Title: GLMM BACI environmental impact analysis shows coastal dune restoration reduces seed predation on an endangered plant

Running head: Dune restoration reduces seed predation

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Abstract: Invasive plants create refuge habitat for predators that results in increased levels of seed predation on native plants. We test the effects of a large-scale removal of the invasive plant *Ammophila arenaria* on the pre-dispersal seed predation rate experienced by an endangered plant in a coastal dune ecosystem. Restoration resulted in a large and lasting reduction in seed predation in this system, and reducing predation could be an explicit goal in future restoration projects. The GLMM statistical model with a BACI (before-after-control-impact) design used here is a useful, flexible model that can be applied to analyze other large-scale restoration activities.

Keywords: *Ammophila arenaria*, apparent competition, BACI, GLMM, *Lupinus tidestromii*, seed predation

Implications for practice:

- This is the first documentation that large-scale removal of invasive plants through habitat restoration reduces the threat of seed predation via refuge-mediated apparent competition for native plants
- Reducing refuge-mediated predation pressure should be a goal incorporated into large-scale invasive plant removal programs
- GLMM is a useful tool for analyzing BACI designs in restoration projects

Introduction

In many ecosystems, invasive plant species change habitat structure, allowing for higher densities of rodents and other seed predators. Invasive plants can harm co-occurring native plants via apparent competition when they increase the density of seed predators and thus seed predation (Dangremond et al. 2010; Orrock et al. 2010; Holt 1977). Small-scale exclosure experiments have demonstrated that apparent competition with nearby invasive plant species threatens native plant establishment in restoration projects (Orrock et al. 2009; Howe & Lane 2004), and these have suggested that restoration projects must reduce seed predation by small mammals to be successful. However, because reducing small mammal seed predation is typically not a major motivating factor for large-scale invasive plant removal (Orrock et al. 2009), restoration practitioners do not typically quantify if and how much predation rates on native plants decline following habitat restoration.

In coastal habitats around the world, *Ammophila arenaria* was intentionally introduced to stabilize dunes. It has dramatically spread from introduction sites, with many negative consequences. Stabilization by *A. arenaria* creates high foredunes and reduces interior sand movement (Hart et al. 2012; Wiedemann & Pickart 1996), which makes dunes susceptible to erosion during strong storm surge (Millington et al. 2009). In western North America *A. arenaria* reduces the availability of early successional dune habitat that sustains rare plants (Dangremond et al. 2010) and animals, such as the threatened Western snowy plover (USFWS 2007). Removing *A. arenaria* to restore plover nesting habitat, native vegetation, and to make dunes resilient to erosion is a top priority for many managers of these coastal ecosystems (e.g., Parsons 2015).

Ammophila arenaria creates dense vegetative cover that houses higher densities of *Peromyscus maniculatu*s than native dune vegetation (Pitts & Barbour 1979). *Peromyscus maniculatu*s consume fruits and seeds of native plants, particularly legumes, in open dune habitat (Pitts & Barbour 1979; Maron & Simms 2001; Pardini et al. 2017). High pre-dispersal seed predation by mice in the presence of *A. arenaria* on the rare legume, *Lupinus tidestromii* has been linked to extinction risk in population viability analyses (Dangremond et al. 2010). Removing invasive *A. arenaria* and the predator refuge habitat it creates should be the most direct method to reduce the threat of seed predation to native plant species. However, it has yet to be demonstrated that large-scale removals of this invasive grass are sufficient to create significant and lasting decreases in seed predation rates.

Here, we use a long-term data set to assess the effect of dune restoration (removing *A. arenaria*) on pre-dispersal seed predation experienced by *L. tidestromii* before and after a large-scale restoration. We use a BACI (before-after-control-impact) design to statistically assess whether dune restoration at a single impact site decreases predation compared to that observed in 6 control sites.

Methods

At our study location, Point Reyes National Seashore (PRNS, Marin County, California, USA), native plants occur in remnant dune basins that are surrounded by near monocultures of the invasive plants *A. arenaria* and *Carpobrotus* spp. *Ammophila arenaria* was introduced to the beaches in the 1940s for sand stabilization and has increased in extent such that it is now a dominant feature of the landscape (Parsons 2015; see Fig. 1). *Lupinus tidestromii* (Fabaceae) is a perennial, herbaceous plant that is endemic to California, and is known from 15 extant populations in Sonoma, Monterey, and Marin counties, seven of which are located at PRNS. It is listed as federally endangered and among its primary threats are habitat loss and encroachment by *A. arenaria* (USFWS 2009). We began monitoring *L. tidestromii* at three sites

(AL, ATT, NB) in 2005 and expanded monitoring at an additional four additional sites (Pop9, DR, BR, and BS) in 2008 (Fig. 1).

Pre-dispersal seed predation on *L. tidestromii* occurs when mice remove entire fruiting racemes (or rarely single fruits) from plants prior to seed dispersal, leaving a visible clip mark (see pictures in supporting information, Fig. S3). On the annual census date (usually ~June 1, when most plants are dehiscing fruits), we counted the number of racemes that were dehisced, predated, or aborted on the entire plant. In 2008-2011, we also measured pre-dispersal seed predation over the entire fruiting season by observing the fate of marked flowering racemes every 3 weeks until fruiting was complete. We calculated pre-dispersal predation as a proportion [clipped/(clipped+intact)] for each plant in all sites for which data were available from 2005-2017. For more details on the methods, see supporting information, Table S1 and Fig. S4.

PRNS completed a large restoration project at Abbotts Lagoon (AL) between the 2010 and 2011 fruiting seasons that removed 32 ha of *A. arenaria* from a 77 ha project area. *Ammophila arenaria* was removed and buried with excavators and bulldozers in foredune and some backdune areas; hand-pulling was used in existing remnant native dune areas that were only sparsely invaded, and herbicide treatment was used in other backdune areas (see supporting information, Fig. S1). Mechanical removal results in complete removal of above-ground biomass so it essentially eliminates rodent refuge habitat immediately, while herbicide treatment effects more gradual change in invaded habitats. Additional treatment of *A. arenaria* occurred near other populations (ATT and BR) between 2014 and 2016; these were smaller in spatial scale and primarily herbicide (due to lower cost). We do not expect these treatments to affect *L. tidestromii* (see supporting information, Fig. S2).

Our analysis here analyzes the effect of restoration at a single impact site compared to 6 control sites. The AL restoration project cost $2-2.5 million, and these high costs prohibited replication of the restoration activity. However, Abbotts Lagoon should be representative of *L. tidestromii* populations at Point Reyes National Seashore, and there are no known confounding variables that should influence our results (e.g., timing of pre-dispersal seed predation sampling, see supporting information, Table S1 and Fig. S4). Further, the long-time series of pre-dispersal seed predation data (13 years) should reduce the probability of Type I and Type II errors.

We used a before-after-control-impact (BACI) design to analyze our data (Underwood 1993). We used a generalized linear mixed model (GLMM) framework due to non-normal distribution of predation data and the presence of random effects (Bolker et al. 2009). GLMMs are robust to unbalanced datasets (Pinheiro & Bates 2000), so they are applicable to BACI analysis with a single impact site and multiple control sites. We constructed a GLMM to determine the proportion of variance in pre-dispersal predation explained by the fixed effects of period (before-after) and treatment (control-impact), and their interaction (the BACI effect). We used a logit link function for proportion data with a binomial distribution. We designated 2005-2010 as "before" and 2011-2017 as "after," and the Abbotts Lagoon site as "impact" and all other sites as "control." To generalize our inference, we included site, year, and their interaction term as random effects. We used an observation-level (individual plant) random effect to account for overdispersion (Harrison 2015). We pooled data across method of data collection (marked racemes versus single point estimates; fitting a model with method as fixed effect did not improve the level of explained variance and did not change the statistical significance of the BACI effect; see supporting information, Fig. S5). We used parametric bootstrap method to test for statistical significance of the BACI effect using the pbkrtest package (Halekoh & Højsgaard 2014). Analyses were performed using the glmer function in the lme4 package (Bates et al. 2015) in the statistical program R (R Core Team 2017). We provide the code for analysis in the supporting information.

Results

Our final GLMM model explained 0.474 of the variance in predation rates. There was a significant BACI period X treatment effect (PBtest=34.832, P=0.014). The restoration treatment at Abbotts Lagoon significantly decreased the amount of pre-dispersal predation observed after treatment compared to before and compared to other control sites. The estimated mean predation rate in the control sites dropped from 0.73 to 0.53 while the mean predation rate in the impact site dropped from 0.78 to 0.03 (Fig. 2, Fig. 3). The BACI effect estimated from the model (the difference of the two changes: $[control_{after} - control_{before}] - [impact_{after} - impact_{before}]$) was 0.548 ± 0.124 (Fig. 3; see also supporting information Table S1 and Fig. S5).

Discussion

Our analysis shows that a large-scale restoration project that removed *A. arenaria* surrounding the remnant dune at Abbotts Lagoon significantly reduced the pre-dispersal seed predation experienced by the federally endangered *L. tidestromii*. The response was almost immediate, and predation levels have remained low for seven years. Apparent competition is becoming a well-documented way that invasive plants threaten native plants in other ecological systems. We concur with others who have suggested that reducing predation should be a restoration goal, and that large-scale restorations are likely needed to sufficiently address the threat posed by predators (DeCesare et al. 2010; Orrock & Witter 2010). Our results document that restoration that is large in spatial extent and complete in removing above-ground biomass is highly effective at reducing the threat of predation. We note that the removal of *A. arenaria* in this restoration by mechanical methods (excavator and burial) results in extremely complete removal. Preliminary results from projects at other PRNS sites where herbicide treatment was the primary removal method suggest that these projects may not have the same immediate result in terms of reducing predation pressure; however, similar reductions may be achieved once standing *A. arenaria* biomass finally decomposes.

From a rare plant perspective, the ultimate goal of restoration is to increase plant population size. It is possible that reductions in pre-dispersal seed predation might not translate into an increase in recruitment of seedlings if there is still high post-dispersal seed predation (Pardini et al. 2017) or if there are other factors that limit seedling establishment and survival. Restoration that removes rodent predator habitat is likely to reduce both pre- and post-dispersal predation if rodent densities are reduced or distributions are highly altered. Census work shows that the reduction in pre-dispersal seed predation observed at Abbotts Lagoon is translating into higher recruitment, expanded distribution, and higher population size for *L. tidestromii*. By summer 2013, there were over 20,000 new plants within restored areas (Parsons 2015; see Fig. S1), and since that time *L. tidestromii* has recruited and reached high abundance and cover throughout most of the mechanically treated backdunes.

We suggest the GLMM framework can be useful for BACI analysis in restoration settings. BACI analyses can be constrained in the analysis-of-variance framework, in which researchers may encounter difficulties transforming data to meet assumptions of normality and homogeneity of variances (Bolker et al. 2009). GLMM can accommodate the random effects, non-normal response variables (Bolker et al. 2009), overdispersion (Harrison 2015), and unbalanced datasets (Pinheiro & Bates 2000) typical in restoration ecology.

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Supporting Information The following information may be found in the online version of this article:

Figure S1. Maps of the restoration activities at Abbotts Lagoon, Point Reyes National Seashore

Figure S2. Pre-dispersal seed predation of *Lupinus tidestromii* at seven study sites from 2005-17 at Point Reyes National Seashore

Table S1. Pre-dispersal seed predation of *Lupinus tidestromii* at seven study sites from 2005-17 at Point Reyes National Seashore

Figure S3. Photos showing pre-dispersal seed predation of mice on *Lupinus tidestromii* at Point Reyes National Seashore

Figure S4. Date on which snapshot estimate of pre-dispersal seed predation is observed does not affect predation level

Figure S5. Method of estimating pre-dispersal seed predation does not influence the results of the BACI statistical model

R code used in statistical analysis

Figure 1. Study location and *Lupinus tidestromii* study sites at Point Reyes National Seashore, Marin County, California, USA. Thin black line indicates dune habitat; blue indicates area dominated by *Ammophila arenaria* prior to restoration (extent derived from 1994 vegetation map and updated with 2007 GPS mapping; data from PRNS); orange indicates area dominated by *Carpobrotus* spp (extent derived from 1994 vegetation map; data from PRNS) .

Figure 2. Pre-dispersal seed predation per plant of *Lupinus tidestromii* at Point Reyes National Seashore before and after restoration (between 2010 and 2011) at the single impact site (Abbotts Lagoon) and averaged across the 6 control sites. Open, blue triangles (impact site) and open, orange circles (control sites) show data points (the proportion of total racemes that were predated) for single plants.

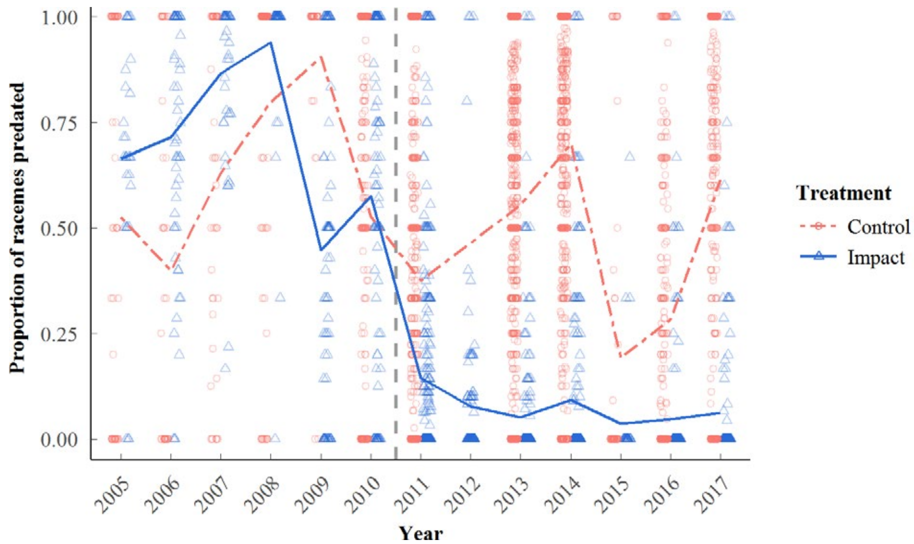

Figure 3. Least square means values of pre-dispersal seed predation of *L. tidestromii* at Point Reyes National Seashore before and after restoration (between 2010 and 2011) at the single impact site (Abbotts Lagoon) and across the six control sites. There was a significant period X treatment BACI interaction effect: restoration significantly reduced predation at the impact site. Error bars show the ± 95% bootstrap CI (1000 runs).