

LONG TERM RESTORATION PROJECTS: INVASIVE SPECIES CAUSED FEEDBACKS
ABOVE AND BELOWGROUND

by

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Long term restoration projects: Invasive species caused feedbacks above and belowground

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ABSTRACT

Invasive species are considered one of the top five threats to biodiversity worldwide and when established can quickly degrade a system. In response to this degradation a large number of ecological restoration projects have been implemented to mitigate the effects of invasion. Using a long term vernal pool restoration project that has documented invasive encroachment over time, I sought to tease apart the mechanisms underlying exotic plant invasion through exploration of positive feedbacks in both above and belowground plant communities. My results demonstrated that a thick litter layer deposited by the invasive species not only decomposed at a slower rate than native litter, but also strongly hindered native species abundance. This invasive litter layer created a positive feedback that allowed for invasive species recruitment while hindering the majority of natives from reaching the aboveground vegetation. However, vernal pool native species were maintained at continually high densities in the seed bank and the abundant aboveground invasives had a much lower presence belowground. These results were corroborated by my comparisons of the existing seed bank community to the historical aboveground vernal pool vegetation (5-8 years prior) versus more recent aboveground vegetation (1-3 years prior). Here I found a strong legacy effect in seed banks; i.e., seed banks had a composition with greater similarity to the historical aboveground vegetation than to the more recent aboveground vegetation. Finally, to test if similar relationships as those observed for

above and belowground vernal pool vegetation were present in other, more widespread systems, I conducted a seed bank study in three Colorado conifer forests types. The forests had all undergone a mechanical fuels reduction treatment aimed at restoring historical fire regimes. Under thick mulch layers of treated woody materials, the seed banks tended toward increased density when compared to the untreated, but seed banks generally did not differ among treatments. These results coupled with the vernal pool findings show that the aboveground vegetation and seed banks are often quite divergent. Therefore when implementing and monitoring a long term restoration project it is important to understand drivers of both the above and belowground responses to fully understand restoration success.

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CHAPTER 1 INTRODUCTION

1.1 RESTORATION ECOLOGY

Due to the far reaching damage humans have caused to ecological systems, restoration ecology has been heralded as one of the most important fields in the coming century (Hobbs and Harris 2001, Omerod 2003, Suding 2011). Despite the recent attention, papers on ecological restoration emerged as early as 1927 (Lisger 1927), which shows that the notion that people can assist degraded systems to a more desirable state is a longstanding way of thinking (Hobbs and Norton 1996). Even so, the field of restoration ecology as a science did not become fully appreciated until the early 1980's when it was proposed that restoration ecology be used as a tool for understanding and dissecting ecological theories in addition to providing aid to degraded systems (Aber and Jordan 1985).

With this shift in thinking the burden of human intervention and designing restoration projects no longer rested solely on the shoulders of land managers and practitioners (Allen et al 1997, Choi 2007). In recent decades the scientific community has developed a more active role in the field implementation portion of projects and as a result restoration ecologists are at the forefront of converging applied and theoretical realms (Allen et al 1997, Palmer et al 1997, Hobbs 2007). Restoration ecology has now reached a point in its development where ecological restoration projects have often benefited from sound applications that are built of rich ecological theory (Young 2000, Young et al. 2005). My research focuses on developing restoration strategies based on a strong understanding of ecological theory that underlies the potential response of these systems to restoration. This linkage facilitates a better understanding of the

relevance of these models in addition to how these concepts can be directly applied to future restoration projects aiding in their success.

1.2 ECOLOGICAL THEORY

1.2.1 Alternative states

Alternative states, or alternative stable states, are defined as multiple combinations of ecosystem states and environmental conditions that will persist and not converge under current conditions (Beisner et al. 2003, Suding et al. 2004, Shroder et al 2005, Hobbs 2007). The pathway of change from one state to another, thus causing alternative states has been attributed to two primary sets of ecological factors: 1) abiotic or 2) biotic (Fig. 1.1, Sousa and Connell 1985, Beisner et al. 2003, Shurin et al 2004). The biotic, or state variable, perspective can be defined by change in species interactions (Sousa and Connell 1985). An example of this state change could be a divergence in community assembly processes leading to different communities (e.g. May 1977, Law and Morton 1993, Collinge and Ray 2009) and thus alternative states. The abiotic, or environmental, perspective suggests that the changes in environmental conditions themselves cause alternative community compositions, and thus alternative states (e.g. Sheffer et al 2001, Didham and Watts 2005).

Restoration projects provide an ideal canvas for understanding how a community can be pushed into alternative states through direct restoration actions and indirect positive feedbacks (Suding et al. 2004). By using long-term restoration projects, it is possible to examine community trajectories, coupled with environmental data, to decipher whether it is the biotic or abiotic components of the system facilitating the alternative states (Beisner et al 2003) or a combination of both (Sousa and Connell 1985). Ultimately, the mechanisms causing these

alternative states must be understood before any management actions can effectively take place. The concept of alternative states can, therefore, be used as a learning tool for what defines restoration success because these states are often symptomatic of the causal mechanisms creating the divergence.

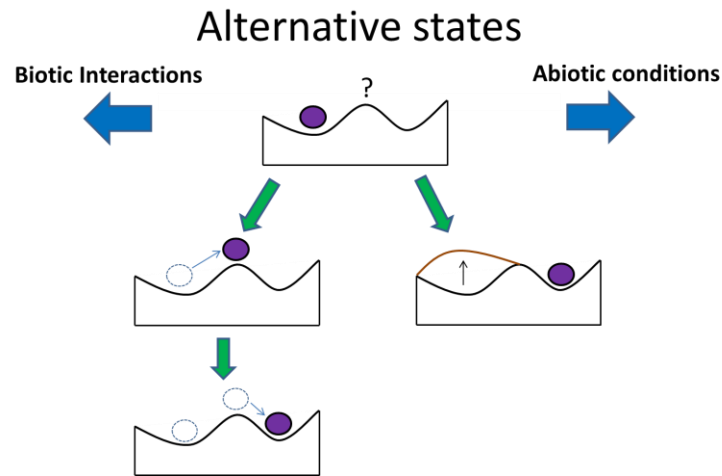


Figure 1.1. Ball-in-cup diagram where the ball represents the biotic state characteristics (variables) and the surface represents the environmental conditions (parameters). The deeper the cup the more difficult it is to move to another state. To reach an alternative stable state the two primary pathways are either a shift in the variables or the parameters (Adapted from Beisner et al. 2003).

1.2.2 Storage effect and ecological filtering

The storage effect describes conditions that allow for species coexistence in the same spatial location over time (Warner and Chesson 1985, Chesson and Huntley 1989, Chesson 2000, Chesson et al 2004, Facelli et al 2005, Angert et al 2009). Three fundamental variables drive this coexistence:

1) First, each species or functional trait must be able to respond to the environment differently (Angert et al 2009). In other words, there must be some sort of environmental filter acting as a sieve that promotes the success of well adapted species and hinders the success of poorly adapted species through their functional and phenological traits (Roy and Blois 2006,

Mayfield et al. 2009, de Bello et al. 2013). If all species responded to environmental stimuli in the same manner (e.g., germinate or remain dormant), then competition could be too fierce and potentially preclude stable coexistence.

2) Second, environmental conditions coupled with plant competition must have a degree of covariance (Facelli et al 2005, Chesson 2000). As environmental conditions, and potential filters, fluctuate, plant competition will occur with varying degrees of intensity and each species would have a varying ability to cope with the changes in competition (Facelli et al. 2005).

3) The third prerequisite is that a species must maintain a safeguard against highly variable environmental conditions to maintain a viable population or community. If a species does not have storage stability within the system (e.g., long lived seed banks), then that species cannot be immediately available when favorable conditions return.

In summary, by coupling strong ecological theory, such as how direct and indirect effects of restoration can be the drivers of alternative states, with long term restoration projects empirically testing those effects a deeper understanding of both the applied and theoretical can be achieved. This same synergism is true when coupling restoration with the storage effect. A key component to a successful restoration projects is to identify, manipulate and assure the most desirable community well after the initial project is implemented. By understanding the dynamics of how species coexist and identifying their associated environmental filters a more tangible method of how to initially implement a project can be initiated. Additionally by using these theoretical frameworks a greater reflection on the the outcomes of long term projects can be better understood.

1.3 STUDY SYSTEMS

Although many research objectives focus on one system and then ask different questions related to that system, my research examines different ecosystems and asks similar questions in a unified manner. Comparing a fundamental question between two study systems, such as: “Does the presence of a physical barrier, formed by plant litter or mulch from mastication, alter the seed bank?” is a novel way of increasing the breadth of my research. Additionally, using multiple systems allows for testing ecological theory and concepts (i.e., alternative stable states and the storage effect) and is an exciting way to assess their generality.

1.3.1 Seed Banks

Seed banks, or the storage of seeds belowground, can act as the reservoir for potential future communities and, when diverse and abundant, can buffer vegetation communities against multiple environmental fluctuations (Thompson and Grime 1979, Templeton and Levin 1979, Leck 2001). Sparse seed banks, on the other hand, can promote future declines of already rare species. For instance, in the context of alternative states: if the seed bank, or biotic condition, is insufficient to replenish the aboveground vegetation in one system, but is highly diverse in another, the system might be considered in alternative states. Alternatively, if a system has a long lasting, highly diverse seed bank that is able to withstand environmental perturbations and emerge under varying systems, the system shows a strong storage effect model (e.g., Facelli et al. 2005) and may not be in alternative states as described by the aboveground. Seed banks are also an excellent medium to look at environmental filters. Seeds found in the seed bank are, by definition, not the individuals that germinated and populated the aboveground vegetation, and thus their positive environmental filter was not yet met. Identifying what environmental filters are needed to germinate illustrates what the related limiting ecological filters may be.

1.3.2 Vernal Pools

Associated with variations in inundation, rapid plant community fluctuations, and a need for restoration due to habitat loss, vernal pools are an ideal system for my research. Vernal pools are shallow ephemeral wetlands found in flat to low slope grasslands. They are defined by abrupt edges delimited by locations of ponding (Keeley and Zedler 1998) and have three clear vegetation zones. The three vegetation zones form in response to different inundation durations and depths and can be described as the 1) pool bottom, 2) transition zone and 3) edge zone (Emery et al 2009). Many vernal pools occur in association in a classic Mediterranean climate with cool wet winters filling the pools and hot dry summers subsequently drying them out and creating distinct seasonal phases (Zedler 1987, Holland and Jain 1981, Keeley and Zedler 1998, Barbour et al. 2005). These predominately annual plants germinate soon after the first seasonal rains and remain in an immature seedling state until the spring dry-down where they quickly grow, flower, disperse their seeds and senesce (Linhart 1974). In short, this system has a highly variable climate, the potential to build large seed banks (Venable and Brown 1988, Pake and Venable 1996, Leck 2001), and easily delineated pool replicates for study.

One known limitation to vernal pool restoration success is the recent encroachment of invasive species (Collinge et al. 2011, Faist et al. 2013). Through strong inundation cycles, vernal pools have historically been able to resist encroachment from upland non-native species (Gerhardt and Collinge 2003, 2007, Tanzentzap et al. 2014). Over the last decades however, broad scale changes in climate regimes and altered precipitation patterns have resulted in wide spread invasion of vernal pools by alien invasive species (Pyke 2005, Collinge et al. 2011). While changes in climate and precipitation have the potential to be the direct drivers that result in invasive encroachment into the pools it is imperative to also understand the impacts of invasion.

Teasing apart how a system is being altered by invasions, both aboveground and belowground, can elicit useful mechanistic answers to the indirect effects of invasion. Ultimately developing a holistic approach of identifying the drivers of invasion and then the associated impacts of the invasion allows for a deeper understanding of the success or failure of vernal pool restoration projects.

1.3.3 Colorado Conifer Forest Mastication

An increase in tree density and fuel accumulation has occurred across the Western United States as a result of fire suppression (e.g., Arno 1980, Fule et al. 2002, Knapp et al. 2005, Stephens et al. 2009). Multiple forest thinning projects have been implemented in response to this increase in density and accumulation. One such thinning treatment is called mastication and involves chipping and shredding the overstory woody biomass and spreading it across the forest floor. Mastication has become a favored fuels treatment and is now widely used because it is cost-effective, easily implemented, and modifies fire behavior to reduce crown fire risk (Stephens and Ruth 2005, Kane et al. 2009, Battaglia et al. 2010). Yet, while fire risks have been addressed in management practices only recently have the ecological consequences of this thinning treatment been researched. Albeit quite different from vernal pools, both biotically and abiotically, masticated conifer forests provide another vantage point to study seed bank dynamics and how they are impacted by restoration practices. The artificially imposed layer of woody biomass I am able to address similar questions as my vernal pool research on a more biologically diverse array of seeds in the seed bank (e.g., grasses, forbs, shrubs and trees). The differences between the systems coupled with the similar need for restoration allows me to continue to ask applicable questions on long term restoration projects specifically in seed bank dynamics.

1.4. RESEARCH OUTLINE

My overarching research goals and objectives are to understand how plant communities are altered in long term restoration projects and ecological theory can guide our understanding of restoration success. By doing so, I focus on how these restoration projects vary in their invasive to native species relationships and whether these variations are facilitated through abiotic or biotic conditions both above and belowground. Through four organized chapters I address specific questions regarding my overarching framework. In my 2nd chapter I consider how a long term vernal pool restoration project has experienced a strong invasion pressure, which has resulted in the formation of alternative states. Within this structure I test both abiotic and biotic responses to the invasion and assess the persistence of these alternative states. For my 3rd chapter I focus on how vernal pool seed banks vary across invasion and inundation gradients. This study complements research looking at environmental filters as I test not only what has not passed through a filter and is maintained in the seed bank but how germination is altered through different inundation levels. My 4th chapter also looks at vernal pool seed banks yet here I ask a more temporal question related to the storage effect. Utilizing a long term annual vegetation dataset I can compare similarities and differences between the current seed bank community and historical vegetation. Finally, my 5th and final research chapter similarly examines seed banks but uses three Colorado conifer forest types to understand how the seed banks of these different systems respond to a masticated physical mulch layer. With these chapters my research is aimed at enhancing both the applied side of ecological restoration and empirically testing the theoretical side of restoration ecology.

CHAPTER 2

POSITIVE FEEDBACKS OF INVASIVE LITTER PROMOTE ALTERNATIVE STATES

2.1 ABSTRACT

Alternative states are present when different ecological communities exist and cannot converge under current conditions. The introduction of invasive species has the potential to push a system from its original native state into an alternative state through positive feedbacks. When attempting to restore a system it is important to understand the mechanistic drivers of these feedbacks before a successful restoration project can occur. My study attempts to dissect the mechanisms behind potential feedbacks in a vernal pool system as a model system to better understand alternative states. My results show that the native dominated vernal pools are deeper and have a longer duration of inundation than the invasive dominated pools. However, once established the invasive species deposit a thick litter layer that is slower to decompose than the natives litter. Native species richness decreases by nearly two fold and the proportion of established native to invasive species decreases by 40% with the presence of a litter layer. Alternatively, a majority of the invasive species do not appear to be restricted by this litter layer and in some cases benefit from it. Soil texture, pH and moisture did not differ underneath invasive and native dominated plant communities under invaded and native vegetation yet microbial biomass and soil carbon:nitrogen were lower under the restored pools compared to the naturally occurring pools. These results suggest that the deposition of litter by invasive species generated positive feedbacks that were strongly associated with their success and with the continued persistence of an invasive alternative state. The native species however are highly vulnerable to invasive litter deposition and in turn are in a more tenuous state than the invasive dominated pools regardless of restoration status. My research also showed that while the

invaded and native dominated pools are in alternative states microbial and soil characteristics may be similar between these two systems and the difference is more likely an artifact of restoration.

2.2 INTRODUCTION

Ecological restoration projects are often oriented at rehabilitating a system that is highly degraded and unable to return to a more desirable state without human intervention (Suding et al. 2004, Hobbs and Norton 2006, Hobbs et al. 2011). A degraded system can become stuck in an alternative, less desirable, state that will not converge with the preferred more pristine environments under current abiotic and biotic conditions (Beisner et al. 2003, Suding et al. 2004). Alternative states are defined as an “alternative combination of ecosystem states and environmental conditions that may persist at a particular spatial extent and temporal scale” (Suding et al 2004). The extent of this state persistence, and thus how stable a state really is, has been debated (Didham and Watts 2005, Didham 2006, Fukami and Lee 2006, Didham and Norton 2007, Mason et al. 2007, Fukami and Nakajima 2011) but it is often agreed upon that systems are in alternative states, while perhaps not entirely stable, if they cannot converge under current environmental and/or biotic conditions (Beisner et al. 2003, Suding et al. 2004).

These disparate ecological trajectories can limit the communities, or states, from returning to a historically shared similar state, or from converging into an entirely new state. It has been theorized that there may be two primary pathways driving these state changes (Beisner et al. 2003). The first potential pathway is that the community itself can push the system to a new state via biotic drivers (Sousa and Connell 1985). Examples of biologically driven state changes may be through plant community interactions (Kulmatiski 2006, Mathews and Spyreas 2010,

Alday et al. 2013, Gerla and Mooij 2014) or multi-trophic species interaction (Norstrom et al. 2009). It is with these biological interactions that the current state can be propelled up and out of the current state and fall into the basin of the alternative state under the newly found biotic conditions. The other potential theorized pathway for a state change is that environmental variables are altered and push the system out of its original configuration into a new alternative state (Beisner et al. 2003, Schooler et al. 2011). Examples of environmental drivers are temperature changes in lake flooding regimes causing zooplankton and phytoplankton to shift in their proportions (Chaparro et al. 2014), lake clarity hindered by turbidity (Kosten et al. 2012) or other external drivers such as sediment deposition and tidal storms on shallow coastal environments (McGlathery et al. 2013).

However, it is often the case that a combination of biotic and environmental factors is what might push systems to a new state. For instance both soil wetness and grazing pressure could be the drivers that shove a tundra system towards alternative states (Saccone et al. 2014). Or perhaps a fire has been excluded from a system and the developing state is dominated by invasive species (Keeley 2006). It is also possible that with the reintroduction of a fire regime, or through forest reduction techniques species invasions can proliferate (Maret and Wilson 2005, Dodson and Fiedler 2006, Kerns et al. 2006, Keeley and McGinnis 2007). In either case without the invasive biotic propagules present the system has a greater potential to return to its pre-fire vegetative state. Therefore, it is the combination of the presence of fire (abiotic), or lack thereof, creating a niche for the invasive species and then the invasive species are able to out compete the natives that in turn cause alternative states that will not converge (Suding et al. 2004, Brooks et al. 2004). Thus, when attempting to restore a system, we must first identify not only if the degraded system is in an alternative state from its “natural” counterparts, but what are the

ultimate drivers causing this state and are the states capable of converging with restoration intervention. In the case of positive feedbacks pushing a state over a potential threshold it is the restoration practitioner's responsibility to target not only what the feedbacks are, but the mechanisms behind the feedbacks and attempt to remove them before any effective restoration project can occur (Suding et al. 2004).

One such system that provides an excellent opportunity to test alternative state theory is temporary wetlands. Temporary wetlands, or vernal pools, are commonly found in Mediterranean-type climates and experience wet winters facilitating ponding, yet also experience dry hot summers that desiccate the soil (Holland and Jain 1981, Keeley and Zedler 1998). During the wet season, distinct ponds, or pools are formed that are visually delineated, have relatively limited connectivity with one another (Ray and Collinge 2014) and often contain discrete vegetation communities (Holland and Jain 1981). These vernal pools also host a large number of state and federally listed endangered species (USFWS Endangered Species Act) as well as endemic vernal pool obligates specially adapted to the extreme climates of winter ponding and dry summers.

Conservation efforts of existing pools and reconstruction through restoration projects in California's Central Valley have been applied in multiple locations in response to a high occurrence of vernal pool habitat loss. Primary reasons for this habitat loss, especially in California's Central Valley are conversion to agricultural lands and land development (Zedler 2003, Pyke 2004). In addition to habitat loss caused by human encroachment, vernal pools in the Central Valley are also experiencing a strong encroachment of invasive plant species (Gerhardt and Collinge 2003, 2007, Collinge et al. 2011, Faist et al. 2013). Historically able to avoid invasion because of their highly dynamic annual ponding cycle (Gerhardt and Collinge 2003,

2007), recent climate variability events and habitat disturbance have allowed invasive encroachment into the pool boundaries (Pyke 2004, Collinge et al. 2011, Collinge et al. 2013, Tanentzap et al. 2014). Studies of a long term restoration project located in the Central Valley of California have reported a substantial increase in invasive species into both restored and nearby native reference pools over the last decade (Collinge et al. 2011, Collinge et al. 2013, Faist et al. 2013).

With the potential for further habitat loss due to invasive species (Pyke 2004, Collinge et al. 2011) it is important to tease apart the effects of invasive species on the vernal pool system. In many cases invasive plants are able to grow faster (Pattison et al. 1998, Daehler 2003, Leger and Rice 2003, Jakobs et al. 2004, Leishman et al. 2007) and often contain higher amounts of nitrogen than their native counterparts (Vitousek and Walker 1989, Witkowski 1991, Ashton et al. 2005, Liao et al. 2008) which provides them the ability to decompose at a quicker rate (Allison and Vitousek 2004, Liao et al. 2008, Spirito 2014) due to higher quality litter (Liao et al. 2008). This cycle of rapid growth and faster decomposition in invasive species can then change the soil chemistry of a system (Allison and Vitousek 2004, Liao et al. 2008) causing positive feedbacks further proliferating invasive species. An example of one such positive feedback is the increase of soil nitrogen after invasion in previously low nitrogen systems (e.g., Lao et al. 2008).

In the case of vernal pool invasion there is a trend towards invasive species encroachment into the pool boundaries (Collinge et al. 2011, Faist et al. 2013). The initial stages of how invasive species enter the pool is thought to be through a lack of inundation depth and duration in the pools facilitating invasive growth (Gerhardt and Collinge 2003, 2007, Tanentzap et al. 2014). However, the success of a restoration project will depend on whether this pathway of

invasion is addressed in the restoration strategies and the mechanisms by which the invasive species might be impacting the native species within the pools are still unresolved.

It is with these dynamics of invaded versus native dominated vernal pools in mind that I asked three primary questions: 1) What are the primary drivers causing invasive and native alternatives states? 2) How does the litter quality and quantity alter nutrient cycling in these vernal pool systems further promoting invasive species populations? And 3) What are the necessary restoration practices needed to return the invaded sites to a more desirable native state?

2.3 METHODS

2.3.1 Study site and experimental design

I conducted my study on a 15 hectare site on the Travis Air Force Base, Solano County, California, USA (38°15'00", 122°00'00"). My site contained both naturally-occurring "reference" pools and pools constructed for restoration. Constructed or "restored" pools were implemented in 1999 to mimic the physical dimensions of the nearby reference pools (Collinge and Ray 2009, Collinge et al 2013). This region is characterized by a Mediterranean-type climate (mean annual temperature of 20.1° C and mean annual rainfall of 500 mm) with the majority of the precipitation falling between the months of November and February (Climate of Sacramento, Report 2010). Because the vernal pools are predominately rain fed, the pool hydroperiod mimics the seasonal rain cycle; pools filled by winter rains which are then followed by a rapid spring dry down and a hot dry summer and fall (Bauder et al. 2005).

A portion of the naturally occurring pools have a high occurrence of invasive species while others have remained dominated by native plants. As a result the experiments were designed to take advantage of these differences in invasive and native community composition

and the pools were parsed out into three pool types: 1) Naturally occurring, invasive dominated 2) Naturally occurring, native dominated 3) Constructed for restoration. I confirmed pool type categories through vegetation surveys. With a dominance cover defined as greater than 50% of total observed area there was high invasive species richness and cover found in both the restored and invasive dominated naturally occurring pools and high native species cover and richness in the native dominated naturally occurring pools (Figure 1a-d). There were significant differences between the designated pool types in invasive total cover (Kruskal-Wallis, chi-square=12.52, $p=0.0019$), invasive richness (Kruskal-Wallis, chi-square=23.60, $p<0.0001$), native total cover (Kruskal-Wallis, chi-square=24.42, $p<0.0001$) and native richness (Kruskal-wallis, chi-squared=13.41, $p=0.001$).

Within each of these three pool types (N=8 replicates per pool type) plots were located at three points along the inundation gradient. Differences in the three inundation and vegetation zones (bottom, transition and edge) were used to locate plots as had been determined in previous studies (Bauder 2005, Emery et al. 2009). Plot inundation gradient placements were situated 1) at the pool bottom, with a relatively flat slope and receives the longest inundation duration of any of the inundation zones and often is host to vernal pool specialists able to withstand long durations of inundation, 2) the transition zone, which is situated along the side slopes of the pools and often fluctuates between submerged and above the inundation line throughout the rainy season, the vegetation is often comprised of a combination of edge and bottom adapted species 3) the edge zone which is just below the delineated boundary of the pool and while can become inundated has shorter and more shallow inundation than the transition or bottom zones and often is where the highest diversity of invasive species are located. This sampling allowed for a balanced design with three pool types (N=8) with dominant (invasive or native) vegetation

(N=24 pools total) and three points along the inundation gradient within each pool (N=72 total plots).

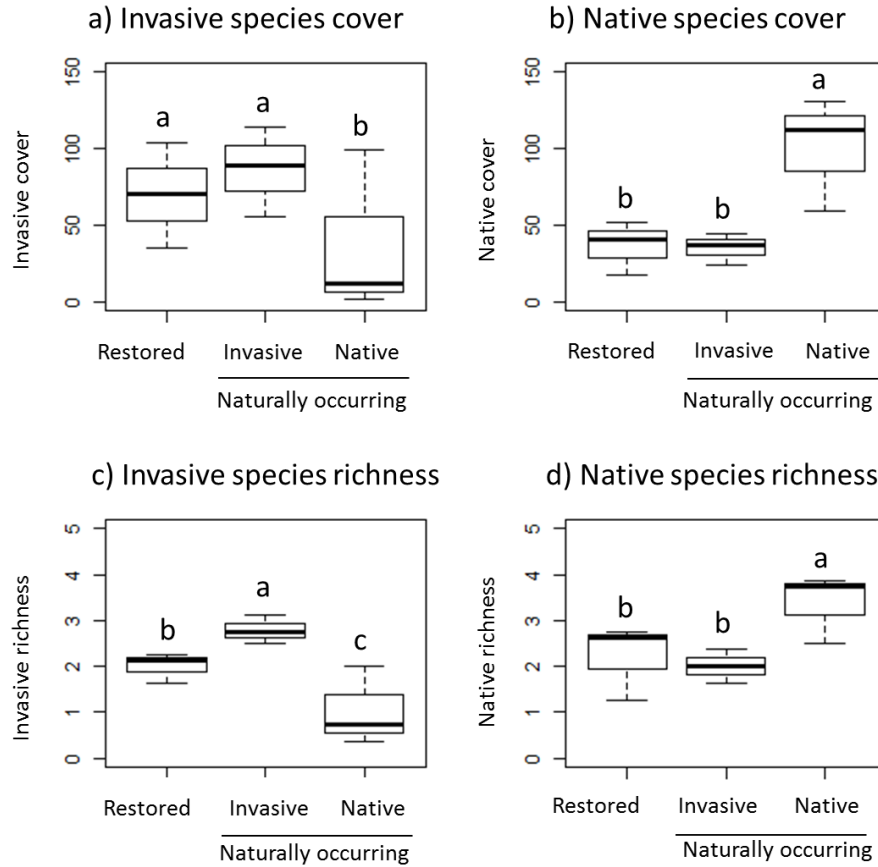


Figure 2.1. Invasive and native species cover and richness by the three difference pool types. Letters indicate significant difference as determined by a non-parametric pairwise Wilcoxon rank sum tests corrected by False Discovery Rate test to reduce type I error. Center line represents the median value of the metric tested and the box surrounding the median represents the first and third quartiles.

2.3.2 Inundation

To ensure that plot locations placed along the inundation gradient reflected different water depths and durations of inundation, and to better understand inundation depths and duration dynamics in and across the pools. I collected weekly inundation depths (to cm) in every

observed pool at the three plot locations (bottom, transition and edge) during the 2011-2012 and 2012-2013 winter wet seasons.

2.3.3 Litter decomposition

I evaluate changes in litter quantity and quality through field implemented litter bags and plant chemical composition to better understand species level decomposition. To determine *in situ* litter decomposition rates I cut foliage from one invasive species and one native during spring peak biomass. “Invasive” litter was composed of pure *Lolium multiflorum*, an exotic annual grass species that is ubiquitous in both restored and reference pools at the study site (Collinge et al. 2011, Faist et al. 2013). The second litter type I used was a vernal pool adapted native annual grass, *Pleuropogon californicus*. After cutting both litter types 2 cm above ground level I air dried the samples at 60°C until the mass was stable (approximately 72 hours). I then clipped litter to a uniform size across types and placed the litter into 10 x 10 cm litter bags (0.8 mm mesh) and recorded the weights of each filled bag. I then placed litter bags of each species at the plot location (edge, transition zone, and pool bottom) in the different pool types with control litter bags not put into the field to account for potential litter loss during transit. To explore effects of deposited leaf litter on decomposition rates I placed litter bags on top of 5 cm of litter and below 5 cm of litter at the soil surface, hereafter referred to as “above” or “below” litter (N=72 litter bags above and N=72 litter bags below the litter). Finally, to account for seasonal variability in decomposition I installed “wet season” litter bags in the fall prior to any substantial precipitation (2011-2012 and 2012-2013) and removed them in the spring. Field incubation for winter wet decomposition studies lasted from September to April. I placed the

“dry season” litter bags (2013) in the field just after spring dry down and removed them before any substantial rains (April-September).

Because it wasn't feasible to install litter bags for multiple plant species I obtained the % nitrogen (N), % cellulose, % lignin and % carbon (C), and calculated C:N and Lignin:N (L:N) ratios for 18 common plant species found at the site (Table 2.1). These metrics are able to serve as a proxy for the potential field decomposition rates (Melillo et al. 1982). Plants with higher cellulose, overall carbon and/or lignin content and lower nitrogen content would be predicted to decompose more slowly in the field than plant species with low cellulose, overall carbon and lignin contents (Taylor et al. 1989). I determined % nitrogen, % cellulose and % lignin in these samples using a modified Goering-Van Soest forage fiber technique (Goering and Van Soest 1970, by EcoCore Analytical services at Colorado State University, CO USA). I processed the overall plant carbon and C:N ratios at the University of Colorado, USA in N. Barger's lab using a combustion technique (Costech analytical Technologies ECS 4010 CHNSO Analyzer, Valencia CA USA).

2.3.4 Litter manipulations

To test how plant litter layer in the field impacts aboveground vegetation I manually manipulated litter depths at each plot location. I manipulated all litter depths in the fall after all plants had dispersed their seeds and fully senesced. To manipulate the litter depths I removed litter from three 20 x 20 cm locations at each plot location to expose only bare soil with no standing aboveground vegetation. To reduce potential seed abundance differences I added 50 site collected *Lasthenia conjugens* seeds (a native vernal pool species of interest, Asteraceae) at the soil surface level. After I had sown seeds I assigned each of three cleared bare soil spots (at the

plot level) to distinct litter manipulation treatments; 1) No litter added, soil left bare 2) Two centimeters of litter and an incomplete physical barrier and 3) Seven centimeters of litter providing a continuous litter layer. I secured litter with garden staples so as to maintain litter over time. I left litter manipulations in place over the winter growing season and returned for vegetation counts the following spring during peak growing season. After litter implementations were in place for a full growing season I then ran vegetation surveys. All vegetation surveys were completed during peak growing season and identified to the species level by counting individual stems which allowed for species richness and individual counts by species or desired grouping. My field litter manipulations and associated vegetation surveys were conducted three years in a row (2012, 2013 and 2014) in April of each year. Litter treatments were reapplied annually. Litter was slightly altered due to environmental variables (e.g., wind and water) changing overall litter depths. At the time of vegetation surveys I measured “actual litter depths.” I then converted these actual measured depths (cm) to a presence/absence binomial response. This presence/absence format aids in better understanding what the presence of litter layer does to functional groups (e.g., native species) and eliminates the uneven statistical replicates of actual litter depths.

2.3.5 Soil properties

I collected soil samples during peak flowering and peak aboveground plant biomass as this is when soil metrics were likely to be most closely linked to the plant growing conditions. My field samples (N=72 at 125 cm³ each) were collected in the field and put into sealed plastic bags so as not to lose soil moisture and then immediately put on ice to hinder any additional microbial activity after collection. I stored the soil samples in a 4° C cooler until samples were

processed. I completed the majority of soil lab analyses (microbial biomass, soil moisture, pH, and C:N) within one month of field collection.

I obtained soil moisture content through the gravimetric soil moisture method by recording an initial weight and drying down the samples at 60° C for 48 hours or until mass was stable. Then to obtain the soil moisture I divided the weight of the water (difference between the wet and dried soils) by the dried soil. I used a portion of the dried soil samples to obtain soil pH (Beckman pH/Temp meter model #340, Abbott Laboratories, Waukegan IL, USA) three times for each sample and reported the mean of the three pH readings. I also used the pool bottom collected samples of the dried soils to obtain soil texture (percent sand, silt and clay) using a modified version of Kettler et al. (2001) protocols for a rapid soil texture analysis. The pool bottoms were used as these locations had the potential for the greatest difference in textures and could provide the most insight into the soil drainage potential. Also, identifying soil textures helps to understand if certain species (e.g., invasive) preferentially chose one texture over another. Finally, I obtained soil carbon to nitrogen ratios using a CHN analyzer in N. Barger's lab at the University of Colorado Boulder (Costech analytical Technologies ECS 4010 CHNSO Analyzer, Valencia CA USA, Sheldrick 1986). For my soil biotic metric I measured carbon microbial biomass per gram of dry soil. To obtain soil microbial biomass carbon I used the chloroform extraction method as described in Jenkinson et al. (2004) which allowed me to determine soil microbial carbon across soil collected from the pool bottoms.

2.3.6 Statistical analyses

To test for initial pool type vegetation differences I ran a non-parametric Kruskal-Wallis test for each four dependent variables (invasive cover and richness and native cover and

richness) with pool type as the explanatory variable. After ensuring all dependent variables differed significantly among pool types, I ran non-parametric pairwise Wilcoxon rank sum tests between each pool type followed by a False discovery rate (FDR) correction to avoid type one error inflation (Benjamini and Hochberg 1995). I ran all Kruskal-Wallis and Wilcoxon-rank sum tests aimed at understanding initial pool type vegetation using JMP statistical software (JMP version 11.1.1, SAS Institute Inc., Cary, NC).

To characterize inundation depth and duration, I averaged individual pools by year, pool type (restored, invasive naturally occurring or native naturally occurring) and plot location (bottom, transition and edge) and then tested for normality. After normality assumptions were met, either with or without square root transformations (Shapiro-Wilk test results at $p > 0.05$), I ran a three factor ANOVA using year, pool type and location as the explanatory variables. To test for pairwise comparisons I ran a post-hoc Tukey's Honestly Significant Difference test (HSD) on each of my variables. I ran all inundation depth analyses and normality assumption tests using R statistical software (R core development team 2014, Vienna Austria).

I characterized litter bag decomposition rates data in a very similar manner as inundation. I averaged individual pools by the three explanatory variables (pool type, location and year), with the addition of litter placement (either above 5 cm or below 5 cm of litter) in the decomposition data. I then checked the appropriate dependent variable for normality (Shapiro-Wilk test results at $p > 0.05$) and square root transformed the data if necessary. I then ran a multifactorial ANOVA and if data were significant I followed the ANOVA by a post-hoc Tukey's HSD test. All subsequent plant litter chemistry analyses were handled in the same manner as the litter bag decomposition analysis (e.g., % nitrogen). I ran all litter bag and plant chemical analyses using R statistical software (R core development team 2014, Vienna Austria).

To understand how the presence of a litter layer impacts the aboveground vegetation I ran a logistic regression (JMP version 11.1.1, SAS Institute Inc., Cary, NC). This logistic regression allowed me to test for how well the presence and absence of litter impacted invasive and native richness and proportions of invasive and native abundance. After determining that native richness had the greatest predictive power I ran a Generalized Linear Model (GLM) with a Poisson distribution and log link function to assure that the presence and absence of litter did not vary between my explanatory variable potentially confounding the results (R core development team 2014, Vienna Austria). I ran a GLM for these data as they were zero inflated and could not be transformed to meet the assumptions of normality. Because pool type was the only primary explanatory variable that varied in its litter presence absence data (GLM, $z=-2.77$, $p=0.006$) I broke up each of the analyses by restored, invasive dominated and native dominated naturally occurring pools and ran non-parametric Mann Whitney U tests for my plant litter manipulation dependent variables (richness and proportions by native or invasive species) against the presence/absence of litter.

I examined data on soil properties to determine if they met the ANOVA statistical assumptions, and ran a square root transformation if necessary (Shapiro test results at $p>0.05$). Each of the individual soil property dependent variables were then subjected to a multifactor ANOVA and if any significance of an explanatory variable was found I ran a post-hoc Tukey's HSD comparison (R core development team 2014, Vienna Austria). For all analyses I determined if a test was significant using an alpha of 0.05.

2.4 RESULTS

2.4.1 Inundation

Regardless of year, the native dominated naturally occurring pools were deeper during the wet season (3.70 cm) than either the restored or invasive dominated naturally occurring pool types (Fig. 2.2; 2.17 cm and 1.89 cm respectively). Because the 2012-2013 wet season was significantly wetter than the 2011-2012 season (ANOVA, $F_{2,126}=6.10$, $p=0.015$) by 0.7cm overall (mean of 2.27 cm for 2011-2012 and 2.90 cm for 2012-2013) I separately analyzed the pool type and location data by year. The plot locations all showed a general trend of the pool bottoms having the greatest mean depths and the edge the least, but in both years the invasive dominated naturally occurring pools did not differ significantly across locations while the pool bottoms of native dominated and restored pools were significantly deeper than the pool edges (Fig. 2.2).

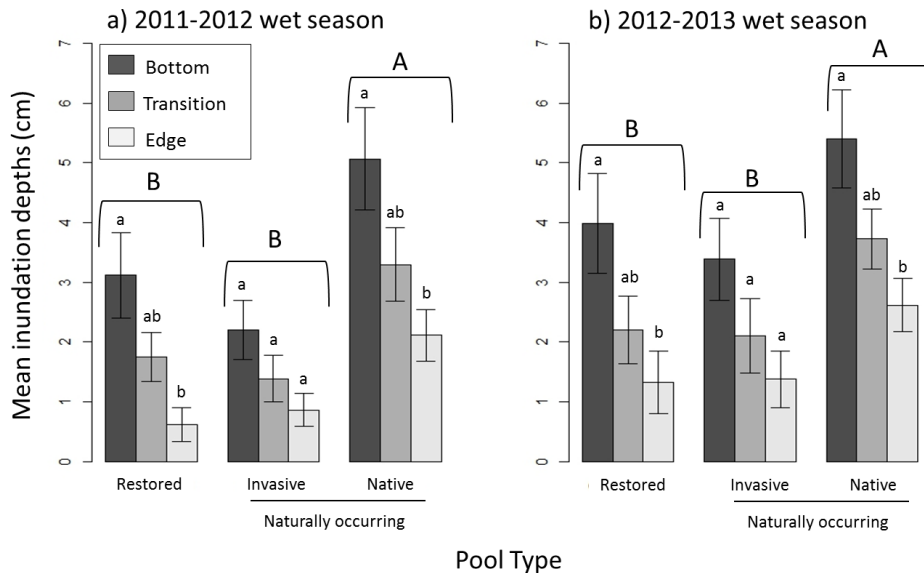


Figure 2.2. Mean inundation differences between pool types and location by winter wet season. Capital letters indicate significant differences between pool types for the associated year and lower case letters indicate significant differences between inundation locations within a single pool type. All results were determined from an ANOVA and post hoc Tukeys HSD test. Error bars indicate a SE of ± 1 .

2.4.2 Litter decomposition

Litter placed in the field during the wet season decomposed nearly 10 times faster than litter exposed during the dry season. Of the two species used in the litter bags, the native grass (*P. californicus*) decomposed at just over 10 percent faster rate with 56% of its original mass lost over a single rainy period where the invasive grass (*L. multiflorum*) lost an average of 50% of its mass over the same duration. Because there was not sufficient quantity of the native grass to collect and use in litter bags for subsequent litter bag trials I used the same placements (plot locations and above and below the litter layer) with only the invasive grass litter to explore plot and pool decomposition differences. *L. multiflorum* decomposition differed between years with the 2011-2012 wet season exhibiting 5% less overall decomposition than the 2012-2013 season and was the most significant determinant of decomposition rates in this analysis (four way ANOVA with year x pool type x plot location x litter placement, $F=88.91_{2,9}$, $p<0.0001$). However I also observed a year by pool type interaction effect (ANOVA, $F=5.14_{2,9}$, $p=0.007$). To account for this interaction effect I analyzed each year separately to test how pool type, location or litter placement influenced decomposition without year driving the results. For the drier year (2011-2012) none of the explanatory variables tested were significant in determining decomposition rates ($P>0.05$), however, in the wetter year (2012-2013) the associated pool types varied significantly in their decomposition rates (Fig. 2.3-a, ANOVA, $F=6.29_{2,9}$, $p=0.002$). Finally, litter bag results showed that in the hot dry summer months pool type and litter placement (whether above or below the litter) were the two significant explanatory variables (ANOVA, $F=3.47_{2,9}$, $p=0.03$ for pool type and $F=106.85_{2,9}$, $p<0.0001$ for litter placement). Yet in the 5 months the litter bags were in place there was generally a very low percent mass lost (often less than 10%) due to decomposition (Fig. 2.3-b).

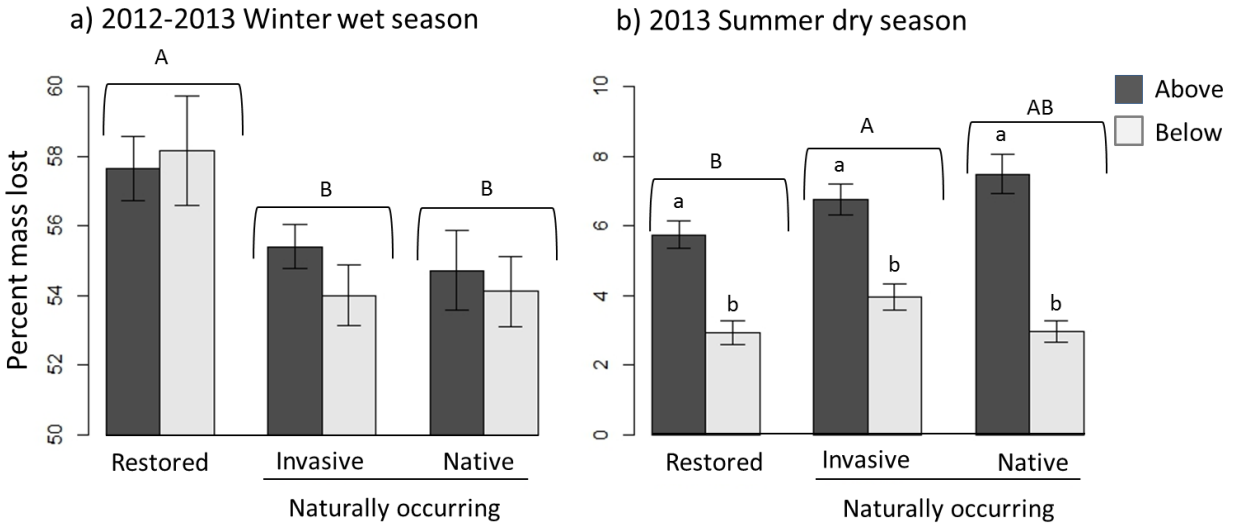


Figure 2.3. Percent mass of *L. multiflorum* lost due to decomposition for the a) winter wet season (2012-2013) and b) summer dry season (2013). Inundation gradient placement was not significant for either of these tests so displayed pool type and litter placement. *Capital letters* indicate significant differences across all pool types and *lowercase letters* indicate differences in litter placement. Litter placement did not have any significance for the winter wet season so no letters are present. All analyses utilized an ANOVA and a post-hoc Tukeys HSD test for significance. Error bars represent a SE of ± 1 .

The two species used in the litter bag trials served as representative species found in the vernal pools; however they do not reflect all of the community variance that could occur. To account for this, I used the chemical constitution of different plant species as a proxy for decomposition rates. I collected 18 species at peak growing season to determine their carbon, nitrogen, lignin and cellulose percentages (Table 2.1). In all of the chemical components tested, except for percent lignin, the plant functional group (forb or grass) significantly determined the chemical composition and in none of the cases did native status appear to be important in the chemical composition. The forbs consistently contained lower percentages, and thus ratios, of the recalcitrant (slower to decompose) materials than the grasses (e.g., % cellulose; ANOVA, $F_{1,18}=9.29$, $p = 0.007$ and L:N ratios; $F_{1,18}=4.82$, $p = 0.044$) and the grasses contained lower percentages of nitrogen than the forbs (ANOVA, $F_{1,18}=12.03$, $p = 0.003$).

Table 2.1. Plant tissue percent nitrogen, carbon, cellulose and lignin and associated carbon:nitrogen (C:N) and lignin:nitrogen (L:N) ratios for common species found within vernal pools and adjacent edges.

Functional group	Native status	Scientific name	% Nitrogen	% Carbon	% Cellulose	% Lignin	C:N ratio	L:N ratio
Forb	Invasive	<i>Convolvulus arvensis</i>	2.85	43.23	23.77	11.36	15.19	3.99
		<i>Erodium botrys</i>	0.99	41.94	32.7	7.1	42.19	7.14
		<i>Lotus sp.</i>	4.9	43.87	28.62	8.84	8.96	1.81
		<i>Rumex sp.</i>	2.84	44.91	30.38	4.35	15.83	1.53
		<i>Sonchus asper</i>	1.46	40.01	28.09	10.64	27.37	7.28
		<i>Vicia villosa</i>	4.39	43.69	28.07	14.86	9.94	3.38
	Native	<i>Achyrrachaena mollis</i>	1.19	40.78	30.06	6.08	34.3	5.11
		<i>Eryngium vaseyi</i>	1.89	41.82	26.77	3.47	22.08	1.83
		<i>Lasthenia conjugens</i>	2.24	43.48	28.5	9.76	19.42	4.36
		<i>Layia chrysanthemoides</i>	1.85	43.75	30.33	6.15	23.66	3.32
Grass	Invasive	<i>Avena fatua</i>	0.59	43.79	28.65	6.4	74.85	10.94
		<i>Bromus diandrus</i>	0.7	41.9	32.44	9.89	59.64	14.07
		<i>Bromus hordeaceus</i>	0.79	42.29	38.54	8.14	53.45	10.29
		<i>Hordeum marinum</i>	0.99	43.01	33.03	3.4	43.3	3.42
		<i>Lolium multiflorum</i>	1.25	42.49	32.81	5.86	33.98	4.69
		<i>Polypogon maritimus</i>	1.88	44.03	33.47	9.26	23.45	4.93
		<i>Taeniatherum caput-medusae</i>	1.41	41.94	41.39	9.14	29.83	6.5
	Native	<i>Pleuropogon californicus</i>	2.59	42.14	27.44	3.96	16.26	1.53

2.4.3 Litter manipulations

The presence of litter strongly predicted native richness (logistic regression, chi squared=98.67, $p < 0.0001$). Because of this strong native response to the presence of litter I binned the litter manipulation studies into a presence absence matrix where litter was either present, regardless of depth, or absent. My logistic regression results with year, plot location and pool type were then added to the model to evaluate whether these variables differed in their litter presence/absence and should thus be analyzed separately. The only variable that demonstrated any difference with the presence of litter across these explanatory variables was pool type (GLM, $z = -2.77$, $p = 0.006$) and so these were then divided for the rest of the litter analyses.

Native richness was reduced by nearly two thirds with the presence of a litter layer (mean 0.8 with a litter layer versus 2.9 without a litter layer). However, for invasive species, litter had no significant effect (mean of 1.9 species with litter and 2.0 without). I observed a consistent reduction in native species due to litter regardless of pool type (Table 2.2) and invasive richness was generally maintained with the presence of litter and showed a reduction in the native dominated naturally occurring pools (Table 2.2). The proportion, or percent, of native species to invasive species generally decreased by 40% with the introduction of litter regardless of pool type (Table 2.2) and inversely increased invasive percent abundance by 40%. These results show that the native species displayed a strong negative response to litter and the invasive species were much less affected.

Table 2.2. Invasive and native richness and percent abundance by pool type as determined by the presence or absence of a measurable litter layer. Each species group, invasive or native, divided by pool type was tested for differences through a Mann Whitney U test.

Pool Type	Species	Mean richness			Percent		
		Absence	Presence		Absence	Presence	
Restored	invasive	1.87±0.1	1.89±0.1	z=0.25, p=0.80	48±3.8	81±2.5	z=-7.25, p<0.0001
	native	2.8±0.17	0.72±0.0	z=9.36, p<0.0001	52±3.9	19±2.5	z=-7.24, p<0.0001
Invasive dominated	invasive	2.3±0.13	2.1±0.08	z=1.14, p=0.25	56±3.6	91±1.4	z=-8.16, p<0.0001
	native	2.56±0.1	0.5±0.11	z=9.00, p<0.0001	43±3.6	9±1.4	z=8.26, p<0.0001
Native dominated	invasive	1.9±0.12	1.69±0.0	z=1.97, p=0.048	27±2.9	70±3.0	z=-7.40, p<0.0001
	native	3.26±0.1	1.13±0.1	z=8.69, p<0.0001	72±2.9	30±3.0	z=7.44, p<0.0001

2.4.4 Soil properties

Soil characteristics (pH, moisture, C:N and Microbial biomass) did not vary uniformly in relation to pool type (Table 2.3). I also did not find a relationship with soil texture (sand, silt and clay) and pool types (p>0.05). Soil microbial biomass did differ between pool types. The lowest microbial biomass was found in the restored pools (4.99 biomass/g of dry soil), which was significantly lower than the invasive dominated naturally occurring pools (Tukey HSD, p=0.02)

and marginally lower compared to the native pools (Tukey HSD, $p=0.07$). The restored pools contained lower soil N and C than the naturally occurring pools and the soil carbon to nitrogen (C:N) ratio followed a similar pattern to the microbial biomass where the restored pools contained a significantly lower C:N ratio than either of the naturally occurring pool types (Table 2.3). For C:N ratios I was able to obtain samples from the pool gradient locations, where the soil microbial biomass and soil texture only were taken at the pool bottoms. I observed a difference in C:N ratios between locations within the inundation gradient (ANOVA, $F_{2,24}=3.32$, $p=0.042$). However, while significant across the plot location inundation gradients the ratios were relatively similar as they did not differ by more than 0.6.

Table 2.3. Soil metrics for field collected soil samples as determined by pool type. Letters indicate comparisons that displayed a significant difference between pool types as determined by a post hoc Tukey HSD test. If no significant differences were observed no letters were added.

Soil metrics	Naturally occurring		Restored
	Invasive	Native	
% Moisture	17.5 ± 1.73	16.8 ± 1.35	13.4 ± 0.68
pH	5.67 ± 0.09	5.84 ± 0.10	5.86 ± 0.04
C:N	11.77 ± 0.22^a	11.71 ± 0.15^a	10.73 ± 0.15^b
Microbial biomass*	7.40 ± 0.83^a	6.84 ± 0.60^{ab}	4.99 ± 0.17^b
Texture			
Sand	19.12 ± 2.91	18.79 ± 2.48	18.91 ± 3.12
Silt	56.10 ± 1.88	59.55 ± 2.39	56.61 ± 2.59
Clay	24.8 ± 3.5	21.7 ± 0.9	24.5 ± 3.1

*Microbial carbon biomass /g of dry soil

2.5 DISCUSSION

Vernal pools at this site are either strongly invaded by exotic plant species or exclude invasive species and maintain a high native component with low levels of invasion. Results demonstrated that the drivers involved with invasion into the pools were primarily through inundation patterns where the deeper the inundation the more likely native species were to occur. Once the invasive species were able to establish through more shallow inundation depths they then created a positive feedback of deeper litter layers that were slower to decompose than the natives dominated pools. In regards to plant decomposition across years: pool types, pool locations and placement above or below the litter the year sampled had a substantial effect on decomposition, yet the strongest variable outside of seasonality was the difference between species decomposition rates. A slower decomposition by the invasive grass allows it to maintain its litter layer in the system longer and the physical presence of the litter layer inhibits native species establishment. The mere presence of any litter layer significantly reduced native richness yet did not reduce invasive richness. The litter layer also strongly shifted the proportion of native species to invasive species. With strong observable vegetation differences with the presence of litter pH, soil moisture and soil texture did not vary greatly across pool type with only the microbial biomass and soil C:N ratio depressed in the restored pools and the naturally occurring pools not differing in any observed soil metric.

2.5.1 Inundation

Vernal pool ecosystems are considered to have shallow inundation depths which rarely exceed a meter and the plant communities are highly attuned to small variations in depth and duration (Holland and Jain 1981, Keeley and Zedler 1998, Emery et al. 2009) as well as

inundation timing (Kneitel 2014). These inundation dynamics within pool boundaries have been shown to exclude invasion of a number of species as they are not often well adapted to such extreme conditions (Gerhardt and Collinge 2007, Tanentzap et al. 2014). My results demonstrated that the native dominated naturally occurring pools contained the deepest inundation depths regardless of variations in year, and thus variations in annual precipitation. These findings provide substantial support for the idea that the driver is inundation, an abiotic constraint, partially responsible for maintaining these alternative community states (invaded and native; Gerhardt and Collinge 2003, 2007). This relationship is further supported by the fact that the restored pools, which contain mostly invasive species, are not different in their average inundation depth or duration than the invasive dominated naturally occurring pools which again is corroborated by previous studies at this site (Collinge et al. 2013). Here, due to similar inundation regimes in both restored and invasive dominated naturally occurring pools, the invasive species have encroached into the vernal pool boundaries regardless of construction status.

2.5.2 Litter decomposition

The strongest determinant of decomposition rates, outside of seasonal variation, was the difference between plant species. The finding that climate and leaf litter chemistry are strong drivers to decomposition is not entirely surprising. These three variables (climate, leaf litter chemistry and decomposition rates) have been longstanding in the literature as a quintessential relationship with warm humid climates having the fastest decomposition and leaf litter with labile, easy to decompose carbon, also allowing for faster decomposition rates (e.g., Aerts et al. 1997). Within the confines of vernal pools the native species decomposed much faster than the

invasive. Numerous studies have shown invasive species often decompose at a faster rate (Allison and Vitousek 2004, Spirito 2014, Liao et al. 2008, Bardgett and Wardle 2010) which then results in higher nutrient release in soils (Witkowski 1991, Ehrenfeld 2003, Allison and Vitousek 2004, Liao et al. 2008). If the invasive species is able to better capture the increase of nutrients it can then grow faster completing the cycle (Perry et al. 2010).

This, however, was not the mechanism observed. For an invasive species to proliferate it has to have some competitive advantage over the native species (Callaway and Aschehoug 2000, Hanfling and Kollmann 2002, Hager 2004, Vila and Weiner 2004) or occupy an empty niche (MacDougall et al. 2009). Within the litter bag study the invasive grass *L. multiflorum* had a slower decomposition rate than its native counterpart (*P. californicus*). However, in an analysis of 18 plant species within the community I observed that it was the functional type, grass or forb that most strongly determined plant nutrient composition. These plant functional group decomposition differences have been previously observed (Godoy et al. 2010) and can play a strong role in litter decomposition dynamics irrespective of the system. The argument can then be made that the invasive versus native decomposition rates are not entirely straight forward and it may be more of a functional group difference causing the feedbacks. That said, the invasive species that are able to best encroach into the vernal pool boundaries are the annual grasses (Gerhardt and Collinge 2007). The invasives that are dominating the pools are slower to decompose in relation to the native species resulting in litter accumulation. With these decomposition dynamics in mind a positive feedback is clearly created that strongly benefits the invasive species, not by the increase in decomposition as is often documented, but rather by facilitating a thick litter layer.

2.5.3 Litter manipulations

The idea that an invasive species caused litter layer likely causes a barrier to native plant establishment is further supported by the litter depth manipulation results. The mere presence of a litter layer decreased the native richness by nearly two thirds compared to when no litter layer was present, yet this layer did not decrease invasive richness. The presence of an invasive litter layer can disrupt a variety of mechanistic pathways that can inhibit establishment and in turn overall community structure (Facelli and Pickett 1991a, Loydi et al. 2015). These inhibitory pathways can include: potential allelopathic disruption (Callaway and Ridenour 2004, Lorenzo et al. 2010, Rashid et al. 2010, Greer et al. 2014, Loydi et al. 2015) including invasive litter chemical leachates hindering germination (Dorning and Cipollini 2006, Loydi et al. 2015) or the mechanical presence of a litter layer causing a physical barrier to germination and establishment (Facelli and Pickett 1991a, 1991b, Vaccaro et al. 2009). In my study it appears that the physical presence of the invasive litter layer is causing the reduction in native diversity and abundance. The areas that contained no litter were able to recruit native species, presumably from the seed bank (Faist et al. 2013) as little as 1-2 cm from an area containing a litter layer that did not produce native species (Faist, *personal observation*). Although not directly addressing the amounts of litter present, cattle grazing in vernal pools has been shown to decrease exotic species and increase natives (Marty 2004) and is used as part of vernal pool restoration plans. Coupling this study with my findings, it can be hypothesized that this act of grazing decreases the biomass into the system and essentially decreases the litter layer that is inhibiting the native species.

2.5.4 *Soil properties*

As established, the presence of an invasive litter layer can dramatically alter soil carbon and nitrogen (Liao et al. 2008) and with differences in soil moisture and soil acidity soil organic matter can also be altered (e.g., Ehrenfeld 2003, Allison and Vitousek 2004). In a broad sense one seminal study showed that invasive species can cause strong positive plant-soil feedbacks and that rare-native species demonstrated a negative feedback (Klironomos 2002). Yet, with the metrics tested in my study the only differences observed were between the restored and naturally occurring pools, not between the two types of invaded pools as I would have expected. My results suggest there is an artifact of restoration present in belowground with the lower microbial biomass and C:N ratios, yet no strong signal of the invasive species altering soil properties was observed. The restored pools -created in 1999- are of a much younger age in their development than the naturally occurring pools and perhaps the C:N ratios have not had sufficient build up time to match those of the reference pools. The same restoration artifact could be true of the microbial biomass observations. If the restored pools, regardless of invasion status, are maintaining higher microbial biomass perhaps the fungal biomass, as opposed to the faster colonizing bacterial biomass (Harris 2008), may not have fully infiltrated the restored pools at the time of sampling. Interestingly however, this lack of difference between invaded pools suggests that the abiotic conditions of inundation coupled with the positive feedback of a physical litter layer are the strong driving forces of the alternative states.

2.6 CONCLUSIONS

My study is a clear example of positive feed backs caused by invasive species that are hindering not only native species habitat, but potential for restoration success. While these and

other results show that pools with greater inundation are generally able to maintain their native species (Gerhardt and Collinge 2003, 2007, Collinge et al. 2001, Tanzentzap et al. 2014) through broad scale climate changes this inundation buffer might not always be present (Pyke 2004, Collinge et al. 2011). Some have speculated that with the prospect of altered precipitation regimes, vernal pools are at a greater risk of extinction from climate change than land development (Pyke 2004). With this potential shift towards invasive species in an increased number of vernal pools, my results presented here directly address the stages that occur after the initial invasion. My study shows that vernal pool native species are highly vulnerable to sustained invasion as invasive species deposit more litter, are slower to decompose and are not adversely impacted by this litter layer.

The invaded pools and those excluding invasion are in fact currently in alternative states caused and sustained both by abiotic and biotic factors. These states will remain if environmental conditions do not change, yet there is evidence of one state being entirely more stable than the other through positive feedbacks. Once established into the pools the invasive species, or “invasive state” are able to maintain their invasion and would require strong human intervention to restore pools to their original state. My research suggests that the invasive species should be considered a more stable alternative state. The native species however, are incredibly vulnerable to moving from one state to another and have a low stability. With the tiniest nudge, through either a poor rain year or a thin litter layer, the natives are flung over to the invaded state and then the two states have converged and are no longer alternative states. This is a clear example of hysteresis where a state moving from A to B is not as easily facilitated as B moving to state A.

From a restoration standpoint clear management recommendations emerge from my study. Deeper pools are more advantageous to the natives and if restored pools are becoming

invaded, every attempt to make the pools deeper should be attained. The invasive litter should not be allowed to build up within the pools and if this is observed a continuous removal of the invasive thatch layer, either through grazing (Marty 2004), burning (Gerhardt and Collinge 2007) mowing or potentially raking the thatch is needed to maintain native species. Fortunately the soil properties do not appear to be adversely impacted by the presence of invasive species and the seed bank communities are dominated by native species (Faist et al. 2013). This demonstrates that while the aboveground vegetation is in an alternative state the belowground community is not. This observation further illustrates that through a twofold restoration plan of deepening the pools and removing litter vernal pools do have the capacity to return to a native dominated system that has little or no legacy effect from invasion.

CHAPTER 3
SEED BANK COMPOSITION VARIES ALONG INVASION AND INUNDATION
GRADIENTS IN VERNAL POOL WETLANDS

3.1 ABSTRACT

Environmental filters are well known influences on aboveground vegetation community structure, however, less is known about their role on belowground seed banks. Understanding the influence of environmental filters on the composition of seed banks can reveal community dynamics across known environmental gradients and facilitate restoration efforts. We examined the influence of environmental filters on seed banks of vernal pools by characterizing seed density and diversity along seasonal inundation gradients. We also sampled seed banks from both naturally occurring and restored vernal pools that differed in their aboveground communities (invasive or native species dominated) in a long-term field study in Solano Co. California, USA. We found the highest seed densities were associated with the longest inundation period and in the naturally occurring pools. Inundation gradients within a pool had little influence on seed bank diversity, yet among the pool types diversity and community metrics varied. The naturally occurring pools, regardless of invasion status, displayed a greater species richness and diversity than constructed pools. Our greenhouse germination trials did not show a strong relationship of inundation depths influencing species and total germination. Overall, we found that local position in the field along inundation gradients within a pool strongly affected soil seed bank density, while seed bank diversity varied more across pool types. Environmental filters may be limiting germination with the pool bottoms having the highest inundation and maintain the densest seed bank, but our lack of difference in the germination trials suggest alternative mechanisms other than inundation may be hindering germination.

3.2 INTRODUCTION

Environmental filters, or abiotic and biotic constraints that characterize a habitat, play a fundamental role in structuring communities (Weiher and Keddy 1995, Díaz et al. 1998, Lebrija-Trejos et al. 2010). Environmental filters limit the success of poorly adapted species and promote those that are well adapted in their functional and phenological traits (Roy and de Blois 2006, Mayfield et al. 2009, Bello et al. 2013). In this context it is well understood that environmental filters affect community structure (Horner-Devine et al. 2007) and substantial work has been aimed at understanding how specific plant functional traits and evolutionary histories can influence community assembly (Weiher and Keddy 1995, Díaz et al. 1998, Lebrija-Trejos et al. 2010, Mayfield et al. 2009).

The storage of seeds in the soil allows plants to cope with unfavorable or unpredictable environmental conditions (Venable and Brown 1988, Pake and Venable 1996, Leck 2001, Brock et al. 2003, James et al. 2007). The existence of soil seed bank communities thus provides an opportunity to consider environmental filtering not only over spatial, but also over temporal gradients. For example, comparisons of the current aboveground vegetation community to the belowground seed bank community may provide insights into which species are passing through the environmental filters and which are maintained belowground (Houle 1996, Weiher and Keddy 1995). An important consideration is that environmental filters can occur after germination has already happened or may prevent germination from occurring (Eriksson and Ehrlén 1992, Davis et al. 2000) and studies aimed at understanding the abiotic constraints on seed bank germination can help parse out when the filter occurs. Teasing apart what stage of the life cycle an ecological filter is influencing may explain the mechanisms behind plant community assembly across time and changing environments.

Inundation gradients in wetlands are an ideal context in which to investigate how abiotic constraints act as a filter between seed banks and aboveground communities and can be used to understand how environmental filters, such as inundation, influence vegetation communities across abiotic and biotic gradients. In wetlands, for example, the most pronounced environmental variations are often spatial or temporal water inundation levels or periods. Additionally, soil seed banks in wetland ecosystems are often dense, and contain a high diversity of species (Cohen 1966, Templeton and Levin 1979, Faist et al. 2013), often with differential germination rates in response to subtle environmental variation leading to distinct spatial patterns of aboveground plant species along inundation gradients (Cassanova and Brock 2000, Webb et al. 2006, Ge et al. 2013). Aboveground vegetation communities in wetland ecosystems are therefore often governed by varying water depths and duration of inundation (e.g. Cassanova and Brock 2000, Campbell et al. 2014) and inundation characteristics in turn may influence soil seed bank composition (Leck 2003, Capon and Brock 2006, James et al. 2007).

Vernal pools, or temporary wetlands, are a model system for understanding seed bank spatial dynamics and environmental filtering mechanisms. Vernal pools undergo an annual cycle of inundation and desiccation, which can lead to variation in inundation levels in space and time, and they also contain an aboveground community that emerges from seed every year (Keeley and Zedler 1998, Barbour et al. 2005, Emery et al. 2009). Rising and falling water levels within the pools create characteristic aboveground vegetation bands of species adapted to particular water depths and duration of inundation (Emery et al. 2009). These inundation-related vegetation bands can be divided into three principal zones: 1) pool bottoms that maintain the greatest inundation depths and duration, 2) transition zones that experience moderate inundation with highly fluctuating levels, and 3) pool edges that have the lowest inundation duration. These

inundation zones appear to directly relate to aboveground biomass and vegetation structure (Emery et al. 2009) and possibly serve as environmental filters for germination from the seed bank, which over the long term could be influencing seed bank composition and density.

In addition to having distinct annual inundation cycles, vernal pools are biodiversity hotspots that host many endemic and often endangered plant species specially adapted to fill these inundation niches (Holland and Jain 1981, Zedler 2003, Emery et al. 2009). These highly sensitive vernal pool habitats have remained relatively uninvaded by exotic species because of the extreme annual variation in inundation and desiccation (Gerhardt and Collinge 2003, Gerhardt and Collinge 2007). However, recent changes in precipitation patterns have allowed invasive species (predominately annual grasses) to dominate a subset of the pools at my long-term study site (Gerhardt and Collinge 2007, Collinge et al. 2011, Faist et al. 2013). With discrete boundaries and only a subset of the pools containing invasive species, this vernal pool system affords the opportunity to understand whether invasive species have altered soil seed banks in addition to aboveground communities, and also to gain insight into whether invasion alters seed bank composition across environmental gradients.

My primary objectives were to spatially characterize vernal pool seed banks associated with different aboveground vegetation types (i.e. pool types) and distinct inundation zones within these pool types. I also sought to better understand how inundation works as an environmental filter that may limit or promote germination of vernal pool species. I studied a network of naturally-occurring reference pools (both invaded and native species dominated) and constructed (restored) pools at a long term field site in Solano Co. California. I asked three questions regarding vernal pool seed banks: 1) How do seed banks vary among different pool types, including invaded, native and constructed, 2) How do seed banks vary along inundation

vegetation zones, and 3) How do seed banks from the different pool types and inundation zones germinate in response to differences in inundation regimes acting as environmental filters?

3.3 METHODS

3.3.1 Study area

My field study was conducted on the Travis Air Force Base, Solano County, California, USA (38°15'00", 122°00'00"). The site experiences a Mediterranean climate, with a mean annual temperature of 20.1° C and mean annual rainfall of 50 cm, with the majority of the precipitation falling between the months of November and February (Climate of Sacramento, Report 2010). Similar to other vernal pool ecosystems, the pools at my site occur in areas with little topographic relief (Bauder 2005) and on poorly draining, high clay content soils that facilitate ponding (Rains et al. 2008). My 15-hectare site contains replicates of both reference or “naturally occurring” pools and constructed or “restored” vernal pools. The restored pools were created in 1999 (N=256) as a mitigation plan to provide viable habitat for vernal pool natives. Constructed to mimic the nearby natural pools or “reference pools” in their physical dimensions of size and depth (Collinge and Ray 2009, Collinge et al. 2013) the restored and naturally occurring pools provide a platform to compare and contrast abiotic and biotic metrics.

3.3.2 Study design

My study design was intended to integrate key vegetation and environmental differences among and within pools. The constructed, or restored, pools at this site are generally dominated by invasive species while only some of the naturally occurring or “reference” pools have shown a high degree of invasion by exotic plant species and others have remained dominated by native

plants (Collinge et al. 2011). To maintain spatial replicates that were not confounded by site characteristics my naturally occurring pools, whether invaded or native, were evenly replicated throughout the site and were not spatially clumped. Additionally my chosen restored pools were those found closest to the naturally occurring pools (<50 m) to assure all pool types were as spatially integrated as possible. Because of these differences in invasion levels, pool origin, and aboveground species composition, the pools were parsed out into three types: 1) naturally occurring, invasive dominated 2) naturally occurring, native dominated and 3) constructed for restoration. Then, within each of these pool types (N=8 replicates* 3 pool types), three locations along the inundation vegetation gradient were chosen for plot placement following Emery et al.'s (2009) designated vegetation zones. These were 1) bottom, 2) transition and 3) edge. The three within-pool gradient locations, hereafter interchangeably referred to as “plot location” were chosen because of differences in duration of inundation (Faist and Collinge, *unpublished data*), but also because of differences in aboveground vegetation. The linear distance between each inundation plot location varied by pool with a range of approximately 0.5 m to 3.5 m between locations due to variable pool sizes (~5 m x 10m to ~15m to 40 m).

3.3.3 Data collection (field and greenhouse)

I collected a soil seed bank sample (125 cm³ for each sample) at the plot location (3 plot locations per pool * 3 pool types * 8 replicates per type = 72 samples total) during peak flowering (April) after the majority of species had germinated. This timing effectively captured the soil seed bank because it corresponds to the time after seeds have germinated, but before the current year's plants have dropped their seeds. Thus, the seeds in the soil at this time provide an accurate depiction of what has been maintained in the seed bank.

After field collection, I air dried the soil samples and split each into three equal parts so that a single sample could be subjected to three different greenhouse inundation regimes: “always inundated”, “intermittently” and “never” to mimic winter wet season field conditions of pool bottom, transition and edge. Because of the high clay content and low debris present in the soil, I did not sieve the samples prior to greenhouse treatments, but soaked them in water for approximately one hour to moisten the clay allowing for more pliable soils. I spread each of the soaked soil subsamples in individual pots over a bed of potting soil (Fafard II, Fafard inc.) allowing for an even soil layer approximately 0.5 cm deep. To mimic the field conditions of initial rainfall wetting the soils prior to full inundation I maintained the samples at an even moisture level (just under soil saturation) for 10 days prior to inundation treatments and maintained germination in a controlled greenhouse environment set to mimic field temperatures (Bliss and Zedler 1997). I conducted the greenhouse emergence trial for 95 days and counted new seedling emergence weekly until there was one full week with no new germination. Seed densities are displayed as seeds per kg of soil as the soil types were not significantly different yet the field collected samples were slightly uneven in volume and weight. The most straightforward method was to correct for densities by sample weight thus correcting for variations in sampling. This method provided an accurate assessment of how many seeds are in a single sample and then scaled up to seeds per kg of soil.

3.3.4 Statistical analyses

To determine if seed bank communities (at the species level) varied in association with pool type, plot location and greenhouse inundation treatment, I ran a PERMANOVA (R core development team 2011) with 4,999 permutations. I then ran a non-metric multidimensional

scaling (NMDS) with a Bray-Curtis distance matrix for visualizing community structure. All community analyses were run in R (R core development team 2011, Vienna Austria) in the Vegan package (Oksanen et al. 2013).

I tested for data normality using a Shapiro-Wilk test within each group (pool type, plot location and treatment). All data were normally distributed ($P > 0.05$) except for invasive species seed densities. The invasive species data were square root transformed, which achieved the assumption of normality (Shapiro-Wilk test, $P > 0.05$). I then ran a three factor ANOVA comparing pool type, inundation plot location and greenhouse inundation treatment to compare overall seed densities and diversities. While the three factors are not entirely independent from one another I chose to run a multifactorial ANOVA over a nested analysis because my categorical variables are fixed effect and not the repeatable random effects required for nested data and allow us to test for interaction effects. After running my multifactorial ANOVA, for pairwise comparison I ran a post-hoc Tukey's Honest Significant Difference (HSD) test for pool types and inundation locations as germination treatment was not a significant variable. I then ran two additional three factor ANOVAs with the invasive and native seed species separated serving as the response variables and the independent variables remaining the same as in total seed densities (pool type, inundation plot location and greenhouse treatments). I then ran Post-hoc Tukey's HSD tests for both invasive and native seed densities looking at pool types and inundation vegetation zones were

I also analyzed the data for four diversity metrics (species richness, evenness, Shannon and Simpson indices) for each of the independent variables (pool type, inundation plot location and greenhouse inundation treatment). Plot location and greenhouse inundation treatment

analyses did not yield any differences in any of the diversity metrics tested, yet pool type did vary significantly and I display these data in the results section.

Because the greenhouse germination treatments did not differ in any of the tested seed density dependent variables (ANOVA, $P > 0.05$) I excluded them from the broad scale results. However, because the greenhouse inundation treatments were aimed at examining environmental filtering at the species level, I categorized species responses to provide insight into how inundation levels impact recruitment. The individual species level data distributions were non-normal and could not be transformed so I ran each species under a non-parametric Kruskal-Wallis test between individual species and the three greenhouse inundation treatments (always, intermittent and never). If I found a significant difference at the treatment level for a species I ran a pairwise non-parametric Wilcoxon rank sum test with a False Discovery Rate correction (Benjamini and Hochberg 1995) to avoid Type I error.

3.4 RESULTS

3.4.1 *Community metrics*

Pool type (naturally occurring native and invasive dominated and constructed for restoration), inundation location (bottom, transition and edge) and greenhouse inundation treatments (never, intermittent and always) all significantly influenced the soil seed bank community. PERMANOVA results showed that when testing the effect of these three factors on seed bank community structure the results were statistically significant with an interaction effect between pool type and plot location (Table 3.1). My NMDS ordination for each of the three factors (Fig. 3.1) showed the most striking difference in the pool type (Fig. 3.1-a) displaying strong spatial clustering between pool types. While statistically significant in the

PERMANOVA the plot location (Fig. 3.1-b) and green house treatment (Fig. 3.1-c) effects did not yield as much spatial clustering.

Table 3.1. Comparison testing through a PERMANOVA to understand the effect of three factors (pool type, plot location and inundation treatment) on seed bank communities.

Source of variation	DF	SS	MS	F	p value
Location	2	2.11	4.31	4.31	0.0002
Pool Type	2	5.15	10.50	10.50	0.0002
Treatment	1	1.02	1.02	4.17	0.0002
Location x Pool Type	4	1.42	0.36	1.45	0.0354
Location x Treatment	2	0.40	0.20	0.82	0.7006
Pool Type x Treatment	2	0.27	0.14	0.55	0.9348
Location x Pool Type x Treatment	4	0.78	0.19	0.79	0.8194
Residuals	197	48.28	0.25		
Total	214	59.43			

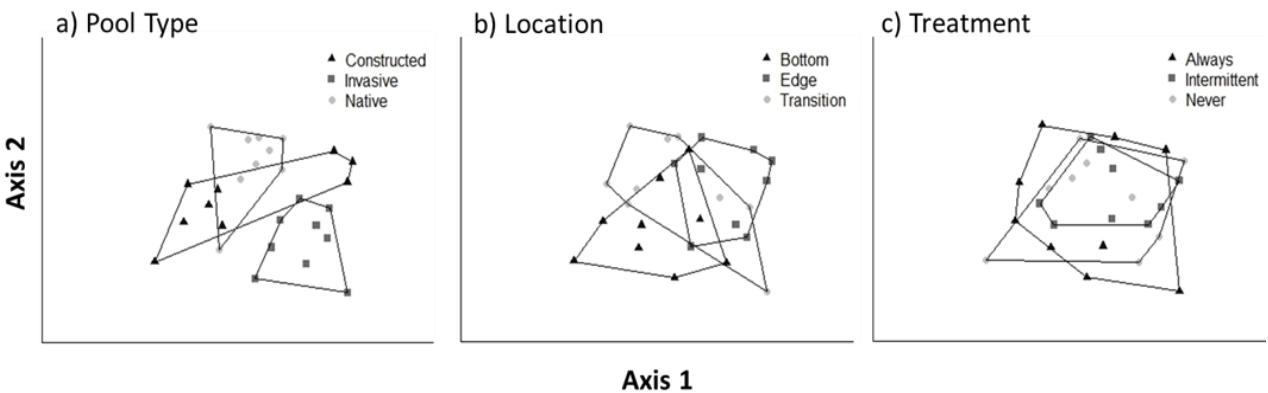


Figure 3.1. NMDS plots showing how vegetation communities vary in relation to pool type, location within a pool and greenhouse inundation treatments. After eight runs the stress solution was reached at 0.196 using a Bray Curtis distance matrix.

3.4.2 Total seed densities

Total seed densities varied by both pool type and location (Table 3.2, ANOVA, $F_{2,9}=7.89$, $p=0.01$ and $F_{2,9}=12.42$, $p=0.003$ respectively) with no significant difference in densities in the greenhouse inundation treatments and no interaction effects observed. The pool type strongly demonstrated the restored pools as having the lowest seed densities (mean = 290 seeds per kg soil) and the invasive and native dominated naturally occurring pools containing higher densities (mean = 456 and 418 respectively). Post-hoc Tukey's HSD pairwise comparisons demonstrated significantly lower seed densities in the constructed pools than in both the naturally-occurring, invasive and native dominated pools ($p = 0.01$ and $p=0.04$ respectively). The two naturally occurring pool types did not significantly differ from one another in seed densities ($p= 0.66$).

Table 3.2. Determining seed density variation with a three factor ANOVA where Pool type, location within a pool and greenhouse treatments were the determining variables and seed density (seeds / kg soil) the response variable. Only Pool type and Location were significant factors and no interaction terms were present ($P \leq 0.05$).

Source of variation	DF	SS	MS	F	p value
Pool Type	2	137582	68791	7.893	0.01048
Location	2	216428	108214	12.416	0.00258
Treatment	1	25505	25505	2.926	0.12131
Pool Type x Location	4	67977	16994	1.95	0.18626
Pool Type x Treatment	2	9142	4571	0.524	0.60891
Location x Treatment	2	2034	1017	0.117	0.89118
Pool Type x Location x Treatment	4	20856	5214	0.598	0.67327
Residuals	9	78441	8716		

The inundation plot locations showed the highest seed densities in the pool bottoms with a mean of 511 seeds per kg from the pool bottoms and 302 and 352 in both the transition and edge zones. Post-hoc Tukey's HSD pairwise comparisons revealed that the pool bottoms

contained over 200 more seeds per kg of soil than the transition zone ($p=0.002$) and more than 150 seeds per kg of soil than the edge zone ($p=0.013$). The transition and edge zones did not differ from one another in their seed densities ($p=0.52$). When the plot locations seed densities were broken down by pool type the same relationship of the highest seed densities in the pool bottom held true for the constructed pools and partially for the invasive dominated naturally occurring pools with the bottom and transition significantly different but the edge not differing (Fig. 3.2). The seeds found in the native dominated pools did not differ by plot location (Fig. 3.2).

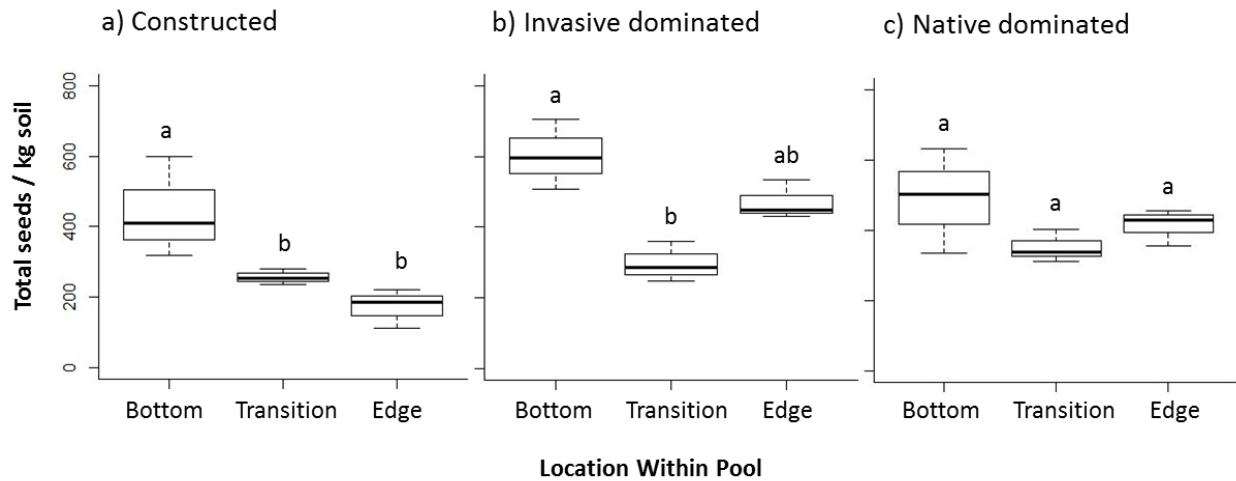


Figure 3.2. Total seed densities as determined by pool type and location. Panel “b” and “c” are both naturally occurring pools with the aboveground dominated by either invasive or native species while the constructed pools “a” were created for restoration purposes and are primarily dominated by invasive species aboveground. Constructed pools contained significantly lower seed densities than either of the naturally occurring pools. Letters indicate significance (ANOVA with Tukey’s HSD pairwise tests, $P<0.05$) with a standard error of ± 1 .

Because pool type was the only variable that demonstrated any differences in total seed diversity I ran my diversity analyses on pool type excluding both plot location and greenhouse inundation treatment. When comparing diversity metrics for the different pool types all metrics other than evenness displayed significant differences with the higher diversity metrics generally found in the naturally occurring vernal pools compared to those constructed for restoration

(Table 3.3., ANOVA, $F_{2,27}=4.15$, $p=0.03$) for richness, ANOVA, $F_{2,27}=5.65$, $p=0.01$ for Shannon diversity and ANOVA, $F_{2,27}=7.27$, $p=0.003$ for Simpson diversity). Through a post-hoc Tukey's HSD pairwise test on seed diversities at the pool type level my results showed that restored pools had lower species diversity than both naturally occurring pool types, as determined by the Shannon and Simpson indices (Table 3.3, $p=0.01$ and $p=0.003$ respectively) but evenness did not vary among any of the pool types. Seed bank species richness was higher in the naturally occurring pools compared to the restored pools, but only significantly so in the naturally occurring invasive dominated pools (Table 3.3). Comparing the relationship between Shannon diversity and seed density I found that there was very little to no relationship between the two (Pearsons Correlation, $t=1.66$, $\text{corr}=0.11$, $p=0.10$).

Table 3.3. Seed bank diversity estimates of pool type. All calculations represent mean diversity indices with letters indicating significant differences (ANOVA followed by a Tukey's HSD pairwise test, $P<0.05$).

Pool Type	Richness	Evenness	Shannon
Invasive dominated	5.80 ^A	0.73 ^A	1.30 ^A
Native dominated	5.79 ^{AB}	0.77 ^A	1.36 ^A
Restored	4.52 ^B	0.73 ^A	1.03 ^B

3.4.3 Invasive and native seed densities

To better understand occurrence of invasive versus native species in the seed bank I analyzed seed densities of native and invasive species separately through a three factor ANOVA with pool type, plot location and greenhouse treatments as the independent variables. Pool type and plot location invasive seed densities were significantly different from one another (Fig. 3.3,

ANOVA, $F_{2,9}=67.99$, $p<0.00001$ and $F_{2,9}=25.92$, $p=0.0002$ respectively) with no observed interaction effects. A post-hoc Tukey's HSD pairwise test showed that each of the pool types were significantly different from one another with the highest densities of invasive seeds (data square root transformed) found in the naturally occurring invasive species dominated pools with a mean of 123 per kg of soil. While significantly more invasive seeds were found in the naturally occurring native dominated pools than the restored pools (63 seeds 30 per kg of soil respectively, $p<0.00001$) both the native dominated and restored pool types contained less than half of the invasive seed densities found in the invasive dominated naturally occurring pools ($p=0.003$ and $p=0.04$). The pool bottoms generally had significantly higher seed densities than the transition zones ($p=0.03$) but not the edge ($p=0.23$) and the transition zone did not differ from the edge. However this relationship varied when broken up by pool type (Fig. 3.3).

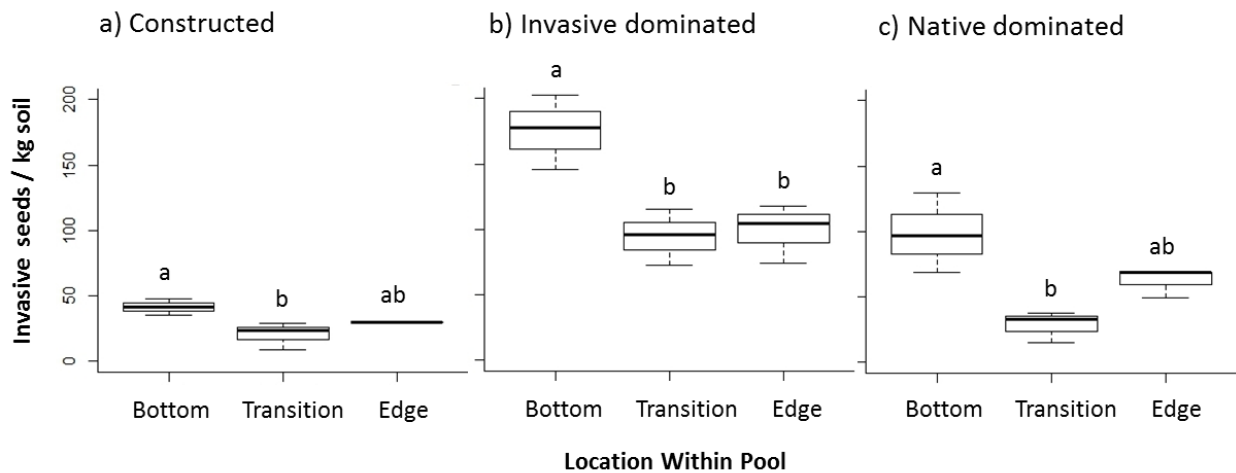


Figure 3.3. Invasive species seed densities as determined by pool type and location. Panel *a* and *b* are both naturally occurring pools with the aboveground dominated by either invasive or native species while the constructed pools were created for restoration purposes and are primarily dominated by invasive species aboveground. For invasive seeds each pool type was significantly different from one another. Lowercase letters indicate significance between pool location within a pool type (ANOVA with Tukey's HSD pairwise tests, $P<0.05$) with a standard error of ± 1 .

Interestingly, the native seed densities did not significantly differ among any of the pool types (ANOVA, $F_{2,9}=3.64$, $p=0.07$) with mean native densities up to 10 times higher than the

invasive species densities (250, 316 and 342 seeds per kg of soil). The native species did however differ in their densities among the inundation plot locations (ANOVA, $F_{2,27}=10.49$, $p=0.004$). A post-hoc Tukey's HSD pairwise comparison showed that the native seeds were highest in the pool bottom (mean=394 seeds per kg) compared to both the transition and edge ($p=0.005$ and $p=0.04$ respectively) with 278 and 237 seeds per kg each. Finally, the transition and edge did not differ ($p=0.65$). Regardless of location or pool type when the invasive and native seeds were analyzed separately there were two to four times higher native seed densities stored in the seed bank compared to invasive seeds.

3.4.4 Greenhouse inundation treatments

Although I found no difference in the number of germinated seedlings in relation to the greenhouse inundation treatments I observed a strong potential for unique species responses to varied inundation levels. Thus, I examined species level responses to inundation treatments (Table 3.4). Surprisingly, the overall densities of dicots or monocots did not differ among inundation treatments. Additionally, none of the dicot species germination counts varied significantly in relation to inundation treatments. The five most abundant native species (*Downingia concolor*, *Crassula aquatica*, *Callitriche sp.*, *Lasthenia conjugens*, and *Juncus bufonius*) showed trends toward favoring specific inundation treatments, but none significantly so. *J. bufonius* (Juncaceae) and *C. aquatica* (Crassulaceae) fared the best when there was no inundation and only saturated soils, while *D. concolor* (Campanulaceae) showed a marginal preference for the always inundated treatment and visually appeared the healthiest in this treatment (Faist, *personal observation*). Two vernal pool indicator species (*L. conjugens*

(Asteraceae) and *Callitriche sp.* (Callitrichaceae) did not show any preference in germination for a particular inundation treatment.

Table 3.4 Species level responses to greenhouse inundation germination trials. Species in bold indicate significant differences in treatment types (Wilcoxon rank-sum test, $P < 0.05$).

Monocots	(mean seeds/kg soil)					
	Inundation treatment					
	Always		Intermittent		Never	
<i>Aira caryophylla</i>	0		0		0.2	±0.2
<i>Bromus hordeaceus</i>	0		0.1	±0.1	0.3	±0.3
<i>Deschampsia danthonioides</i>	0		2.6	±1.2	3.4	±1.0
<i>Hordeum marinum</i>	2.9	±0.8	11.8	±3.1	15.3	±4.7
<i>Juncus bufonius</i>	10.9	±5.5	38.4	±10.4	49.0	±16.1
<i>Lolium multiflorum</i>	2.2	±0.7	6.3	±1.3	4.1	±0.9
<i>Phalaris minor</i>	0.7	±0.7	0.9	±0.6	0.2	±0.2
<i>Pleuropogon californicus</i>	1.6	±0.9	3.2	±0.4	2.1	±0.7
<i>Polypogon maritimus</i>	0.7	±0.7	0.7	±0.5	1.3	±1.3
<i>Vulpia bromoides</i>	0.4	±0.3	2.8	±1.3	1.5	±0.6
Unknown Monocots	9.9	±2.5	2.0	±0.9	1.9	±0.7
Mean Total Monocots	29.1	±6.4	68.9	±12.4	79.4	±17.0
Dicots						
<i>Callitriche sp.</i>	28.4	±7.9	17.1	±4.3	32.8	±10.0
<i>Cerastium glomeratum</i>	0		0		0.5	±0.3
<i>Cicendia quadrangularis</i>	0.8	±0.5	0.8	±0.4	7.6	±7.0
<i>Cotula coronopifolia</i>	0		0.4	±0.3	1.0	±0.5
<i>Crassula aquatica</i>	66.6	±18.7	75.9	±12.1	100.8	±14.5
<i>Downingia concolor</i>	115.2	±22.5	70.1	±12.1	91.5	±28.2
<i>Erodium botrys</i>	0		0		0.2	±0.2
<i>Eryngium vaseyi</i>	8.0	±2.0	9.4	±2.1	5.7	±1.8
<i>Geranium dissectum</i>	0		0		0.1	±0.1
<i>Hemizonia congesta</i>	0		0.1	±0.1	0.5	±0.3
<i>Hemizonia pungens</i>	10.9	±2.5	11.2	±2.8	10.1	±2.1
<i>Layia chrysanthemoides</i>	0		0.1	±0.1	0.2	±0.2
<i>Lasthenia conjugens</i>	30.8	±10.1	34.8	±11.1	42.7	±10.5
<i>Lasthenia glaberrima</i>	0		0.3	±0.3	0	
<i>Leontodon taraxacoides</i>	0		0.1	±0.1	0	
<i>Lotus corniculatus</i>	0.3	±0.3	1.0	±0.9	0.8	±0.5
<i>Lythrum hyssopifolium</i>	66.1	±13.3	44.1	±9.9	47.9	±11.2
<i>Myosurus minimus</i>	0		0.8	±0.8	3.4	±2.3
<i>Picris echioides</i>	0		0		0.1	±0.1
<i>Plagiobothrys stipitatus</i>	1.8	±0.8	1.5	±0.6	5.9	±1.6
<i>Psilocarphus oregonus</i>	0.5	±0.5	0.4	±0.3	1.8	±1.4
<i>Psilocarphus tenellus</i>	0		0.8	±0.4	1.0	±0.6
<i>Rumex crispus</i>	0.2	±0.2	0.9	±0.4	0.2	±0.2
<i>Sonchus asper</i>	0		0		0.1	±0.1
<i>Veronica peregrina</i>	6.1	±3.9	2.1	±0.7	3.7	±1.2
UNK10	7.6	±4.4	2.2	±0.9	5.9	±2.4
Unknown Dicots	1.1	±0.5	2.5	±1.0	4.8	±3.1
Mean Total Dicots	344.281	±44.4	275.709	±27.3	369.377	±42.6
Mean Total Counts	373.400		344.630		448.780	

The most common invasive species found in the seed bank, and most common seed bank species overall (though uncommon aboveground; Faist et al. 2013), was *Lythrum hyssopifolium* (Lythraceae). *L. hyssopifolium* did not display any preference for inundation treatment maintaining high numbers regardless of inundation level. The invasive species with the one of the highest abundances aboveground (*Lolium multiflorum*) did not have large quantities of seeds stored in the seed bank, which is consistent with previous findings from California vernal pools (Bliss and Zedler 1997, Faist et al. 2013). That said, *L. multiflorum* had low germination numbers overall, but did trend toward lower mean counts in the always inundated treatment than both the intermittent and the never with a mean of six and four seeds per kg of soil in the intermittent inundation and two seeds per kg soil in the always inundated.

3.5 DISCUSSION

3.5.1 Community metrics

While the PERMANOVA showed all three categorical factors (pool type, plot location and greenhouse treatment) significant when visualizing the data (Figure 3.1) pool type has the greatest difference. With the invaded dominated naturally occurring pool parsing out to be on its own where the restored and native dominated naturally occurring pools clumped together there is a potentially straightforward explanation. This difference could be that the invasive dominated pools have the invasive seeds in the seed bank that the other two pool types don't, thus separating it out. Interestingly, while the pool types differed in their community structure this pattern was not observed in the seed densities as location was the most striking.

3.5.2 Total seed densities

Aboveground species composition is often quite divergent from its associated seed bank (Hopfensperger 2007), partially due to environmental filters limiting the success of seed bank species poorly adapted to germinate in existing environmental conditions (Cingolani et al. 2007, Ge et al. 2013, Hedberg et al. 2014). With lower seed densities in the restored pools than the invasive dominated naturally occurring pools and trends toward lower densities in the native dominated pool type, I surmise that this discrepancy could be an artifact of restoration (Seabloom and van der Valk 2003) rather than aboveground vegetation signals dictating seed bank community compositions. Over the 11-year duration of the restoration project the restored pools may not have had sufficient time to build up a comparable seed bank to the naturally occurring pools due to dispersal and connectivity limitations (Seabloom and van der Valk 2003, Ray and Collinge 2014).

The low species diversity estimates found in the restored pools may also be an artifact of restoration similar to the patterns observed for seed densities. Because the overall seed densities were lower in restored pools there may be less opportunity for greater diversity within the seed bank. However, another explanation could be that the presence of invasive species in the seed bank inflates the diversity metrics (e.g. increasing mean richness). Invasive species have even been shown to decrease seed bank species richness and biodiversity with increased invasion (Holmes and Cowling 1997, Holmes 2002, McGeoch et al. 2010) or conversely, to not significantly alter seed bank diversity (Vilà and Gimeno 2007, Gioria and Osborne 2010). However, I found that the presence of invasive seeds in vernal pool seed banks increases or maintains diversity rather than reducing it. My results suggest that the persistent and dense seed

banks found in vernal pools (Faist et al. 2013) appear to be able to admit the introduction of invasive species and increase overall seed bank community diversity.

My inundation plot gradient, and proxy for environmental filter, results were consistent with previous studies showing that the inundation gradient in wetlands (or in similar ecosystems with fluctuating inundation) can alter seed bank densities and composition (Cassanova and Brock 2000, Baldwin et al. 2001, Peterson and Baldwin 2004, Capon and Brock 2006, Capon 2007, Robertson and James 2007, James et al. 2007). Prior to my study, however, this relationship had not been determined in vernal pools or temporary wetlands, yet these ecosystems are of rising concern as climate change and land development threaten their existence (Zedler 2003). With the highest densities of seeds in pool bottoms, which experience the longest duration of inundation, my findings are in contrast to the observation that highest densities occur in the intermediately flooded habitats (James et al. 2007) or in those with the greatest degree of disturbance. The high densities of seeds found in the pool bottoms could also be due to the fact that fewer seeds were able to be recruited because of winter inundation and were maintained belowground rather than being filtered through germination, which is similar to studies in other wetland habitats (Capon and Brock 2006).

3.5.3 Invasive and native species densities

Although the invasive dominated naturally occurring pool types contained the highest densities of invasive seeds and this generally mimicked their aboveground vegetation composition, the same was not true for native seed densities. For native species, there were no differences in seed densities for the three pool types suggesting the aboveground vegetation signal does not appear to transfer to the belowground seed bank. A common recruitment

limitation is low seed density (Clark et al. 2007, Seabloom et al. 2003, Myers et al. 2009), yet, the native species at this site do not appear to be limited in that manner, but rather they may be undergoing a filter that limits either germination or their upward mobility to reach maturity.

With regards to environmental filtering, my study illustrated an intriguing difference between invasive and native species strategies. Although dissimilarity between the aboveground vegetation and the belowground seed bank has been found in mine and other studies (Parker and Leck 1985, Hopfensperger 2007, Faist et al. 2013), the degree of dissimilarity varied when considered according to invasive status. The high abundance of natives in the seed bank regardless of aboveground vegetation suggests that there is a filter limiting the dense native seed bank from getting to the aboveground community. This was further enhanced by my findings showing that only the pool type mattered in invasive species differences, which suggest propagule availability.

Another possibility could be related to the functional type of the species, whether invasive or native. While there is only one perennial species located at this site the invasive species aboveground are most commonly graminoids while the natives are predominately forbs. This relationship of graminoids as invasives and natives as forbs does not necessarily hold true in the seed bank (Faist et al. 2013). The invasive species that are found in the seed bank are comprised of graminoids (e.g. *Lolium multiflorum*) but these numbers are at a much lower density than its aboveground presence. In contrast the non-native forb *Lythrum hyssopifolium* is the most common seed banking species and is rarely found aboveground (Faist et al. 2013). This instance of a high number of forbs in the seed bank, regardless of native status, may indicate that the invasive species in the seed bank have similar functional traits to the natives, thus allowing them to be maintained in the seed bank. This could also indicate that the invasive forb species in

the seed bank are responding to the environmental filters in a similar manner to the natives, thus hindering their aboveground vegetation.

Finally, the restored pools had lower overall seed densities yet they had the highest *proportion* of native seeds. From a management perspective, this may provide guidance for future restoration efforts in that when attempting to inoculate a new vernal pool project the aboveground vegetation may not be the best indicator of what is in the seed bank. For instance at my site the restored pools would transport the lowest number of invasive species to the restoration project if used as soil inoculum.

3.5.4 Greenhouse inundation treatments

The spatial relationships I observed from the field-collected samples showed the pool bottoms as having the highest seed bank densities. With these findings I predicted that the germination trials would mimic the field and the “always inundated” treatment would produce the lowest germination because of unfavorable environmental filters causing seeds to be stored in the soil rather than emerging aboveground (Bliss and Zedler 1997, Cassanova and Brock 2000). When broken down by functional group, such as graminoid (monocot) and forbs (dicot), there still were no observable trends leading me to believe that the responses may be species specific. However, when I examined individual species responses, the majority of the species did not exhibit significant relationships as to which inundation treatment they preferred for germination (Table 3.4). This lack of observed difference suggests that in this system the strongest environmental filter for germination may not be inundation.

Previous research has suggested that the common invasive grass species entering the pools *Lolium multiflorum* and *Hordeum marinum*, are most inhibited by inundation depths, rather

than biotic competition (Gerhardt and Collinge 2003, 2007). This research suggests that there are strong environmental filters placed on the system and thus limiting the introduction of invasives into the pools (Gerhardt and Collinge 2007). My lack of germination difference may be explained by my experimental greenhouse inundation depths. My study maintained a maximum inundation depth of 2cm and the maximum field inundation depths are up to 10-20 times that. A greater inundation depth may be needed to inhibit germination of these inundation adapted species. The highest seed densities found in the pool bottom may still suggest that inundation is actually a mechanism, or environmental filter, limiting germination with the pool bottom seeds maintained in the seed bank rather than germinating. While the exact inundation depth needed to inhibit germination was not uncovered in this study I have illustrated that the environmental filter of inundation depth may be at play in constructing seed banks yet these filters are not as strong as originally predicted.

3.6 CONCLUSIONS

Understanding how seed banks are influenced by their aboveground vegetation, whether invasive or native, is an important step in understanding seed bank dynamics and thus environmental filtering. Vernal pool wetlands provide a unique opportunity to see how the abiotic filters of inundation duration can influence not only the aboveground vegetation, but the belowground seed banks as well. My findings show that vernal pool seed banks have a strong spatial signal with the highest density, but not diversity, of seeds in the pool bottoms. I also found that the invasive dominated pools differed in their total invasive seed banks, but the native seeds showed a strong persistence regardless of pool types and maintained high densities of native seeds across all conditions. My results also suggest that a strong environmental filter is at

work limiting the recruitment and aboveground establishment of native vernal pools species, but at my study site this filter may not be at the germination level for inundation times. These findings can be directly applied to ecological restoration projects as knowing where the highest density of seeds and highest proportion of native seeds exist helps when using soil inoculum for new restoration projects. Vernal pool seed bank knowledge is also enhanced as my study showed that seeds stored in the seed bank are able to germinate under a variety of different inundation types, displaying strong resilience and persistence.

CHAPTER 4
BANKING ON THE PAST: SEED BANKS AS A RESERVOIR FOR RARE AND NATIVE
SPECIES IN RESTORED VERNAL POOLS

4.1 ABSTRACT

Soil seed banks serve as reservoirs for future plant communities, and when diverse and abundant can buffer vegetation communities against environmental fluctuations. Sparse seed banks, however, may lead to future declines of already rare species. Using collected soils and germination trials, we assessed species diversity and density in seed banks of restored, ephemeral wetlands (vernal pools) in California's Central Valley, USA. Using long term vegetation surveys, we compared the community structure of seed banks to that of aboveground vegetation and assessed the temporal links between below- and above-ground communities. We also compared the proportional abundances of different cover classes as well as abundance of native plants in seed banks to aboveground communities. Proportional abundances of both rare and native species were greater in seed bank samples than aboveground, yet the seed bank had lower species richness than aboveground vegetation. However, the seed bank had greater levels of differentiation among pools, (beta diversity; β) than aboveground samples. Additionally, the seed bank was more similar to the earlier (2003-2006) aboveground community than the more recent (2007-2010) aboveground community. The correlation of species composition in the current seed bank to an earlier aboveground community suggests that seed banks exhibit storage effects while aboveground species composition in this system is not driven by seed bank composition, but is perhaps due to environmental filtering. We conclude that the seed bank of these pools is neither prone to the same temporal rates of invasion as the aboveground community, nor is seed abundance presently a limiting factor in the aboveground frequency of native species or a promoting factor in plant invasions of these restored habitats.

4.2 INTRODUCTION

The existence of dormant, soil seed-banks can allow different vegetation communities to occupy the same space at different periods of time (Chesson and Huntley 1997, Chesson 2000a, 2000b, 2004, Angert et al 2009). Defining a vegetation community based on the active, aboveground species composition at a site can thus be incomplete, as there may be other species waiting to emerge following environmental or structural shifts (Warner and Chesson 1985). By allowing plants to store propagules belowground until adequate germination conditions are met, seed banks can buffer communities against perturbations (Thompson and Grime 1979, Templeton and Levin 1979, Leck 2001) and their composition can be a useful metric for estimating the *potential* future composition of the vegetation community (Major and Pyott 1966). Different rates of compositional turnover in belowground seed banks than in aboveground communities might lead to dramatic shifts in vegetation community structure following disturbances—shifts that could either enhance or limit native species abundance and diversity depending on seed bank assembly rates and processes.

Systems documented to build dense and diverse seed banks, such as wetlands, may be those that are disproportionately impacted by highly fluctuating environmental conditions over time (Brock and Rogers 1998, Facelli et al 2005, Aponte et al 2010). The changing availability of water in wetlands, for example, may cause plant propagules to lie dormant during moisture poor or overly saturated conditions, and then germinate when their appropriate moisture regime is present (Cohen 1966, Templeton and Levin 1979, Venable and Brown 1988, Pake and Venable 1996, Bliss and Zedler 1997, Leck 2001). If the species- or community-specific environmental conditions for germination are not met, native species could be adversely impacted over time.

My study focuses on vernal pools, which are ephemeral wetlands that are intermittently inundated over annually repeated cycles of flooding and drying in California's Mediterranean climate zone. Thus, vernal pools often experience greater variability in micro-climate than other wetland ecosystems. Also, vernal pools are populated almost exclusively by annual plants that are known to persist and propagate from seed (Keeley and Zedler 1998). The annual plants in this community (along with one common perennial species, *Eryngium vaseyi*) generally emerge during the wet-season's early rainfall and persist in immature states during periods of water inundation. During the relatively short, warm spring, the pools dry and these annual species quickly bolt to reach reproductive maturity and disperse their seeds before the onset of the hot dry summer (Linhart 1974, Keeley and Zedler 1998). The combination of highly variable water depths and inundation periods, as well as dominance by annual plants tends to promote a substantial seed bank in vernal pools, making them ideal models for studying seed bank structure and the links between seed banks and the aboveground vegetation community over time.

Vernal pools are found in Mediterranean climates worldwide, but are also present wherever strong seasonal precipitation patterns occur on flat or depressed surfaces that allow for prolonged inundation (Keely and Zedler 1998, Deil 2005, Aponte et al 2010). Globally, 50% of wetland habitats have been lost (Zedler and Kercher 2005) and vernal pools in particular have been disproportionately affected with losses of over 90% of their total area. Much of this habitat loss in vernal pools has been along the west coast of the United States, particularly in California's Central Valley, as a result of land development and agriculture (Zedler 2003, Zacharias and Zamparas 2010, Rhazi et al 2013). Because of their highly variable and unique environmental conditions, vernal pools historically hosted many rare and endemic annual plant species (Croel and Kneitel 2011) that are now threatened due to habitat loss (Zedler 2003). Thus,

the preservation and restoration of vernal pools is of great interest as they are critical habitats for the maintenance of biological diversity.

In addition to loss of habitat area, vernal pools have been negatively affected by invasive species—creating an additional threat to native biodiversity (Gerhardt and Collinge 2007, Collinge et al 2011, Collinge et al 2013). Historically, California’s vernal pools were assumed to be buffered from invasive species due to inherently variable environmental conditions—primarily annual flooding—which prevented the establishment of exotic plants less adapted to annual inundation (Gerhardt and Collinge 2003, 2007, Marty 2005, Price et al 2010b). Nevertheless, vernal pools have experienced exotic species encroachment as invasive plant species have flourished across California’s Mediterranean landscapes and as historic environmental buffers have weakened due to recent, shorter inundation times (Gerhardt and Collinge 2003, 2007, Collinge et al 2011, Collinge et al 2013). Understanding how this recent influx of invasive species into the vernal pool system has altered the seed bank, and thus the *potential* of the community is of great interest for the restoration and conservation of these threatened habitats.

To understand how exotic plant invasions might influence soil seed banks, and ultimately how they might influence success of restored vernal pools, I compared long-term records of aboveground plant communities to co-located samples of the belowground (seed bank) communities of a vernal-pool restoration site established in 1999 in California’s Central Valley. I compared species composition and diversity, proportional abundances of rare, intermediate, and common occurrence categories; and the proportional abundance of native and invasive species in both communities. My primary goal was to understand how the aboveground vegetation compared to its seed bank counterpart below ground. Specifically, my three objectives were: (1)

to determine if the community structure of the seed bank matches that of the aboveground community, and if so, over what sampling period, (2) to determine if the abundances of rare species in the aboveground community are constrained by their abundances in the seed bank, and (3) to compare the abundances of invasive and native species in the aboveground versus seed bank community to understand how levels of invasion differ between the active and dormant community.

4.3 METHODS

4.3.1 Study site and experimental design

I conducted my study on the Travis Air Force Base, Solano County, California, USA (38°15'00", 122°00'00"). The 15-hectare site contains both naturally-occurring, "reference" and restored vernal pools with 256 experimental pools that were constructed in 1999 to mimic nearby reference pools (Collinge and Ray 2009, Collinge et al 2013). My original restoration experiment involved the establishment of a permanently marked, 50 cm x 50 cm square sampling plot in each of the 256 pools into which different seeding treatments were imposed, to track community trajectories within and among pools in relation to the initial seeding treatments. I have sampled plant species composition in these 0.25 m² sampling plots annually since 2002, when all of seeding treatments were completed. For the present study I selected a subset (n= 57) of permanent plots in restored pools evenly distributed among size classes (5 x 5 m, 5 x 10 m, and 5 x 20m) and spatially dispersed across the entire restoration site.

My research site experiences a Mediterranean climate, with a mean annual temperature of 20.1 °C and mean annual rainfall of 500 mm, with over half of the precipitation falling between the months of November and February (Climate of Sacramento, Report 2010). The vernal pool

hydroperiod closely follows the seasonal rain cycle; pools are filled by winter rains, followed by rapid spring drying, and a subsequent hot, dry summer and fall. Because vernal pools are fed by precipitation, they predominately occur in areas with low to no-slope topography (Bauder 2005) and on soils with high clay content that facilitates flooding through low soil infiltration (Rains 2008).

4.3.2 Seed bank sampling

I characterized the seed bank at each sampling location within a pool by collecting soil samples adjacent to permanently established vegetation sampling plots in restored pools (Collinge and Ray 2009) that had an observed peak inundation depth of 5-10 cm. I collected one soil sample from each of 57 sampling locations, comprised of a 125 cm³ cube from the soil surface to a 5 cm depth. I collected soil samples in March of 2010 to coincide with the period when aboveground vegetation had reached peak germination, but prior to seed set, which ensures that samples were representative of the seed bank and not a measure of recently dispersed seeds. I air-dried samples in paper bags and then split them into equal parts by mass, storing one half of each sample for use in future studies.

4.3.3 Seedling emergence

To determine seed abundance in the seed bank, I used a standard germination emergence method (Gross 1990, Bernhardt et al 2008, Price et al 2010a). In November 2010, I soaked the greenhouse-designated soil samples for 12- 24 hours to loosen the highly compact clay-rich soils and spread a thin layer (approximately 0.5 cm deep) of the mixture in round pots (7.6 cm diameter × 3.8 cm deep) with a base of potting soil (Fafard II, Fafard Inc., Agawam, MA). I

maintained soil moisture at saturation in each pot for approximately 60 days, or until there were 14 consecutive days with no new germinants observed. I repeated this process three additional times with each soil sample, allowing soils to dry down for a minimum of two weeks between trials. I then counted germinants and identified individuals to species in each of the four trials. If I could not identify the species in the germinant phase, I allowed the individual to flower for identification purposes. I observed little to no mortality across germinated samples (Faist, *unpublished data*) so did not evaluate patterns of mortality in my analyses. I could not identify nine possible species in the seed bank observations; because of their low presence (accounting for 0.03 of the total counts) and to avoid inflation of diversity estimates they were removed from the total species count and the rest of the analyses.

To accurately assess what seeds were found in the soil and because seed bank studies in different systems have used soil sorting as a common assessment tool (Gross 1990, Price et al 2010a) I manually sorted seeds from the remaining half of each soil sample for community comparison to the emergence trials. I found that manual sorting did not reveal additional species not already present in greenhouse emergence trials, and also that the community identified by sorting did not significantly differ in species composition and abundances (PERMANOVA $P > 0.05$) or diversity (Shannon H' index; $P > 0.05$) from the community found in greenhouse emergence trials (Faist *unpublished data*). Because I found no evidence that manual sorting improved my characterization of the seed bank community, I included only the data from greenhouse emergence trials in the analyses presented here.

4.3.4 Aboveground plant community

For the present study I used plant species composition data gathered annually (2003-

2010) from permanent plots, one plot per vernal pool, directly adjacent to where soil samples were extracted. For each aboveground plot (50 cm × 50 cm = 0.25 m²) I recorded frequency of each plant species during peak flowering (April) using a 100-cell sampling grid (5 cm × 5 cm per cell). I divided the aboveground data into two temporal subsets: early aboveground (2003-2006) and late aboveground (2007-2010). I chose the division of aboveground data between 2006 and 2007 to (1) create even aged community cohorts, (2) to account for an above-average precipitation spring (2005 – 2006) that significantly altered the vegetation community after 2006 (see Collinge and Ray 2009, Collinge et al 2011), and (3) to facilitate test of the paired relationship between aboveground community structure and soil seed bank community structure—*i.e.*, the rapid change in the aboveground community provided me a natural experiment to test for temporal links between the structure of above- and below-ground communities.

4.3.5 Data analysis

To assess whether my sampling intensity effectively captured species composition in my samples, I ran a species accumulation curve. Given the different scales of measure (frequencies versus counts) used for above- and below-ground data collection, I chose not to use sample rarefaction methods (Simberloff 1972). Instead, I standardized aboveground vegetation frequency data and seed bank count data to proportional abundances for all analyses and examined curves relating species accumulation to sampling effort (sometimes considered as ‘individual rarefaction curves’)—a method used to assess the effectiveness of sampling effort for describing ecological communities (Gotelli and Colwell 2001). I then estimated the sampling efficiency of the three community groups from the ending slope (*i.e.* the slope of the line

between the final two sample points) of each group's species accumulation curve.

I compared measures of alpha (α) diversity (species richness and Shannon H') via Kruskal-Wallis tests followed by Steel-Dwass nonmetric means comparisons in SAS-JMP 9.0.0 (JMP 2010). To avoid violating assumptions of sample independence, I analyzed beta (β) diversity (measured here as Bray-Curtis dissimilarities) using ADONIS followed by the permutation method of 'betadisper' in the Vegan package for the R platform (Oksanen et al. 2011; Team RDC 2011).

To visualize the community structure of the aboveground vegetation groups and the seed bank I used non-metric multidimensional scaling (NMDS) ordinations using Bray-Curtis distance and 50 runs with real data, followed by 50 runs with randomized data in PC-ORD (MJM software; McCune and Medford 2011). I compared the community structure of the three groups via PERMANOVA (a non-metric analysis that enables comparisons of species assemblages with an output similar to ANOVA) with 4999 randomizations in PC-ORD and with pairwise comparisons subjected to a Bonferroni sequential correction. To examine the relationships between the aboveground community sampled from 2007 - 2010 to the previous aboveground community from 2003 - 2006 and the seed bank community; as well as the relationship between the seed bank community and both aboveground communities, I used Mantel tests completed with PC-ORD. Mantel's r and P -values ($P = 1 + \#$ of runs with Mantel $Z \geq$ observed $Z / 1 + \#$ of randomized runs) generated by PC-ORD with 5000 randomized runs.

I compared the proportional abundance of invasive and native vegetation, as well as the proportional abundances of rare, intermediately abundant, and common species via χ^2 tests. I defined the rare category for the purpose of this study as species that represented $< 2\%$ of the aboveground community's (2003 - 2010) total abundance, intermediate species were those $\geq 2\%$

to $\leq 10\%$ of aboveground abundance, and common species were those making up $> 10\%$ of total aboveground abundance.

4.4 RESULTS

4.4.1 *Species accumulation and diversity*

I found high seedling abundance from soil seed bank samples; emergence trials indicated that seed banks had a mean of 21,750 seeds/m² of soil surface with a range from 1,600 to 121,200 seeds/m². Combining aboveground and seed bank communities, there were 68 plant species found among all sampled pools (Fig. 4.1). Landscape-level or gamma (γ) diversity in the early (2003 – 2006) aboveground community was 62 species (Fig. 4.2a), while γ -diversity in the late (2007 – 2010) aboveground community dropped to 48 species. Seed bank γ -diversity included 32 species. Species accumulation rates were similarly rapid across all three community groups (Fig. 4.3), with final slopes of the species accumulation curves indicating that overall sampling effort was more effective in the seed bank community than in the aboveground communities (Fig. 4.3). Specifically, finding an additional, unique species was estimated to require 10 additional samples (0.1 species per sample) in the seed bank community and 5 additional samples (0.2 species per sample) for both aboveground communities.

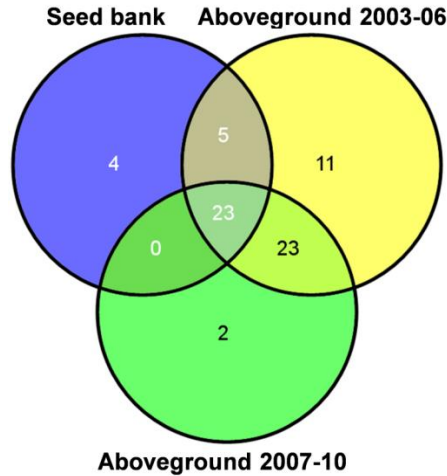


Figure 4.1. Plant species' occurrence within and among seed bank samples (blue circle), and aboveground vegetation community samples from 2003-2006 (yellow circle) and 2007-2010 (green circle). Overlapping areas represent shared species; non-overlapping areas represent species unique to a community.

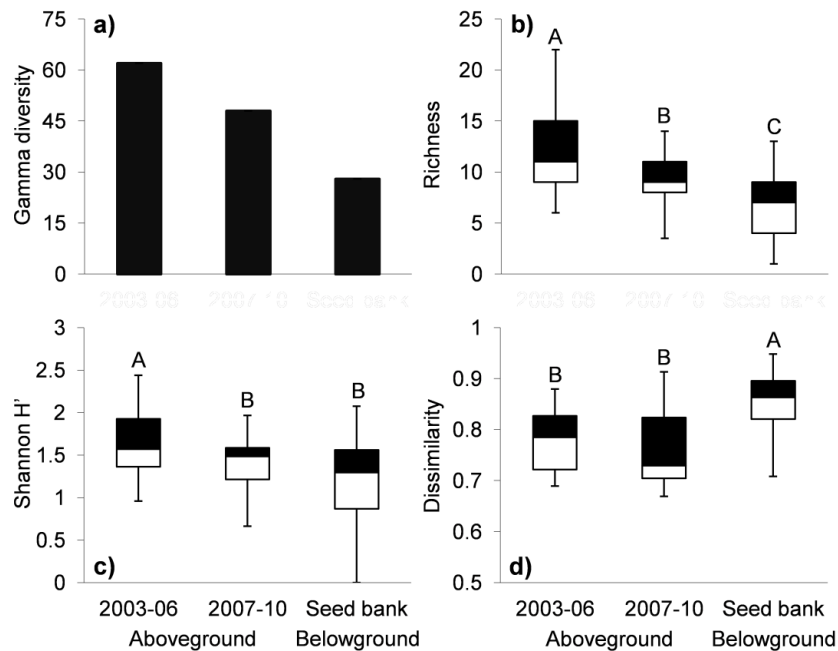


Figure 4.2. Above- and below-ground species diversity measures from early- (2003-06) and late- (2007-10) aboveground vegetation community, and from the soil seed bank; (a) gamma (landscape-level) diversity, (b) species richness, (c) Shannon-Weiner diversity index (H'), and (d) beta diversity measured as Bray-Curtis dissimilarity (a value of 1 indicates completely different communities, 0 indicates equivalent communities). Lettering above box and whisker plots represents significant differences ($P < 0.05$) from sequentially corrected pair-wise comparisons; error bars show $1.5 \times \text{IQR}$ (Inter-Quartile range), black shading indicates the third quartile, white indicates the first quartile, the dividing line the median.

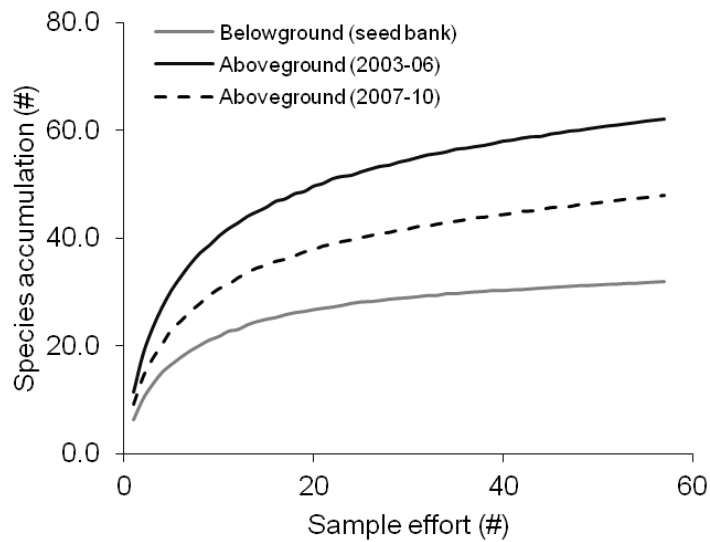


Figure 4.3. Species accumulation curves for early aboveground (2003-2006), late aboveground (2007-2010) and seed bank observations as a measure of sampling effort.

Sample-level or alpha (α) diversity measured both as species richness and Shannon diversity (H') was lower for the seed bank than for aboveground samples; mean seed bank species richness of $6.4 (\pm 0.32 \text{ SE})$ was significantly lower than that of aboveground vegetation in both the early and late sample periods (11.5 ± 0.54 , and 9.1 ± 0.38 respectively) ($P < 0.0001$; Fig. 2b). Shannon diversity (H') of the seed bank (1.2 ± 0.06) was significantly lower than H' in the early aboveground (1.6 ± 0.06) vegetation ($P = 0.002$; Fig. 2c), but was not significantly different than the late aboveground (1.4 ± 0.04). Beta (β) diversity (the amount of pair-wise dissimilarity for samples) was significantly greater ($P = 0.003$; Fig. 2d) in the seed bank (0.85 ± 0.008) than in the early- and late- aboveground community (0.78 ± 0.008 and 0.75 ± 0.009 , respectively), which did not significantly differ ($P > 0.05$).

4.4.2 Community structure and species abundance

Structure of the three community groups (seed bank, early and late aboveground) was

significantly different (PERMANOVA $F_{2,168} = 20.2$, $P < 0.001$; Fig. 4.4). The results of my mantel test showed a high similarity between the seed bank and the early aboveground observations (2003-2006, $r=0.22$, $P=0.0002$) yet a low similarity with its more temporally related late aboveground (2007-2010) observations ($r=0.11$, $P=0.010$) (Table 4.1). The early and late aboveground compared observations were the most closely related groups within these mantel test results ($r=0.38$, $P=0.0002$).

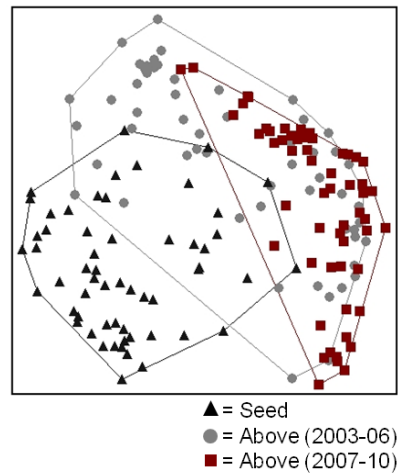


Figure 4.4 NMDS ordination comparing the within-sample, community composition of the seed bank (black triangles), the aboveground vegetation sampled from 2003 to 2006 (grey circles), and the aboveground vegetation sampled from 2007 to 2010 (red squares). NDMS axes = 4; final stress =14.9

Table 4.1. Mantel test for association between seed bank and aboveground vegetation communities.

Dependent matrix	Independent matrix	r	P
Seed bank	Aboveground (2003-06)	0.22	0.0002
Seed bank	Aboveground (2007-10)	0.11	0.010
Aboveground (2007-10)	Seed bank	0.11	0.009
Aboveground (2007-10)	Aboveground (2003-06)	0.38	0.0002

Mantel tests were completed on Bray-Curtis distance matrices; values of r and P are based on 5000 permutations.

When the community was separated into dominance categories, I parsed 55 out of the 68 species in the aboveground community into the rare category (species representing $\leq 2\%$ of total

abundance), while 8 species fell into the intermediate category ($> 2\%$ to $\leq 10\%$ of total abundance) and 2 species were considered common ($> 10\%$ of total abundance). Proportional abundances of rare, intermediate, and common species in the seed bank were significantly different from their abundances in both the early (2003-2006) and late (2007-2010) aboveground communities ($P < 0.00001$ for both comparisons; Fig. 4.5), while abundances for the two time periods of the aboveground community were not significantly different ($P > 0.05$). Combined rare species represented 21% and 14% of the total community abundance in the aboveground samples (early and late respectively), but represented a larger portion (54%) of the seed bank community (Table 2; Fig. 4.5). Intermediate species represented 48% and 41% of the early and late aboveground respectively, and 39% of the seed bank. Finally, the two dominant species made up 31% and 45% of the early and late aboveground abundance but only 8% of the seed bank.

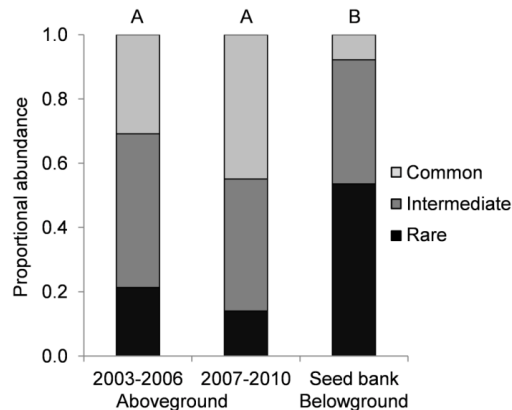


Figure 4.5. Proportion of the aboveground and seed bank vegetation community comprised of common, intermediate abundance, and rare species. Dominance categories were determined by combined 2002-2010 aboveground cover. Species with $< 2\%$ total cover were considered rare ($N = 55$ species), $> 2\%$ and $< 10\%$ intermediate ($N = 8$ species) and if a cover of $> 10\%$ was found the species was considered dominant ($N = 2$ species). Letters above bars indicate significance differences from a χ^2 test followed by Bonferroni sequential correction.

The contributions of individual species to the relative abundances of the common and intermediate species categories were highly variable among the early and late aboveground and

in the belowground seed bank communities (Table 4.2). In particular, the two most dominant species, *Eryngium vaseyi* (native and the only perennial in the system) and *Lolium multiflorum* (invasive annual grass) were found in very high frequencies in the aboveground communities, but their belowground abundance was substantially lower (Table 2).

Table 4.2. Percentage of the community comprised of native and introduced plant species categorized as rare (species combined), intermediate abundance, and common in aboveground and seed bank vernal pool vegetation.

Abundance category	Aboveground (2003-2006)	Aboveground (2007-2010)	Seed bank	Functional type*	Native (yes/no)
Rare (< 2% of total community; all combined)	21.34	13.98	53.58	na	na
Intermediate ($\geq 2\%$ to $\leq 10\%$)					
<i>Lythrum hyssopifolium</i>	3.12	1.06	28.6	Forb	no
<i>Deschampsia danthonioides</i>	4.68	1.7	0	Gram	yes
<i>Vicia villosa</i>	1.71	5.05	0	Forb	no
<i>Downingia concolor</i>	6.01	1.92	8.35	Forb	yes
<i>Plagiobothrys stipitatus</i> (v. <i>micranthus</i>)	9.06	2.19	0.16	Forb	yes
<i>Hordeum marinum</i>	4.15	12.01	0.35	Gram	no
<i>Lasthenia conjugens</i>	12.85	4.66	0.76	Forb	yes
<i>Bromus hordeaceus</i>	6.19	12.42	0.4	Gram	no
Dominant (> 10%)					
<i>Eryngium vaseyi</i> **	8.46	16.07	2.82	Forb	yes
<i>Lolium multiflorum</i>	22.41	28.86	4.97	Gram	no

*Forb or graminoid, **Perennial life form, all others annuals

4.4.3 Introduced and native species abundance

The early (2003-2006) and late (2007-2010) aboveground communities had significantly different proportions of invasive and native species with a marked decrease in native species from the early- to late-aboveground community ($P = 0.0002$; Table 2, Fig. 6). The proportions of invasive and native species in the seed bank community were most similar to the early-aboveground community, with no significant difference ($P > 0.05$) between the two groups;

while the proportions of invasive and native species in the seed bank community were significantly different than those of the late- aboveground community ($P = 0.0007$, Table 4.2, Fig. 4.6). Introduced species abundances represented 49% and 73% of the early and late aboveground community respectively, and 46% of the seed bank composition. Natives represented 51% and 27% of the overall abundances in early- and late- aboveground communities respectively, and 54% in the seed bank.

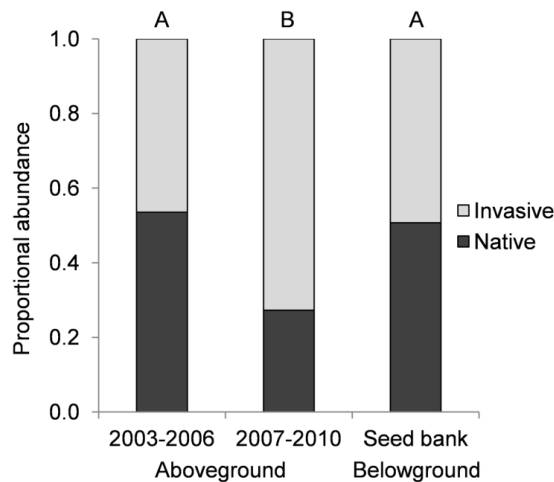


Figure 4.6. Proportion of the aboveground and seed bank vegetation community composed of native (black bar) and introduced (gray bar) species. Letters above bars indicate significant differences from a χ^2 test followed by Bonferroni sequential correction.

4.5 DISCUSSION

California's Central Valley, as with other Mediterranean climates, was historically the site of a vast network of seasonal, ephemeral wetlands (vernal pools) that have now largely been converted for agriculture and urban development. The remaining vernal pools harbor a large number of threatened native plants, thus there are active restoration efforts that address not only wetland habitat conservation and construction, but also the increasing levels of exotic plant invasions. In this above- versus belowground plant community study my findings revealed that

the aboveground abundances of rare and native plants in these restored vernal pools are not necessarily limited by their abundances in the belowground soil seed bank. Also, my results indicate that invasive plant encroachment in restored vernal pools has proceeded faster in the aboveground community, than in the belowground seed bank community. This pattern of invasion is likely linked to a temporal disconnect in species composition between aboveground vegetation and the soil seed bank; indeed I found that the community structure of the seed bank in these restored pools is more similar to aboveground plant community structure from five to eight years prior (2003 - 06) than to aboveground community structure from only one to four years prior (2007 -10). The higher proportional abundances of viable native and rare species in the soil seed bank than are currently found in aboveground vegetation suggests the potential for the community of these critical habitats to return to a more desired state—one replete with native plants and supportive of rare species—with changes in local environmental conditions.

4.5.1 Species accumulation and diversity

My results show that landscape level (gamma; γ) diversity in the aboveground community appears to be decreasing over time (Fig. 4.2) possibly due to changes in seed banks. Mean sample-level (alpha; α) diversity was lowest in the seed bank, while sample-level dissimilarity (beta diversity; β) was highest in the seed bank (Table 4.2; Fig. 4.2). Lower α -diversity and greater β -diversity in seed bank samples suggests high spatial variation and species turnover belowground, possibly due to dispersal limitation or priority effects, or a combination of both, which have been shown to influence patterns of aboveground plant community assembly in these vernal pools (Collinge and Ray 2009, Collinge et al. 2013). Yet it is important to note that similar to measures of landscape γ -diversity, the median β -diversity of the aboveground

community has decreased over time (though not yet significantly), suggesting a disparity between plants that germinate and emerge into the aboveground community and what is maintained in the seed bank. This pattern might be due to environmental filters (*i.e.* niche-based processes) strongly influencing the composition of aboveground communities in vernal pools, which could alter seed banks over time (e.g. Houle 1996, Benech-Arnold et al 2000).

Importantly, my sampling effort does not appear to significantly influence measures of species diversity and dissimilarity in either the seed bank, or the aboveground community characterizations. I found a similar, early rate of species accumulation in all three communities (Fig. 4.3), but with a decline toward an asymptotic value of species richness occurring more rapidly over the 57 independent samples in the seed bank than in the aboveground communities. This result is not surprising given the relatively low variation (*i.e.* standard errors of the mean values) in diversity measures across my study. Also, the low overall level of α -diversity in my study system, in conjunction with the high levels of β -diversity, indicate that a greater number of independent samples is likely preferable to an increase in each sample's size (either soil core volume, or surface coverage aboveground); a result previously reported for other ecosystems dominated by annual plants with high seed densities like mysystem (*e.g.*, Thompson 1986, Bigwood and Inouye 1988, Csontos 2007). Also, Bigwood and Inouye (1988) compared sampling methods for describing vegetation communities of ecosystems dominated by annual plants and characterized by high amounts of β -diversity over relatively small spatial scales, with their results leading them to recommend making seed bank samples smaller with a concomitant increase in sample number—an approach I attempted to employ.

4.5.2 Community structure and species abundance

The differences observed at the community level between the seed bank and the aboveground categories were marked. The mantel test (Table 4.2) illustrated that the seed bank similarity between the two aboveground groups (early and late) were not uniform. The higher seed bank similarity with the early aboveground data was initially surprising as there was a greater temporal lag between the two categories compared to the more recent 2007-10 aboveground-seed bank comparisons. This temporal lag is discussed further in the following paragraphs, but an initial explanation could be that the seed bank received an influx of persistent seeds during the early observation period (2003-2006) and as the aboveground community patterns changed –whether due to plant competition, environment or another variable- (Collinge and Ray 2009, Collinge et al. 2011, 2013) the seed bank has remained intact from the earlier years, causing a greater dissimilarity between the more recent aboveground monitoring periods.

Further examining this above- and belowground dissimilarity, the aboveground dominance categories (Fig. 4.5, Table 4.2) display striking differences when compared to the seed bank. I found that these seed banks contained an abundance of vegetation considered rare in aboveground communities and lower frequencies of species that are highly common aboveground. Possible explanations for this disparity could be that the common aboveground species may have different seed bank strategies than the rare aboveground species. For instance my sole perennial species (*E. vaseyi*) has the ability to return from established roots rather than relying on seeds to propagate. Also, some of the more common aboveground species may germinate nearly all of their seeds annually, depleting those species in the seed bank at the time of collection (Thompson et al 1995). Inversely, species whose recruitment conditions are not met (*i.e.* rare species) may not germinate and are then maintained in the seed bank until appropriate

conditions occur in the future (Leck and Simpson 1995). Other work in temporary ponds has found high seed densities and similar disparities between rare species abundance above- and below-ground (Aponte et al 2010). Together, these studies show that the definition of “rarity” when only pertaining to aboveground presence can be misleading or quite different than seed bank “rarity”. Regardless, my results would seem to indicate that aboveground rarity is not determined by limitations in the seed bank, and that storage effects might promote future aboveground communities with higher abundances of ‘rare’ species.

4.5.3 Introduced and native species abundance

In addition to my finding that the structure of the seed bank community more closely resembled the community structure of the early aboveground vegetation, I also found that the introduced-to-native species abundance ratios of the seed bank more closely resembled the early (2003-2006) aboveground community rather than the late (2007-2010, Fig. 4.6). Possible explanations for this pattern are that the native species have a higher longevity in the seed bank than the introduced species allowing them to “wait out” conditions that are not adequate for survival (Templeton and Levin 1979). Alternately, more natives in the seed bank might reflect limited germination rates of natives in response to competition with invasive annual plants—*i.e.* invasive plants may establish earlier in the season and out-compete natives which could deplete seed banks of invasives while maintaining natives (Tognetti and Chaneton 2012). Or lastly, because introduced species have only recently invaded these constructed vernal pools (Collinge et al. 2011), they may not have had sufficient time to build a substantial seed bank. In any case, introduced species have an ever-increasing presence in the aboveground community and now dominate each year’s standing vegetation, yet, they do not appear to be infiltrating the belowground community at similar rates and proportions.

4.6 CONCLUSIONS

My study revealed marked differences in aboveground vegetation and seed bank communities from restored vernal pools. I observed that the seed bank hosts a higher proportion of native and rare species, and has a community composition that more closely matches the composition of aboveground vegetation from five to eight years prior than the composition from only one to four years prior. This discrepancy offers support for a ‘storage effect’ where different communities can occupy the same spatial location by being active versus dormant at different times. In addition, the relatively low representation of invasive species in the seed bank despite high aboveground frequencies, suggests a legacy of native species maintaining populations in the seed bank. The seed bank composition, or *potential* of the community, indicates that this system could return to a native-dominated community with either an environmental shift, or alternative management actions. Although many studies regarding restoration and invasion conclude with a sombre story, my research shows that the seed banks of restored vernal pools have not been overwhelmed with invasive species and that native species may once again thrive in these communities. The return of natives may occur in response to subtle environmental changes such as deepening the pools to create longer inundation periods or other management efforts geared toward the promotion of conditions favoring native-plant life histories.

CHAPTER 5

IMPACTS OF MASTICATION, A FUEL REDUCTION TREATMENT, ON SOIL SEED BANKS IN THREE COLORADO (USA) CONIFER FOREST TYPES

5.1 ABSTRACT

Mastication is a relatively novel forest fuel reduction treatment that involves chipping or shredding unwanted woody biomass and depositing the material across the forest floor. By decreasing forest density mastication has been shown to lessen crown fire risk, yet other impacts of this treatment are only recently being studied. My study focuses on how mastication treatments alter the density and composition of soil seed banks. I implemented the study in three Colorado conifer forest types: lodgepole pine, ponderosa pine, and pinyon pine – juniper. Results across all forest types showed that treated sites tended to have higher seed bank densities than untreated sites yet not significantly so. The aboveground vegetation and seed bank plant functional groups were highly divergent from one another. While the seed bank was dominated by forbs regardless of forest type or treatment, forbs never dominated the aboveground vegetation. Although plant functional group mastication treatment effects were not observed the identified non-native species only occurred in the treated ponderosa sites suggesting a potential belowground invasion. These results give credence to the fact that when undertaking a mastication project it is important to look at what forest type is being treated to better understand what will allow the release of the seed bank through germination.

5.2 INTRODUCTION

Following decades of fire suppression and global change processes recent reports note an increase, or alteration, in forest wildfires across multiple ecosystems (Grissino-Mayer et al. 2004, Westerling et al. 2006, Stephens et al. 2009, Seidl et al. 2011, Stephens et al. 2012, Rocca et al.

2014). A change of reported fire risk and forest fuels density coupled with human encroachment into the wildland urban interface has prompted managers to implement fuels reduction treatments to reduce potential crown fire risk and threat to personal property (Stephens and Ruth 2005, Stephens et al. 2009, Knapp et al. 2012, Stephens et al. 2012, Ryan et al. 2013). One such fuels reduction treatment is mastication, or the chipping or shredding of woody biomass followed by broadcasting the woody material, commonly called mulch, across the treatment area. Mastication has recently become a favored fuels treatment across many forest types and is now widely used because it is cost-effective, easily implemented, and has been shown to modify fire behavior to reduce crown fire risk (Stephens and Ruth 2005, Vitorelo et al. 2009).

Because fuels are not removed from the site following mastication, the treatment results in greater woody biomass distributed into the forest floor (Reiner et al. 2009, Kreye et al. 2011, Knapp et al. 2012, Battaglia et al. 2010, Rhoades et al. 2012) which can in turn effect understory composition (Kane et al. 2009, Wolk and Rocca 2009). Furthermore, the heterogeneous deposition of masticated materials, or mulch, can lead to variable responses in understory plant cover (Kane et al. 2009, Wolk and Rocca 2009, Kane et al. 2010, Ross et al. 2012). At broad spatial scales, such as over an entire thinning project, it has been shown that mulching can increase diversity and plant cover due to an increase in light and maintenance of soil moisture (Wolk and Rocca 2009, Kane et al. 2010). However, when looking at the system at a finer spatial scale, the high heterogeneity of forest floor fuel depths can have a range of effects on the understory vegetation community (Collins et al. 2007, Wolk and Rocca 2009, Kane et al. 2010, Battaglia et al. 2010, Ross et al. 2012, Young et al. 2013, Redmond et al. 2014). However, defining a vegetation community solely on the active, aboveground species composition may be incomplete as other species may be waiting to emerge belowground following environmental or

structural shifts (Warner and Chesson 1985) such as a fuels thinning treatment. While the presence of masticated material can elicit a variety of responses from the aboveground vegetation community, we have yet to understand how the seed bank, and the potential future colonizers of a community, might be altered by this process.

Many plant species rely on seed banks, or the storage of propagules belowground, as a buffer to weather unfavorable conditions (Ooi 2012, Brock and Rogers 1998, Facelli et al 2005, Faist et al. 2013). An unfavorable condition is often classified as a disturbance creating a niche for the seed bank to colonize the aboveground vegetation immediately after the disturbance has occurred (Lavorel and Labretton 1992, Jutila and Grace 2002, Luzuriaga et al. 2005, Pakeman and Small 2005). Whether human induced or naturally caused, seed banking strategies are commonly correlated with types of disturbances (Thompson and Grime 1979), thus, eliciting highly variable seed bank release responses. However, ecological disturbances often relate directly to the removal of biomass, such as through a forest fire (e.g., Whelan 1995), or through redistribution and loss of soil layers (e.g., Belnap 1995). These types of disturbances regularly leave large areas of exposed soil that facilitates the release of the stored seed bank adapted to exploit gaps (Thompson and Grime 1979, Pakeman and Small 2005). In the case of mastication, the spreading of the chipped material over the forest floor provides a unique opportunity to understand seed bank dynamics and general treatment effects on the understory community with the addition of material.

Multiple potential seed bank responses could occur due to mastication. For instance, the treatment happens all at once and is comprised of chipped or chunked woody debris (Kane et al. 2010, Battaglia et al. 2010, Rhoades et al. 2012) without a gradual buildup of a surface litter layer, as often happens in forest floor buildup (Varner et al. 2005, Hiers et al. 2007, Banwell and

Varner 2014). The mastication layer may act as a slow-to-decompose complete barrier for all seeds that were in place prior to the mulch deposition or limit new seeds coming in. This barrier would then favor long lived seeds in the seed bank that are able to “wait out” the poor conditions and lower the instance of transient seed banking species over time (Thompson and Grime 1979, Bakker et al. 1996). Alternatively, as mastication is a thinning treatment it opens up the canopy allowing more light to enter the system (Battaglia et al. 2010) which has been shown to stimulate understory vegetation (Owen et al. 2009, Rhoades et al. 2012, Huffman et al. 2013, Ross et al. 2012, Redmond et al. 2014). More available light coupled with a mulch layer creating greater stability in soil moisture (Rhoades et al. 2012) and an increase plant available Nitrogen may enhance the seed bank through a vegetation surge under treated sites if seeds are able to penetrate the barrier. These responses are further complicated as the amount of basal area treated, and consequentially mastication depths, varies by forest stand structure (Reiner et al. 2009, Battaglia et al. 2010, Rhoades et al. 2012). Different conifer forest types may then undergo unique seed bank structural responses.

To explore how mastication affects seed bank density and composition, I initiated a study in three common Colorado conifer forest types – lodgepole pine (*Pinus contorta*), ponderosa pine (*Pinus ponderosa*) and pinyon pine – juniper (*Pinus edulis – Juniperus spp*). I asked two questions. (1) How does mastication impact seed bank densities, both in total and by plant functional groups (i.e., graminoid, forb, shrub, tree)? (2) How do the aboveground functional group responses caused by mastication match the seed bank shifts? I hypothesize that the presence of masticated material will diminish the number of seeds in the seed bank as it will provide a physical barrier hindering the transfer of seeds from above. Conversely the seeds maintained below this physical barrier are not released from the soil and are maintained in the

seed bank. This loss of seed transfer could potentially enhance species that rely on seed banks to persist such as forbs and hinder those that exhibit low seed bank longevity such as many trees and grasses.

5.3 METHODS

5.3.1 Study area and design

My study utilized nine Colorado sites originally established by Battaglia et al. (2010). Three sites were located in each of three conifer forest types: 1) *Pinus edulis* and *Juniperus sp.* or pinyon pine-juniper (hereafter referred to as pinyon-juniper), 2) *Pinus ponderosa* or ponderosa pine and 3) *Pinus contorta* or lodgepole pine. The warmest and driest forest I utilized was the pinyon-juniper type, that spanned an elevation of 1915 to 2170 m with an annual precipitation between 254 and 483 mm and average maximum and minimum temperatures 13-18° C and -6-2° C respectively. The ponderosa sites were designated as the mid elevation sites (2100 to 2360 m) and were generally dominated by ponderosa pine, but also often contained a high occurrence of Douglas-fir (*Pseudotsuga menziesii*). The ponderosa sites receive an annual precipitation between 406 and 560 mm and average maximum and minimum temperatures are 14-17° C and -2-2° C respectively. The highest elevation sites were in the subalpine with lodgepole pine as the dominant overstory species (elevation 2700 to 2818 m). These lodgepole sites receive an annual precipitation of 508 mm and average maximum and minimum temperatures 11 and -8 C respectively. All climate data were obtained through WRCC, 2009 (Battaglia et al. 2010).

Mastication treatments at the sites were conducted from 2004-2006 and treated using a HydroaxTM, masticator with a rotary axe mower and vertical shaft (Battaglia et al. 2010, Rhoades et al. 2012). Within each site I located three replicate 50 m transects in both masticated

(treated) locations and in nearby untreated reference areas (N=6 transects/site). Previous research on these sites has demonstrated that tree basal area and shrub cover was reduced 47-89% by mastication and that understory vegetation cover increased with mastication, particularly for grasses and forbs (Battaglia et al. 2010; Rhoades et al. 2012). Additionally, the three forest types experienced different mean mulch depths as their treated woody biomass varied. Lodgepole forests contained the highest median mulch depth (3.8 cm), while ponderosa had an intermediate mulch depth (3.3 cm) and the pinyon-juniper had the lowest observed depth at 1.4 cm (Battaglia et al. 2010).

5.3.2 Field sampling

In the summer of 2012, 6-9 years following treatments, I observed vegetation cover estimates according to plant functional group for three 1-m² quadrats along each of the pre-established transects (3 quadrats per transect * 6 transects per site* 3 sites per forest type * 3 forest types = 162 samples). I determined aboveground functional groups as: graminoid, forb, shrub and tree. Quadrats were located in areas that represented the average or above average mastication depths for each forest type (Battaglia et al. 2010) to test for the potential barrier a mastication layer may cause. To verify this I collected five forest floor depth measurements (litter, duff and/or masticated material) within these same vegetation 1-m² quadrants. The composition of masticated material contained more woody debris than control transects (Battaglia et al. 2010, Rhoades et al. 2012) and was often interspersed with the litter and duff deposited prior to mastication so I obtained an average forest floor depth for both treatments and controls.

To ensure I was only collecting what was directly in the soil seed bank I obtained soil samples at peak aboveground biomass. By collecting at peak growing season I had confidence a majority of seeds had germinated leaving only the stored propagules (i.e., the seed bank) belowground. I collected each soil seed bank sample in the center of the 1-m² quadrats after removing all material down to mineral soil to obtain only what was being stored in the soil seed bank, rather than seeds potentially deposited after the treatment ((3 per transect + 6 transects)* three sites per forest type *three forest types) = 162 samples). Using a soil corer I obtained 130.5-cm³ of soil at each sampling point to a depth of 5cm below the mineral soil surface. I maintained samples on ice while transporting from the field. Upon arrival at the lab I then stored the samples for 5 months of cold stratification in a ~2-4° C cold room which has been shown to break seed dormancy (Baskin and Baskin 2001).

5.3.3 Greenhouse methods

After I completed the cold stratification I sieved soil samples through a 2 mm soil sieve to remove rocks and large debris and to break up soil clumps and carefully sorted through all material greater than 2 mm for any potential seeds. Because I only observed one seed that did not pass through the sieve I am confident that we captured all seed sizes present. Due to the highly variable amount of debris and rocks within the original sample I sifted samples to allow for 100-cm³ of mineral soil noting the volume if a sample contained less than this desired amount. This allowed me to standardize the number of seeds in a set volume of sifted mineral soil at the sample level. I then scaled up all samples (100 cm³) to report number of seeds found in mineral soil to a depth of 5cm within a 1 m quadrat (reported as seeds/m²) as has been done in previous studies (Jurand 2012, Brooks et al. 2013).

To determine viable seeds in the soil seed bank we conducted a greenhouse emergence trial at the University of Colorado 30th Street Greenhouse, Boulder, CO, USA. I randomized the sieved soil seed bank samples on the greenhouse benches. I ensured that the soil samples were evenly spread (~0.5 cm depth) over 500 ml of sand in well-draining pots. I placed four additional control pots alongside the samples to observe if seed dispersal from nearby greenhouse plants was potentially contaminating our samples. I observed one contaminant species from the greenhouse (*Fatoua* sp. (Moraceae)) and removed this species from the data. I watered soil samples daily with our initial germinant observations also conducted daily then as germination began to decrease we switched to every three days, then to weekly observations. I counted, recorded and gave an unknown name to all new morphologically distinct germinants as they emerged. Finally, when I had two weeks with no new germination we terminated the trials (120 days). For identification purposes I left all unknown germinants to develop for an additional 60 days after monitoring had ceased. In an effort to ensure I depleted the seed bank after germination I manually sorted a subset of the soil samples (N=36) under 10x magnification. I did not find any seeds left over in the soil samples after germination. At the termination of the experiment unknown germinants were pressed and dried.

5.3.4 Molecular methods

Because a majority of the unknown specimens did not have identifying morphological characteristics I implemented a DNA sequencing approach to identify or confirm identification of all unknown germinants. I removed approximately 1 cm² of leaf tissue from the specimen and pulverized using a Spex Geno/Grinder in the molecular laboratory of E. Tripp (University of Colorado, Boulder). From these samples, I extracted DNA following a modified CTAB protocol

(Doyle and Doyle 1987). For each sample, I sequenced one of three molecular markers that are among the fastest evolving Sanger loci in plants and that have excellent reference databases represented in GenBank, for the purposes of later identification: nuclear ribosomal ITS+5.8S, the chloroplast *psbA-trnH* spacer region, and the chloroplast *trnL-trnF* spacer. DNA was amplified via PCR using Qiagen kits, with an annealing temperature between 54-60 degrees C. Resultant products were purified enzymatically and sequenced unidirectionally by QuintaraBio Company (Albany, California). To identify sequences to taxonomic group, between 200-250 bases of the resulting sequences were run through the National Center for Biotechnology Information's n-BLAST algorithm. I only considered the top 10 BLAST hits as well as hits that were $\geq 90\%$ sequence similarity. In general, hits $\geq 95\%$ sequence similarity BLASTED the sequence to genus whereas hits between 90-94% sequenced similarity were resolvable only to plant family. Following molecular identification, I re-examined specimens morphologically to confirm the sensibility of molecular IDs. Our sequencing methods readily confirmed the identification of a majority of my unknown germinants to either family or genus. In several cases, based on the known above-ground flora, I was able to further identify unknown germinants to species. I used the USDA Plants Database (www.plants.usda.gov) to confirm the most current nomenclature, native status and growth habits of specimens.

5.3.5 Statistical analyses

To better understand variations among my explanatory variables (forest type, mastication treatment and site location) I obtained the mean number of seeds/m² at the transect level for all analyses. I then tested differences between plant functional group seed densities (grass, forb, shrub, tree) using a non-parametric Kruskal-Wallis test using R software (RDC, 2014, Vienna

Austria). After finding a difference between functional group densities I analyzed each functional group (grass, forb, shrub) separately using the GLMMIX procedure (SAS Institute Inc., Cary, North Carolina, USA). The MODEL statement combined with the DISTRIBUTION option was defined for each attribute. Before analyses were conducted I rescaled the data according to Stahel (2002) to accommodate 0 values. After functional groups were analyzed I modeled the total seed densities following the same GLMMIX procedure as with the functional groups.

To compare aboveground vegetation cover and seed bank densities functional groups were divided for both aboveground and seed banks data into proportions (e.g., proportion of both aboveground vegetation cover and seed bank density comprised of shrubs). Through a series of Kruskal-Wallis tests I checked to see if plant vegetation cover varied by treatment, forest type or site location. My average forest floor depths were also compared for differences in these three explanatory variables using a Kruskal Wallis test. I then ran a chi squared test to look for differences in proportions of functional groups. All significance tests were based off of an alpha of 0.05 and a standard error of ± 1 .

5.4 RESULTS

5.4.1 Seed banks

My seed bank results yielded 12 known plant families across all samples yet no single family, genus or species, dominated the seed bank. For instance, while Brassicaceae contained the highest seed densities of any family these results were driven by two annual forb species; *Draba cuneifolia* and *Descurannia pinnata* which consisted of 67% and 20% of the seeds observed in Brassicaceae. Most of the taxonomic clarity was to family or genus (Table 5.1) yet a

prominent identified annual/perennial forb species observed across multiple locations in all three forest types was *Androsace septentrionalis* (Primulaceae). I also found *Campanula rotundifolia* (Campanulaceae), and *Heuchera parvifolia* (Saxifragaceae), both perennial species, mostly occurring in ponderosa forests but also located in lodgepole forests. My only identified non-native forb species were *Verbascum thapsus* (Scrophulariaceae), *Linnaria vulgaris* (Plantaginaceae), and *Carduus nutans* (Asteraceae). I only observed these non-natives in the treated ponderosa sites and aside from *V. thapsus* they occurred in relatively low numbers (Table 5.1). I identified a single invasive grass species (*Bromus tectorum*, Poaceae) in a treated ponderosa site, yet with one observed individual of this species a relatively low seed banking capability was observed. A low occurrence of graminoids was a general trend throughout the seed bank. Although two genera did contain noticeably more seeds than the other graminoids with *Carex sp.* as the most common graminoid found in all forest types regardless of treatment and *Agrostis sp.* as the most common identified grass (Poaceae) species (Table 5.1). I did not find any shrub seeds in the pinyon-juniper forest types and only very sparsely spread out shrub seeds in the ponderosa forests. The shrub Genera/Species I observed in low numbers were *Rubus sp.* and *Ribes sp.* and the dominant shrub species found was *Jamesia Americana* (Hydrangeaceae) and this occurred at similar numbers in a lodgepole site regardless of treatment.

I then assigned the seed bank individuals into their associate functional groups (grass, forb, shrub and tree) to see if the treatments and forest types helped facilitate seeds in one functional type over the other. Overall the densities of seeds in each functional group significantly differed from one another (Kruskal Wallis, chi-squared = 108.478, $p < 0.00001$) with forbs making up the majority of the seed bank (mean 977 ± 183 seeds / m^2) and grasses and shrubs with nearly 7 times lesser seeds (127 ± 28 and 150 ± 91 seeds / m^2 respectively). The

Graminoids were the only functional group that displayed a higher density of seeds in the treated sites than the untreated (GLMM, $p = 0.04$) where all other functional groups did not differ across treated and untreated. The functional group that differed by forest type was the forb group (GLMM, $p = 0.0006$) in which there were less forb seeds found in the lodgepole forests than the ponderosa.

Grouping the seeds further and looking at total seeds/m² by mastication treatment we observed that the treated sites contained nearly 400 seed/m² more than the untreated sites (1435 ± 333 and 1076 ± 219 seeds / m² respectively) yet these results were not significantly different ($p > 0.05$). The ponderosa forests containing the highest densities (GLMM, $p = 0.01$) of the three forest types (1793 ± 410 seeds / m²) and the pinyon-juniper and lodgepole much lower densities (Fig. 5.1; 1260 ± 317 and 733 ± 264 respectively).

Table 5.1. Raw counts of seeds segregated by family and genus (identified to species if positive identification was available). Each family has the total number of seeds observed including individuals that were identified further. *Denotes identified non-native species.

Family	Genus/Species	Seeds
Alsiniaceae		5
Asteraceae:		21
	<i>Carduus nutans</i> *	1
	<i>Cirsium</i>	4
	<i>Erigeron</i>	14
	<i>Gnaphalium/</i>	
	<i>Pseudognaphalium</i>	1
	<i>Symphytotrichum</i>	1
Boraginaceae	<i>Mertensia</i>	1
Brassicaceae:		75
	<i>Alyssum</i>	1
	<i>Boechera</i>	9
	<i>Descurainia pinnata</i>	15
	<i>Draba cuneifolia</i>	50
Campanulaceae	<i>Campanula rotundifolia</i>	17
Caryophyllaceae	<i>Silene</i>	2
Cyperaceae	<i>Carex</i>	16
Fumariaceae	<i>Corydalis</i>	1
Grossulariaceae	<i>Ribes</i>	1
Hydrangeaceae	<i>Jamesia americana</i>	41
Juncaceae	<i>Juncus</i>	1
Plantaginaceae:		28
	<i>Linaria vulgaris</i> *	3
	<i>Penstemon</i>	4
Poaceae:		24
	<i>Agrostis</i>	15
	<i>Bromus tectorum</i> *	1
	<i>Elymus</i>	1
	<i>Poa</i>	7
Primulaceae:	<i>Androsace septentrionalis</i>	43
Rhamnaceae:		5
Rosaceae:		18
	<i>Potentilla</i>	15
	<i>Rubis</i>	3
Saxafragaceae	<i>Heuchera parvifolia</i>	18
Scrophulariaceae	<i>Verbascum thapsus</i> *	29
Unknown Forbs		22
Unknown Gramioids		14

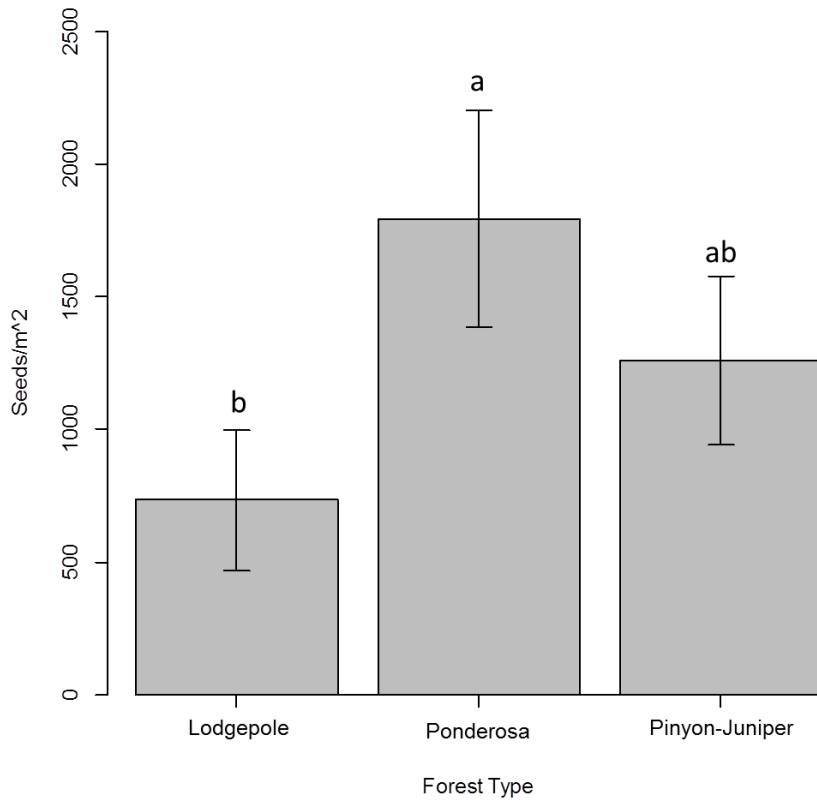


Figure 5.1. Seed densities/m² by forest type and treatment. Letters indicates significant differences between pairwise comparisons with the standard error at ± 1 .

5.4.2 Aboveground vegetation and mulch depths

The overall percent aboveground vegetation cover, including tree cover, did not vary between treated and control sites with the mean percent for treated and control sites both at 16% and 18% respectively. However, the vegetation cover did vary by forest type (Kruskal Wallis, chi-squared=14.16, p=0.00008) with the pinyon-juniper sites having the highest overall vegetation cover ($28 \pm 6\%$ treated and $27 \pm 6\%$ untreated) the ponderosa sites containing the next highest cover percentages ($15 \pm 3\%$ for treated and $21 \pm 4\%$ for untreated) and the lodgepole displaying the lowest percent cover ($7 \pm 2\%$ for treated and $8 \pm 3\%$ for untreated). With no observable difference in treatment and multiple interaction effects determining overall vegetation

cover in I then divide cover into functional groups. An overall comparison of the two vegetation assemblages showed a significant difference between the aboveground and seed bank functional groups (chi squared= 76.162, df=3, p<0.00001). This difference is most apparent in how the proportion of aboveground vegetation was relatively evenly dispersed when contrasted with the seed bank functional group proportions (Fig. 5.2). Finally, across all forest types the aboveground vegetation composition was significantly different in its functional group proportions than its seed bank counterpart (Fig. 5.2).

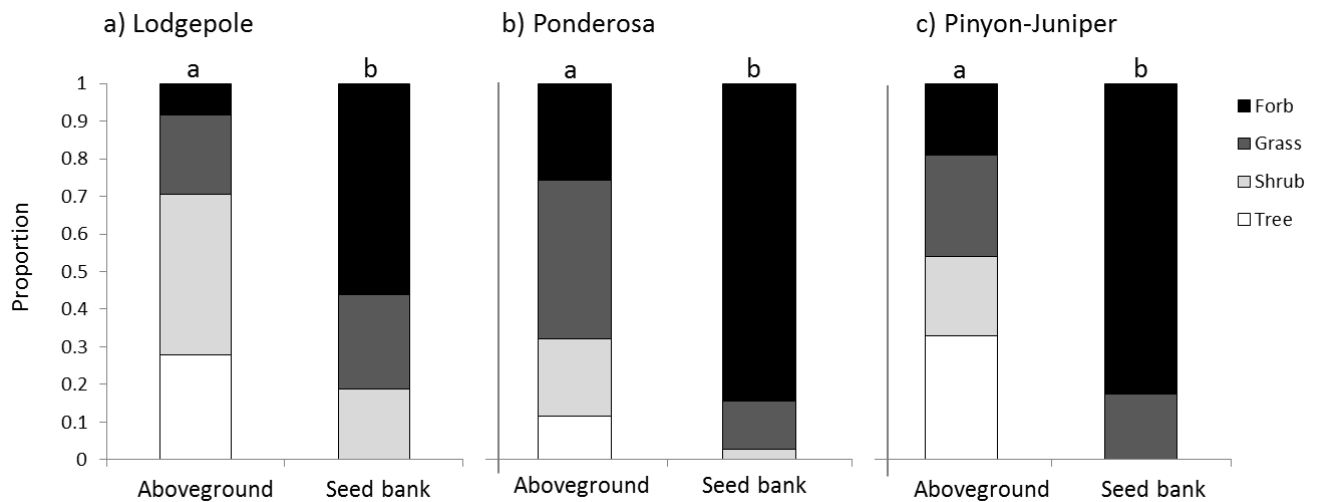


Figure 5.2. Plant functional groups of the aboveground vegetation and seed banks by forest type. Letters indicate significant differences (p<0.05) between the aboveground and seed bank through a chi squared test.

Because I actively sought out equal to or higher average masticated depth treatments (by forest type) for my seed bank collections and then took the average forest floor depths (litter through 100hr fuels) I did not observe a significant difference between treated and untreated average forest floor depths (Kruskal Wallis p>0.05). However, as to be expected the forest types

did differ significantly in their average forest floor depths (Kruskal Wallis, chi-squared=31.6, $p=0.001$ and chi squared=23.6, $p<0.00001$).

5.5 DISCUSSION

Through morphological and genetic analyses a diverse number of families, genera and species were observed in the seed banks for the different forest types and across treatments. However when broken down into functional types the only functional type that showed a treatment effect was the graminoids with a higher seed density in the treated areas than the untreated. While not differing by treatments the forb functional group did have a higher density of seeds in the ponderosa forest type than the lodgepole and marginally more than the pinyon-juniper types. When observing total seed densities the ponderosa forest type had the higher density of seeds compared to the lodgepole type and following a similar trajectory to the forb functional group only marginally higher densities than the pinyon-juniper forest types. The functional group proportions of the aboveground and seed bank comparisons were highly divergent. While the aboveground had a more even representation of the plant functional groups the seed bank was strongly skewed towards a higher density of forbs over any other functional group.

5.5.1 Seed banks

Because seed banks often are called upon after a disturbance to quickly colonize an area (Bakker et al. 1996) my results showing annual and annual/biannual forbs as common seed bankers agrees with current seed bank literature (Wienk et al. 2004, Korb et al. 2005). The pinyon-juniper annuals (*D. cuneifolia* and *D. pinnata*) were clearly the driving species in

Brassicaceae. As disturbance colonizing species, their higher abundance in the seed bank is as expected. However, while not very high in numbers, the identified non-native species were solely found was in the treated ponderosa sites. As thinning projects in ponderosa forests have been shown to invite invasives (e.g., Miller and Seastedt 2009) and it is interesting to note that this invasion is also transferred belowground to the seed bank. Additionally, the observation of Carices (*Carex* sp.) occurring in high numbers in the seed bank has been demonstrated across the genus regardless of species (Shutz 2000) and in this study was not altered by a mastication treatment.

The only functional group that showed a difference across the treated and untreated sites was the graminoids. While highly species dependent, grass seeds are generally thought to be less persistent in the seed bank as forbs and shrubs (Faist et al. 2013, Thompson et al. 1993). This discrepancy between seed persistence and functional groups may elicit a treatment effect in grasses that isn't being swamped out by the existence of seeds that were present and viable prior to the treatments as could be the case with many highly persistent forb seeds (Thompson et al. 1993). Alternatively, grasses often colonize forest openings and fuel thinning treatments are design to open up the canopy allowing more light. This increase of grasses, whether invasive or native, in fuel treatments has been documented in a variety of thinning treatments (e.g., Griffis et al. 2001, Rhoades et al. 2012).

When looking at forb densities across forest types the observed higher densities of forbs in the ponderosa seed banks compared to that of the lodgepole forest type could be related to the differences in adaptation to disturbance. The high elevation densely packed lodgepole pine forests often contain high litter depths and a low understory cover (Battaglia et al. Rhoades et al. 2012) and their fire return interval is much lower than the ponderosa forests that are better

adapted for more frequent disturbances ((Veblen et al. 2000, Schoennagel et al. 2003, Schoennagel et al. 2004). This difference in disturbance regimes could potentially be responsible for skewing the density of forbs in favor of the ponderosa forests. Additionally, the observed difference in overall seed densities varying by forest types are likely driven by the forb densities as they are dominating the seed bank. Even with the highly variable densities observed across the forest types this study displayed a moderate seed density compared to seed bank studies conducted in similar ecosystems (Halpern et al. 1999, Wienk et al. 2004, Zobel et al. 2007, Abella and Springer 2012).

5.5.2 Aboveground vegetation and mulch depths

While the plant functional group proportions varied greatly across the aboveground vegetation and seed banks, neither category demonstrated a difference according to the mastication treatment. Previous studies in these systems have found that higher vegetation cover, especially in grasses and forbs, occurred in the masticated sites yet this relationship varied by mulch depths (Rhoades et al. 2012). A sampling effect could describe why the aboveground vegetation did not differ between treated and untreated sites. Because I chose the average, or higher than average, mulch depths to test for mulch as a potential seed barrier I did not observe a difference in forest floor depths across the treated and control sites. This lack of difference between forest floor depths could in turn not allow distinct differences in the aboveground vegetation. Regardless, my study finding a lack of differences across treated sites, other than in the graminoid seed densities that are discussed above, demonstrated that the seed bank may have a high resilience to mastication over time. Originally hypothesized that the presence of the mastication layer will not allow the transfer of seeds appears to not be the case. While the

mastication treatment may create an open canopy, sufficient soil moistures and higher plant available N increasing vegetation cover (Rhoades et al. 2012), the thick forest floor depths did not appear to be creating a seed barrier for either the deposition or germination of seeds, even with the higher mulch depths preferentially chosen. The limited differences observed are useful to land managers as it can be surmised that regardless of forest types or treatment the impacts of mastication on the seed bank under the mastication layer is minimal.

CHAPTER 6 CONCLUSIONS

Long term restoration projects offer a unique perspective on what facilitates restoration success over time. It is through long term restoration projects my dissertation sought to understand the persistence of alternative states caused by invasive species, how the seed bank varies both spatially and temporally within a restoration framework and how seed banks be used to explain environmental filters. I also sought to understand seed bank dynamics in multiple systems.

For my first research chapter (chapter 2) I observed the maintenance of dramatically different invasion levels regardless of restoration status. This research found that the invaded pools generally contained lower inundation depths and shorter inundation durations than the native dominated pools. However, once established the invasive species caused positive feedbacks that benefitted them and greatly hindered the native species. These positive feedbacks kept the invasive species in a more stable state than the highly tenuous native species which could be pushed to an invasive state with only a small nudge (e.g., poor rainfall year (Collinge et al. 2011, 2013)). Unfortunately, this invasive caused positive feedback and resulting high native species vulnerability creates a difficulty when attempting to remove the invasives through restoration. I have however, unraveled the mechanisms that caused the invasive positive feedbacks. From my research results, if invasion is occurring I would recommend making the pools deeper and maintaining a management strategy to minimize a litter layer (e.g., Grazing (Marty 2005) or annual burning (Gerhardt and Collinge 2007)).

Interestingly, while the aboveground vegetation appears to be in highly divergent alternative states the soil seed bank is not following the same pattern. My research observed a

very high occurrence of native species found in the seed bank and a generally low abundance of invasive species. While low in numbers the invasive dominated aboveground vegetation in naturally occurring pools did maintain an overall higher abundance of invasive seeds than the native dominated aboveground vegetation in naturally occurring pools. The restored pools contained the lowest overall seed densities, yet although they did have a high instance of invasive species in their aboveground vegetation this was not found belowground. A strong environmental filter (such as inundation or invasive litter layer) may be preserving the native seeds belowground until the proper conditions are met. This research suggests that with a high abundance of native seeds found in the seed bank a soil inoculum could be highly effective for new restoration projects. Additionally, it might be most beneficial to take an inoculum from a previously restored pool bottom as the pool bottoms are where the highest density of seeds are stored and the restored pools have the highest proportion of native seeds.

My fourth chapter compared the current seed bank to its historical and more recent aboveground vegetation compositions. My results displayed a strong storage effect of seeds in the seed bank that were highly disparate from its aboveground vegetation. Here the seed bank community more closely mimicked historical vegetation (5-8 years prior) as opposed to the more recent aboveground vegetation (1-3 years prior). This illustrated not only a legacy effect of the native species being maintained over time, but that the aboveground vegetation and belowground seed bank can occur within a similar spatial location under different temporal conditions.

Finally, tying my previous seed bank chapters together (chapters 3 and 4) my conifer seed bank study was able to utilize a long term forest thinning restoration project called mastication. Through mastication this artificially deposited layer of woody biomass had the strong potential to decrease seed bank density as it could essentially halt the transfer of seeds to

and from the soil. However, my findings did not show a decrease but rather a generally higher density of seeds in the treated sites as opposed to untreated. The density of seeds was strongly determined by the forest type the mastication occurred in and overshadowed the overall effects of treatments. As found in my vernal pool system, and in other seed bank studies in different systems (Hopfensberger 2007), the aboveground vegetation did not match its belowground seed bank composition. Upon looking at the invasive species present in the seed bank, while their numbers were not extraordinarily high, the only identified invasive species were found in the treated ponderosa sites. Thinning treatments have been shown to invite invasive species (Miller and Seastedt 2009, Korb et al. 2005) and it appears that this invasion may also be transferred to the seed bank as well.

In summary, my research compliments the restoration ecology literature in that it provides empirical tests of alternative state and storage effect theoretical frameworks, as well as environmental filter dynamics. Future work in these and other systems on these topics could benefit from continuing the long term work observing how the relationships last over an even greater time span. For instance, if our vernal pool seed bank study is conducted over the next 5 and 10 years comparing it to its historical vegetation (as in chapter 4) the persistence of the native seeds in the seed bank can be tested since we know they are being maintained under the litter layer. This would also allow for a greater understanding of how the native species track behind aboveground in their storage capabilities. Another necessary study is to test how the litter layer coupled with the deepening the pools can help the native species over a longer time period. My research looked at the effect over a single growing season, and a factorial design looking at the compounded effects of litter removal and greater inundation could help aid vernal pool restoration efforts.

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