time (GF) ($p = 0.16$).

At least 17 non-native plant species established within our mesocosm plots (Table 2). We found significant effects of both site and priority treatment on non-native abundance (site: $F_{2,68} = 15.16$, $p < 0.0001$; treatment: $F_{4,68} = 4.17$, $p = 0.004$; Figure 1C). Specifically, while non-native cover did not differ significantly between plots in which only forbs were seeded (F) and plots in which forbs were given one-year priority over grasses (FtG) ($p = 0.99$), plots in which grasses and forbs were seeded at the same time as forbs (FG) had significantly less cover by non-native species than did forb priority plots (FtG) ($p = 0.047$), and marginally lower invasive cover than did forb-only plots (F) ($p = 0.06$). Providing grass with one-year priority over forbs (GtF) did not increase invasion relative to plots in which forbs and grasses arrived at the same time (GF) ($p = 0.9998$).

Table 2. Non-native species found within the study plots.

Species	Functional Group
Avena barbata/fatua	grass
Bromus diandrus	grass
Bromus hordeaceus	grass
Convovulus arvensis	vine
Hordeum murinum	grass
Lolium multiflorum/perenne	grass
Malva parviflora	forb
Phalaris aquatica	grass
Plantago sp.	forb
Taeniatherum caput-medusae	grass
Triticum aestivum	grass
Unknown forbs	forb
Unknown grasses	grass
Vicia villosa	N-fixing forb
Vulpia myuros/bromoides	grass

4. Discussion

Priority effects tend to advantage early-arriving species [2]. As such, manipulation of species arrival has been offered as a possible restoration technique, allowing land managers to prioritize select species by planting them earlier than others [9]. However, we find that, at least in some cases, this can leave restored communities vulnerable to invasion. Specifically, in our California grassland restoration study, we found that adding native grasses one year after native forbs during experimental restoration plantings left these plots vulnerable to invasion relative to plots in which grasses and forbs were seeded at the same time. Adding native forbs one year after native grasses, on the other hand, did not result in an increase in invasion.

Priority effects were largely successful in promoting target functional groups. In the 2015 dataset presented here, grasses were more abundant in all treatments in which grasses were added in the first year of study. Likewise, forbs were also most abundant when they were seeded in the first year of study. Considering cover data in the same plots collected in 2014, we found even clearer evidence for the power of priority effects. In the 2014 survey of plant cover, one-year priority of grasses significantly increased grass cover relative to plots in which grasses and forbs were added at the same time and one-year priority of forbs significantly increased forb cover relative to plots in which grasses and forbs were added at the same time [9]. These findings are in agreement with others that have found that alterations to the order of arrival of plant species within a community shape community composition [5,7,13,14].

Native grasses, with or without the presence of native forbs, seem to confer a greater level of biotic resistance to invasion, at least at very early stages of community development in these California grasslands. Grasses in particular have been found elsewhere to offer a high level of biotic resistance to invasion [15]. Competition between the resident community and invaders for resources such as light, water, and/or nutrients is a major factor regulating biotic resistance [16]. As such, priority

effects have the potential to increase a system's susceptibility to invasion if they reduce its capacity for biotic resistance in some way [13]. The decrease in invasion by early planting of grasses in our study may be due, in part, to the ability of native California grasses to establish and occupy space quickly, thereby making it more difficult for non-native species to establish. Previous work has suggested that dense native grass cover, more than biodiversity per se, can limit invasion by non-native grasses in California grasslands [17]. However, this difference is likely not the whole story in our study system. After one growing season, native grass cover in the grass-only plots was 50%, while forb cover in the forb-only plots was similar, at 43% (unpublished data). Functional group matching of native and non-native species may also play a role in enhancing biotic resistance in our study as non-natives have been shown to have more difficulty invading systems that are dominated by the same functional group [15,18]. California grasslands are heavily invaded by annual grasses, primarily from Eurasia [19]. In our surveys, grasses constituted 65% of invasive cover. We found evidence that non-native grasses responded more strongly to the priority treatments than did forbs, with marginally less grass invasion in plots in which grasses arrived earlier (Appendix A and Figure S1). The findings of our study bolster the evidence of others that early colonization by native grasses may help limit the establishment of non-native plants [20].

Dynamics during community assembly may play a role in the success of non-native species. In particular, the ability of non-native species to insert themselves into communities at later stages of community assembly may contribute to their success [21]. Here we find that this ability is likely uneven across ecological contexts. In particular, non-native species were more successful at inserting themselves into communities in which forbs were the sole initial colonizers, while they had much more difficulty invading communities in which grasses were early colonizers. Higher biodiversity (as accomplished in plots in which both grasses and forbs were added in the first year), did not resist invasion better than plots in which grasses were added alone, suggesting that, in this case, functional group is more important that biodiversity in warding off invasion.

Forbs are often of particular interest in the restoration of grassland systems. Both aesthetically pleasing and ecologically functional [22,23], they are a desirable group in restored grasslands, and their promotion during restoration can lead to increased biodiversity [24]. However, perennial grasses are often competitively dominant and have historically dominated the composition of restored grasslands [25,26]. As such, there is growing interest in promoting forbs in grassland restoration. Of late, restoration seed mixes tend to favor forbs over grasses [27]. Another means to this end has been to provide forbs with priority during planting [9,25].

Priority effects still have an important place in restoration. Insertion of native species into a restoration, before non-native species invade, can promote native cover while depressing non-native cover [10,28,29]. In addition, priority effects can be used to promote certain native species when seeded earlier than other native species [4,7,9], but these effects are likely often contingent on factors, including species identity [7,11,18], environmental context [10,12,30], and background levels of invasion. As such, it can be important to evaluate the specifics of a particular system to determine when and where priority can be a useful restoration tool, and when it may leave a system vulnerable to unintended consequences such as invasion.

We find a signature of priority treatments on non-native cover in our plots after four growing seasons. Community dynamics set up during assembly do seem to have the potential to have lasting impacts, not only for seeded species [7,8,31] but also, as evidenced here, for non-target species including non-natives. Here, we find that four growing seasons after planting initiation, order of species arrival still drives the relative abundance of the target functional groups, but has also set up important variability in the abundance of non-native species across planting treatments. Specifically, delaying the planting of native grasses increased susceptibility to invasion by non-native species in the regional species pool, and may require greater attention to post-restoration weed control.

While manipulating assembly dynamics is still an important and underutilized tool in restoration, unintended consequences involving non-target species warrant further study. Restoration outcomes

can be highly case-specific, as can the long-term outcomes of priority effects. Restoration ecology should work toward building a more predictive framework, including a better understanding of when priority effects will likely yield desirable restoration outcomes, and when they may drive unintended consequences.

Appendix A

To assess the impact of priority planting treatments on invasion by 1) non-native grasses and 2) non-native forbs we used ANOVA, blocked by site, with priority treatment as the explanatory variable. A Tukey's HSD test was used to determine differences between priority treatments.

We found that priority treatments influenced native grass cover in our study plots (site: $F_{2,68} = 5.81$, $p = 0.005$; treatment: $F_{4,68} = 2.68$, $p = 0.04$; Figure S1A). However, Tukey's HSD failed to detect any significant pairwise differences among priority treatments ($p > 0.05$). Priority treatment did not influence plot susceptibility to forb invasion (site: $F_{2,68} = 50.85$, $p < 0.0001$; treatment: $F_{4,68} = 0.72$, $p = 0.58$; Figure S1B).

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