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**ENVIRONMENTAL CONTINGENCY OF SEED-FUNGI INTERACTIONS IN COEXISTING INVASIVE  
PURPLE LOOSESTRIFE AND NATIVE CATTAIL**

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## ABSTRACT

Purple loosestrife (*Lythrum salicaria*) is a highly invasive species able to quickly take over entire wetlands, especially after disturbances. Bountiful seed production and a persistent and prolific seed bank play a key role in loosestrife's ability to invade. However, some competing native species, such as cattails (*Typha spp.*) have comparable seed production rates but less abundant seed banks, suggesting that there may be a difference in belowground seed survival. I investigated the abundance of loosestrife and cattail seeds in soils at roadside sites relative to above-ground stem densities. Given the importance of fungal pathogens to seed viability, I asked whether soil fungi differentially affect seed germination rates of purple loosestrife and cattail species under a variety of soil moisture conditions (dry, well-watered, and saturated). I also examined the proportion of seeds with microbial infections. I found that purple loosestrife is ~20 times more abundant in the soil than cattail in sites with varying aboveground dominance. Fungicide provided a protective effect (i.e. yielded more germinants) for both purple loosestrife and cattail in moist soils, but benefitted only cattails in saturated soils. When I examined the microbes that infected seeds, I found a diverse array of fungi and bacteria, which may explain some of the trends in the fungal/seed bank interactions. Overall, this study indicates that fungal interactions with the seed bank vary between species and are contingent on soil moisture. The results are consistent with the idea that under some environmental conditions, soil-fungi may influence competitive outcomes between invasive loosestrife and native cattails.

## INTRODUCTION

Purple loosestrife (*Lythrum salicaria*), an invasive species introduced from Eurasia, is currently threatening wetlands across the United States and southern Canada. Though it was introduced in the 1800's on the eastern coast of North America, loosestrife only began problematically invading wetlands in the 1930's, forming dense monospecific stands which alter wetland ecology (Thompson et al. 1987). It is now present in all states except Alaska, Florida, Louisiana, and South Carolina (Natural Resources Conservation Service 2014).

Loosestrife's vast and rapid spread has been aided by its capacity to quickly adapt to local environments (Coluatti & Barrett 2013), its increased competitive ability in North America relative to its native range (Blossey & Notzold 1995, Joshi et al. 2014) and its ability to form persistent seed banks (Mullin 1998). Though much is known about the dispersal and establishment of loosestrife, fewer studies examine the specific ecology of seed bank persistence in comparison with competing species. For this reason, this study focuses on comparing the influence of soil fungi on seed germination in three different soil moisture environments, and between two species: purple loosestrife and cattail (*Typha spp.*).

Cattails are the most common associate of purple loosestrife, and are frequently displaced upon invasion (Thompson et al. 1987). Once purple loosestrife establishes in a broad-leaved cattail (*Typha latifolia*) stand, it consistently outcompetes the cattail (Weihe & Neely 1997). The replacement of cattails with purple loosestrife can dramatically alter the ecology of a wetland. For example, it reduces food sources for muskrats and certain bird species (Rawinski 1982),

alters the composition of wetland bird species (Tavernia & Reed 2012), and reduces success of plant colonizers and plant diversity (Hovick et al. 2011).

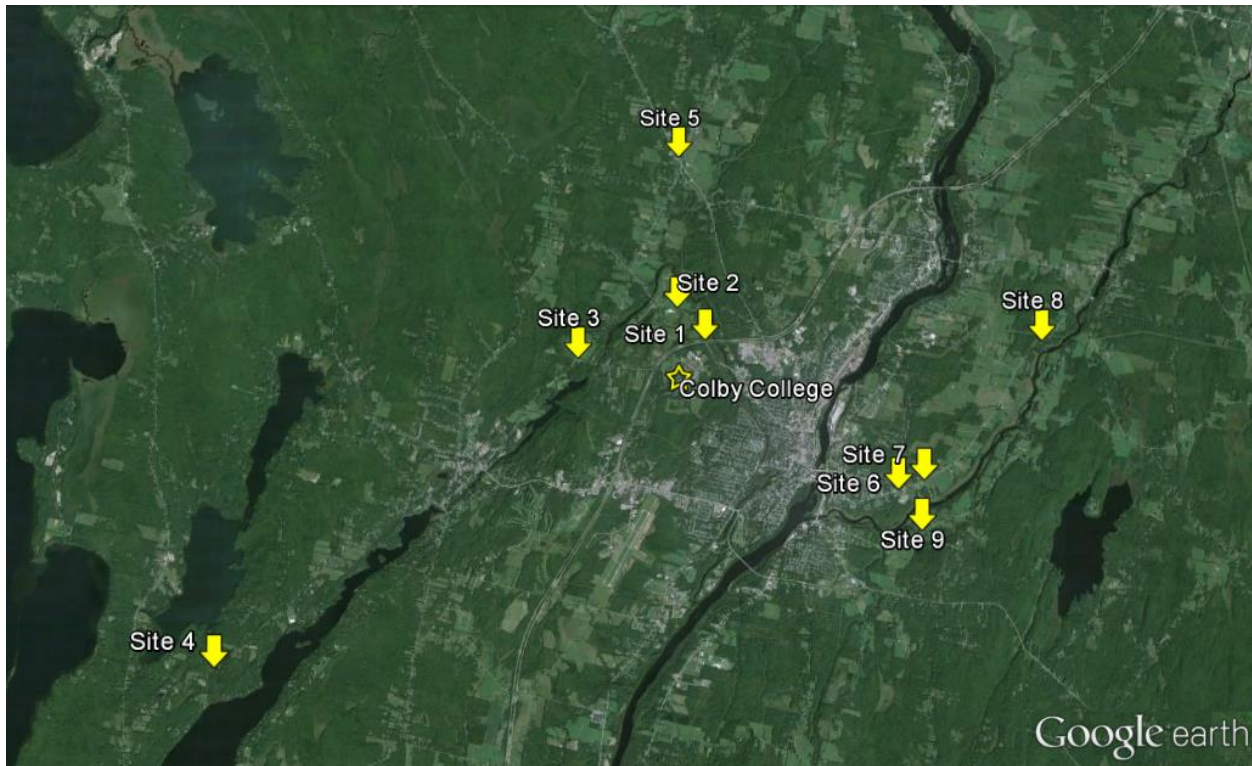
The dramatic shift from cattail to loosestrife is partially caused by the establishment of a dominant purple loosestrife seedbank (Mullin 1998, Gioria et al. 2012, Thompson & Moloney, 2013). In areas where cattail and loosestrife coexist, loosestrife is much more dominant in the seed bank (Welling & Becker 1993). Broad-leaved cattail seed bank densities have been found to be <1,000 seeds/m<sup>2</sup> in areas with >90% cattail coverage (Tu et al. 1998), whereas purple loosestrife seeds densities have been found to be 10-20 times greater (Rawinski 1982). The difference in seed bank density may be due to the abundant seed production of purple loosestrife, ~100,000 seeds per flowering stalk (Shamsi & Whitehead 1974). However, cattails are also known for abundant seed production, with broad leaved cattails yielding ~222,000 seeds per flower stalk (Yeo et al 1964). The discrepancy in seed bank abundance may therefore be partially due to seed bank survival.

Differential seed bank survival of cattail and purple loosestrife may partially be due to differences in susceptibility to fungal pathogens. Soil fungal pathogens are known to reduce the seed viability of many species (Wagner & Mitschunas 2008) and play an influential role in determining biodiversity in some regions (Augsburger 1984). It is possible that, following the predator escape hypothesis (Elton 1958, Crawley 1986), seeds of purple loosestrife are less susceptible to fungal attack than coexisting native species, having not coevolved with the soil fungi.

Previous studies have found that there is not a general trend of invasive species being subject to less fungal attack than native cogenetic pairs (Blaney 2001, Blaney 2002). However, a study comparing selected invasive species and rare natives did find that invasive plants interact differently with soil fungal pathogens (Kilronomos 2002). The comparison of these studies indicates that specific invasive species may gain an advantage over coexisting natives by escaping soil fungal pathogens of their native range. Given the differential seedbank survival rates of purple loosestrife and cattail, as well as loosestrife's known chemical defense to biotic decomposition (Hendry et al 1994), I hypothesized that the invasive species may be less susceptible to native fungal pathogens than its primary native competitor.

The effects of fungal pathogens are contingent on soil moisture (Wagner & Mitschunas 2008). Higher soil moisture generally increases fungal pathogen effects, whereas dryer soils reduce fungal pathogen growth (Moredecai 2012, Schafer & Kotanen 2003). However, with the saturated conditions found in many wetlands, fungal effects may be decreased due to anoxic conditions (Griffin 1972).

In order to more fully understand how the seed bank ecology of cattails and purple loosestrife differ, I conducted a study based on nine roadside sites in Central Maine where cattail and purple loosestrife co-occur. To better understand the existing seed bank dynamics, I conducted an observational study in which I surveyed aboveground and belowground densities of both species at each site. I also conducted an experimental study in which I examined how soil moisture differentially affects seed bank/fungal interactions of cattail and loosestrife by



**Figure 1:** Map of study sites: All study sites were within an 8km radius of Colby College (43°33'50"N, 69 ° 39'49"W) and contained either roadside drainage ditches or culverts.

conducting a greenhouse experiment burying local seeds in field soil for 12-13 weeks exposed to varying moisture levels and treating half with fungicide. Finally, I grew the bacterial and fungal infectors out of a subset of the buried seeds after 5 weeks of burial to determine the percentage of seeds infected with bacteria and fungi.

## METHODS

### *Research approach*

To better understand the differences in seed bank dynamics of purple loosestrife and cattail, I conducted two complementary studies: one observational study and one experimental study. The observational study aimed to determine what the existing seed bank dynamics were in the study site and the experimental study sought to better understand what may be causing the trends I observed. Though different samples and data were collected, both studies used the same field sites.

### *Study sites*

I chose to focus the study on roadside sites. This area of loosestrife habitat is particularly crucial and suitable for this study for several reasons. First, loosestrife uses highways as dispersal corridors (Wilcox 1989), so its establishment and dominance over cattail in these regions can lead to an increase in dispersal and invasion to new wetlands. Second, roadsides, particularly roadside ditches, are areas known for high disturbance as they are frequently cleared of

vegetation and sediment to allow for better drainage. This disturbance regime likely increases the similarity between the purple loosestrife-rich seed bank and above ground composition, as the seed bank has been shown to be a colonizing source after disturbances (Luzuriaga et al 2005, Roberts et al 2014). Finally, soil moisture levels of these regions vary greatly and could potentially be altered with road construction.

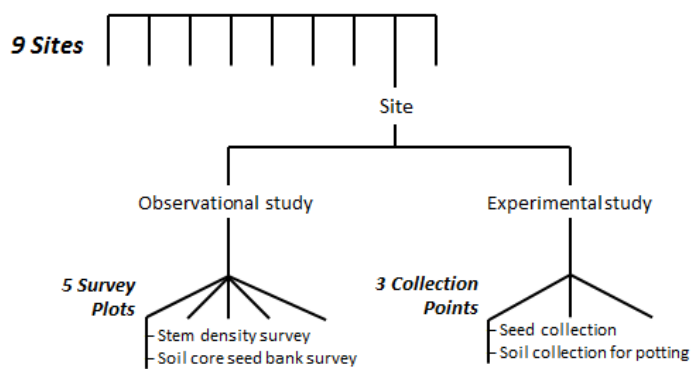
I selected nine sites for seed and soil collection around Waterville, ME (Figure 1). All sites were in roadside areas and contained populations of purple loosestrife and cattail. I determined the sampling area of each site as the area where one or both of the species occur. Sampling areas ended where neither species occurred or where there was a clear interruption (i.e. a road, driveway, culvert, open water, etc). Sampling areas varied from approximately 150 – 1342 m<sup>2</sup> and bordered the road for 49 to 284 m.

When selecting sites and through the rest of the study, I did not distinguish between broad-leaved cattail (*Typha latifolia*), narrow-leaved cattail (*Typha angustifolia*) or their hybrid (*Typha x glauca*) due to unreliability of species level identification at the time of collection. Estimates of species identification did not relate to variation in germination rates between sites. Two collection points (one at site 6, one at site 9) may have contained only hybrid seeds, as the germination rate was 0% for all treatments. These points were kept in the analysis and their presence did not alter trends or statistical significance. Potential presence of hybrid seeds in other locations was controlled for by adding equal numbers of seeds from each seed head to packets from each collection point. Thus, if hybrid seeds were present, their sterile presence would decrease germination rate across all treatments in a single replicate, and the random variance between replicates which this may have caused was statistically controlled for.

*Field Methods*

At each of the nine sites, I collected one site-level metric (estimated percent cover) and took replicate samples nested within each site (Figure 2). For the observational study I used five selected plots. From each of these plots I took two soil cores and measured stem density. For the experimental study, I had three random collection points at each site where I collected seeds and soil.

For the observational study I collected two metrics to estimate aboveground density. To gather a site-level metric of purple loosestrife and cattail relative dominance, I estimated percent cover of purple loosestrife, cattail and “other species” in early December. I also selected five 1x1 m survey plots at each of the nine sites. I selected two survey plots with high purple loosestrife density and low cattail density, two survey plots with low



**Figure 2.** Diagram of collected items and data at each site. Note that the soils from each collection point were homogenized by site before the burying seed packets in the experimental study.

purple loosestrife density and high cattail density, and one with equal densities of both. At each of these 1x1 m survey plots, I surveyed the stem density of cattail and purple loosestrife to gather a local metric of purple loosestrife and cattail relative dominance. Both of these metrics were used to describe aboveground relative dominance.

To examine the belowground seed bank density, I took two 15 cm soil cores in mid-December from random points within each of the 1x1 m survey plots. I air dried the soil cores and stored them at room temperature for three weeks.

For the experimental study, I collected seed heads from three collection points randomly selected along the roadside length of each site in early October (Figure 2). The seeds were stored at 4°C for 1-2 weeks before being removed from the seed head and transferred to paper envelopes and stored at 4°C.

In early November, I collected ~12 liters of soil from the top 15 cm of soil at each collection point. The soil was stored outdoors until late November. The average high temperature during this period was 8°C and average low was -3 °C; no day reached warmer than 14°C or cooler than -11°C. During this time, I removed large roots and homogenized the soil by sieving through a 1x1 cm wire grid, which was washed in water and sterilized with 95% ETOH and 10% bleach solution between processing soil for each site. During the homogenization I checked soil for earthworms and removed any that were found. I mixed all the homogenized soil from each site before potting. I filled 3.5x3.5x5 in pots with field soil to 1 cm from the brim (at field saturation). Once the soil lost moisture, the volume in some pots was reduced, and the soil filled the pot to approximately 5 cm from the brim. In the center of each pot, I made a 1-2 cm depression to help reduce runoff during watering.

#### *Sampling of existing seed bank*

To examine the belowground density of purple loosestrife, cattail, and other species, I germinated seeds from the soil cores. I homogenized the two air-dried soil cores from each survey plot and took the combined dry weight. I spread the soil cores across 450 cm<sup>2</sup> of Fafard® extra fine germination mix. The trays were kept in a greenhouse and were watered every 3 days. Seedlings were marked, counted and removed once they were identified to reduce competition. Seedling density was calculated as (seeds)/(g of dry soil core).

#### *Seed burial experiment*

To determine whether effects of fungi on germination depend on soil moisture, I buried packets of 30 seeds for 12-13 weeks in field soil. I made seed packets from 10x10 cm squares of fine nylon mesh folded in half with triply-folded, stapled edges. I filled these packets with 30 seeds of a single species and 4.5g of sterile sand to reduce risk of seed-to-seed infection (Mourick et al. 2005). I stored seeds at 4°C for the eight weeks between seed collection and the initiation of the experiment.

Pots were subjected to two levels of fungicide treatment (with fungicide (F) or without fungicide (NF)) and three levels of watering (dry, well-watered, and saturated). Thus, one seed packet of each species from each collection point was exposed to one of six treatments 1) F\*dry 2) F\*well-watered 3) F\*saturated 4) NF\*dry 5) NF\*well-watered 6) NF\*saturated. The fungicide

treated pots received 5 ml of a 0.2% Southern Ag® Captan Fungicide solution (mixed as recommended by the manufacturer) seven days before seed burial and were retreated during week three and week seven of burial. The untreated pots received no fungicide. Fungicide was only applied on days that all pots were watered. All “dry” pots were watered only right before fungicide application, which allowed sufficient time between watering for the soil to dry out. “Well-watered” pots were watered every 3 days, which kept soils moist. “Saturated” pots were lined with plastic bags to prevent drainage, and were watered enough to maintain standing water to the edge of the pot, which was usually every 3 days, but occasionally more frequently. The saturated conditions were designed to mimic the saturated conditions found in many of the field sites.

The seed packets were kept in the potted field soil in a greenhouse. The mean greenhouse temperature was  $14.5 \pm 3.86(\text{SD})$  °C with a maximum of 37 °C and a minimum of 6 °C. I randomly placed the pots on two greenhouse benches, one containing fungicide treated pots and the other containing untreated pots. I put 3.8 cm of space between each pot to prevent contamination. I rotated pots between tables part way through the experiment and re-randomized pots on each table after one month. After twelve to thirteen weeks of burial, each seed packet was emptied onto a 3.5 inch round petri dishes containing one moist Anchor Paper Co. 3 1/2” crocker blue blotter circle and incubated in a Percival Scientific (Perry, IA, USA) Model AR66L3C9 growth chamber set to 80% humidity with a 12 hour light-dark cycle at 30 °C (light) and 20°C (dark). These conditions were set to optimize germination of the more sensitive cattail species based on Lombardi’s study (1996).

Seeds were incubated until an asymptote was reached in both species (10 days for purple loosestrife and 12 days for cattail). Purple loosestrife seeds were kept in the growth chamber for 14 days. A randomly selected 12% of loosestrife samples were incubated for 10 more days, during which time total germinants only increased by 0.2%. Cattail seeds were kept in the growth chambers for 25 days. A randomly selected 11% of cattail samples were incubated for an additional 13 days, during which time there was an 8% increase in total germinants.

### *Seed microbiomes*

To better understand the fungal and bacterial communities inside seeds, I buried packets similar to the ones used in the seed burial experiment but containing only six seeds (two from each collection point in a site) and no sand (to enable me to find individual seeds in packets upon retrieval). Two seed packets (one of each species) were buried side by side in potted soil from their respective site 1-2 cm below the surface. I used field soil and seeds from six of the nine sites. Half of the pots were treated as F\*well-watered and half were treated as NF\*well-watered.

After four weeks I exhumed half of the seed packets, and selected two random seeds from each packet. The other half of the seed packets were processed after five weeks. I surface sterilized each seed by placing it in 90% ETOH for 10sec, 0.525% NaOCl for 1 min, 70% ETOH for 1 min, and sterile water for 30sec. Seeds were rinsed in sterile water between each soak and forceps were flame-sterilized between each transfer. Seeds were placed on sterile paper towels to



dry for ~1 min before being placed on 2%MEA plates (sterilization methods modified from protocols by M.S. Benitez and A.E. Arnold.)

Two control seeds of each species from each site which had been stored in envelopes at 4°C were also surface sterilized and placed 2%MEA plates. All plates were incubated at room temperature and fungal cultures were re-plated until pure cultures were established. Fungi and bacteria were visually grouped by morphotype.

### *Data Analysis*

Data analysis for all methods was performed in R 3.2.3 (R Core Team 2015).

***Sampling of existing seed bank:*** To determine differences between species' belowground density, I used a paired Wilcoxon-signed rank tests). To determine relationships of aboveground and belowground density, I used Spearman's signed rank correlation tests (n = 45, 5 survey plots at 9 sites)

***Accumulation of Buried Seed Germinants:*** In order to ensure that I observed the majority of seed germinants, I created accumulation curves of germinants of each treatment and species. To create the curves, I first adjusted all data points for each sample so that "Day 1" represented the day that each sample was exhumed from the soil, processed, and placed in the growth chamber. Due to the random staggering of exhumation across ten days, the date of exhumation differed from the date of the start of the experiment for many samples.

I calculated the germination rate as the number of germinants per the number of buried seeds. The average germination rate on each day represents the (total number of known germinants in a treatment)/ (total number of samples in that treatment). The known germinants on any given day may have not represented the actual count of total number of germinants on that day as not all samples were checked for germination daily, and selection of checked samples was random. For example, on any given sampling day, I may have checked a pseudo-random third of the samples and within these samples some may be marked as "Day 5" whereas the other was marked on "Day 7" because their exhumations were staggered by two days.

***Germination rate of Buried Seeds:*** To assess whether the influence of fungi on the germination rates depended on soil moisture levels, I used generalized linear mixed-effects models (GLMM) with binomial error distribution. I specified fungicide, water treatment, and the fungicide\*water interaction as fixed effects. I included site of collection as a random effect to acknowledge and quantify the variability among sites, and because no specific hypotheses were linked to individual sites. Because each site contained three collection points, I nested collection point within site in the model (Figure 2). To account for over dispersion which can inflate estimates of explained variance, I added an observation level random effect (Harrison 2014).

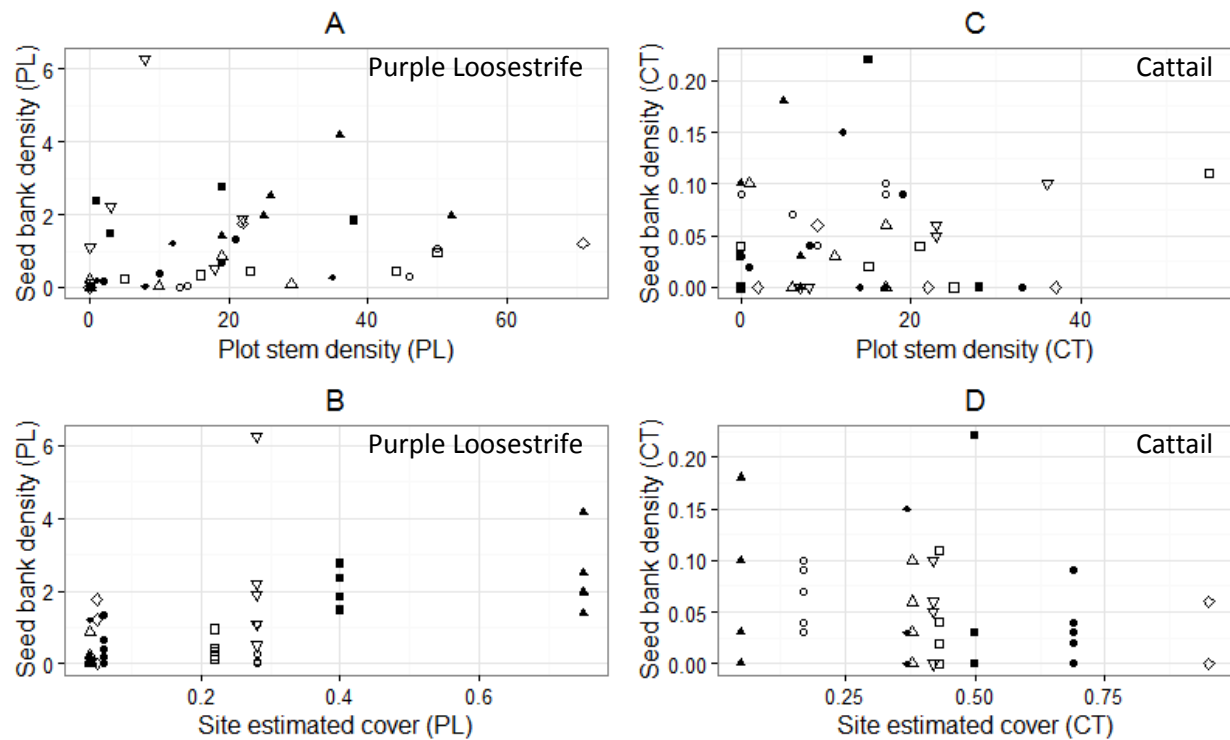
I generated separate models for purple loosestrife and cattail because the primary interest of this study was the effect of the treatments on germination for each species and not differences in germination between species across treatments. Separating the models by species also best reflected the structure of the study, in which purple loosestrife and cattail seeds were incubated in separate pots and did not interact.

**Seed Microbiome:** To better understand how the plant species and fungicide treatments affected the infection rate of seeds, I used chi-squared tests to compare counts of fungal and bacterial seed infection of species/treatment combinations. Several seeds were co-infected with multiple fungal morphotypes. However, for the sake of simplicity and due to the unreliability of visual morphotyping, these seeds were counted as one infected seed, the same as a seed infected with a single morphotype.

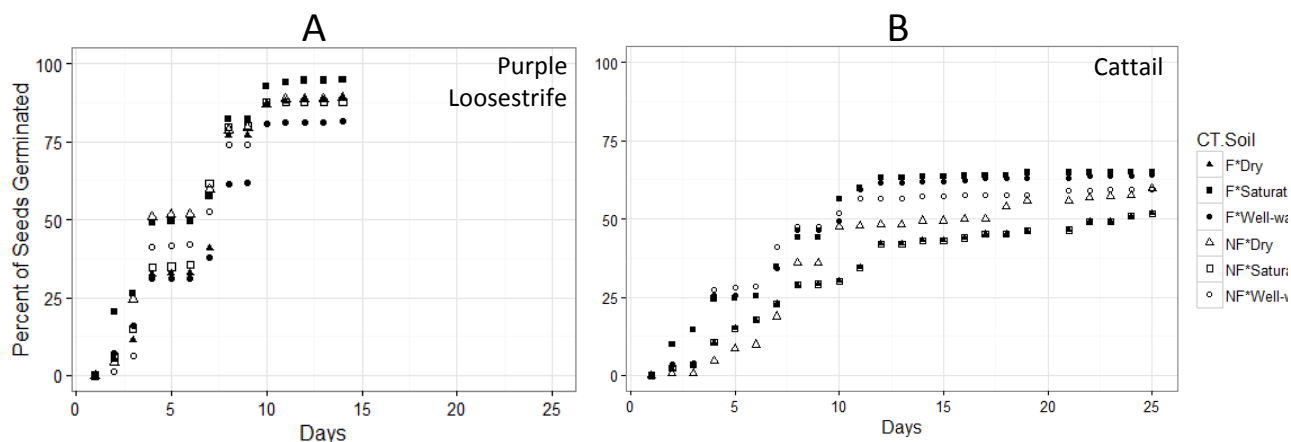
## RESULTS

### *Sampling of existing seed bank*

Germination rates of seeds in the soil cores indicate that purple loosestrife seed bank density was significantly higher than cattail density ( $n = 45, V = 795, p < 0.001$ ; Paired Wilcoxon tests). Average purple loosestrife seed bank density was  $0.997 \pm 0.128$  (SE) seeds/gram of dry soil, whereas cattail seed bank density was only  $0.045 \pm 0.005$  (SE) seeds/gram of dry soil. Purple loosestrife seed bank density is correlated to aboveground density (Figure 3). This correlation exists both at the local survey plot scale of aboveground cover (stems/ $m^2$ ) ( $n = 45, \rho = 0.523, p < 0.001$ ) and at the site-level scale, estimated percent cover of purple loosestrife ( $n = 45, \rho = 0.585, p < 0.001$ ).



**Figure 3.** Relations of above ground and below ground density. Panels A and B display the aboveground/belowground relationships for purple loosestrife (PL) and panels C and D display the relationships for cattails (CT). Panels A and C display the relationship between seed bank density (germinated seedlings/gram of dry soil) and stem count of each selected species in the  $m^2$  survey plot from which soil cores were taken. Panels B and D display the relationship between seed bank density and estimated percent cover at the site level of each selected species.



**Figure 4.** Accumulation curves of the mean percent of seedlings germinated. Showing the mean percentage of seedlings of purple loosestrife (A) and cattail (B) that were known to have germinated on each day after exhumation. Field soil water treatments are indicated by shape (triangle = dry, square = well-watered, circle = saturated). Mean germination rates of seeds buried in fungicide treated soils (F) are represented by filled shapes and mean germination rates of seeds buried in untreated soils (NF) are represented by open shapes. Error bars are removed for ease of interpretation.

#### Accumulation of buried seedlings

In the experimental study with buried seed packets, I found that after 10 days in the growth chamber, approximately 99% percent of the total measured purple loosestrife germinants (Figure 4A) had germinated, and after 12 days, 89% of cattail total measured germinants were germinated (Figure 4B). All treatments within the species reached an asymptote after the same

length of time.

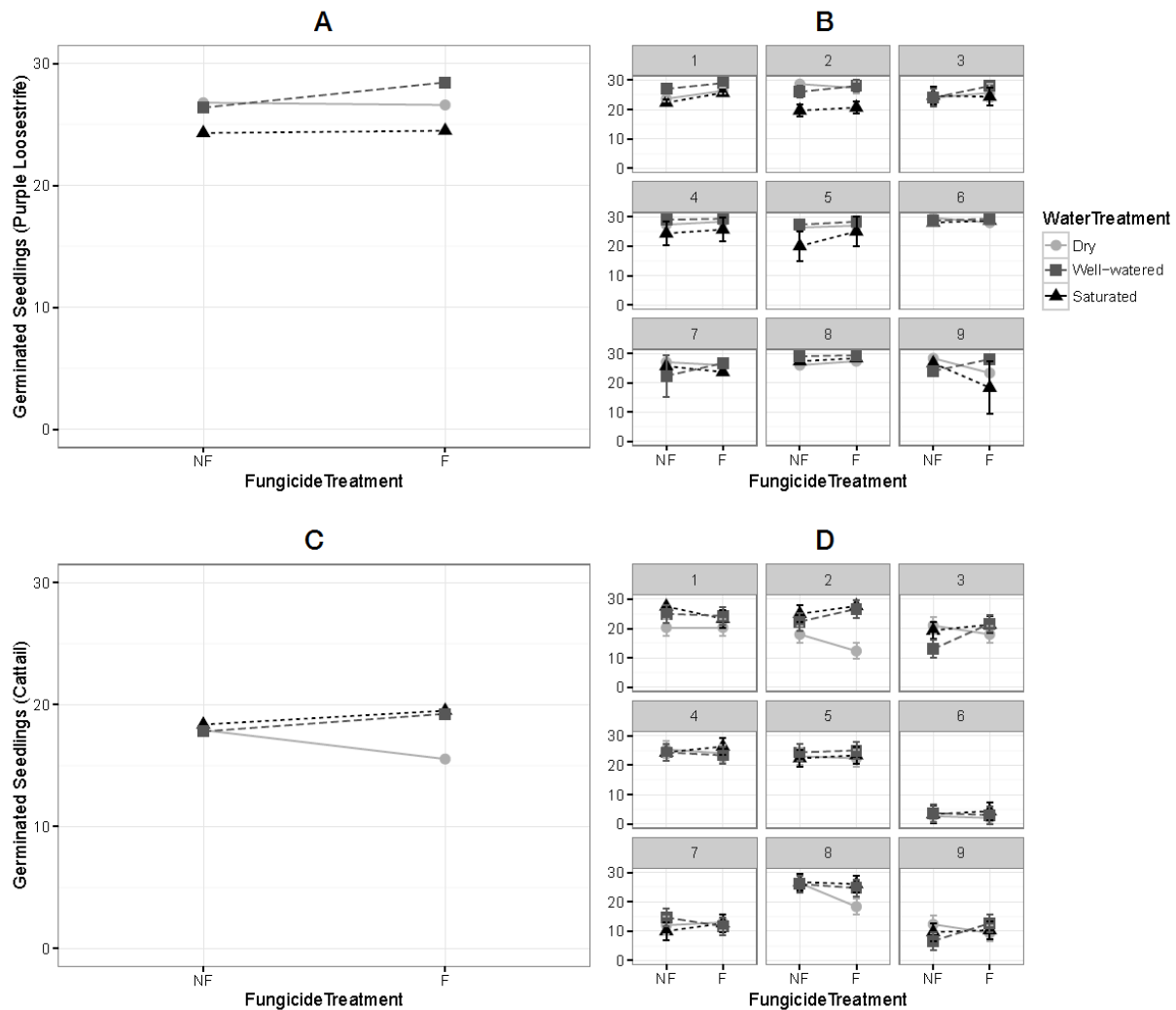
Differences in some of the water\*fungicide treatments emerged early and were sustained until the end of the germination trial.

**Table 1.** Effects of fungicide and water treatments on purple loosestrife and cattail seed germination using a generalized linear mixed model. Statistics are calculated relative to the dry water treatment. Significance values are indicated as follows: \*\*\* =  $p < 0.001$ ; \*\* =  $p < 0.01$ ; \* =  $p < 0.05$

Term	Estimate	SE	z-value	
<b>Purple Loosestrife</b>				
Main Effects				
Intercept	2.389	0.250	9.550	***
Fungicide	0.102	0.298	0.341	
Well-watered	0.940	0.323	2.912	**
Saturated	-0.536	0.289	-1.854	.
Interactions				
Fungicide x Well- watered	-0.936	0.442	-2.117	*
Fungicide x Saturated	-0.172	0.409	-0.420	
<b>Cattail</b>				
Main Effects				
Intercept	-0.199	0.512	-0.389	
Fungicide	0.464	0.121	3.846	***
Well-watered	0.749	0.123	6.065	***
Saturated	0.807	0.124	6.501	***
Interactions				
Fungicide x Well- watered	-0.772	0.174	-4.427	***
Fungicide x Saturated	-0.715	0.177	-4.040	***

#### Germination rate of buried seeds

The results of the GLMM (Table 1) indicate that for both species, the effect of fungicide depended on soil water treatment. Fungicide application did not significantly affect purple loosestrife



**Figure 5.** Mean count of germinated seedlings in each fungicide and water treatment. 320 replicates contained 30 total seeds and 3 replicates contained 31 total seeds, thus the average count of germinated seedlings represents an approximate proportion of germinated to ungerminated seedlings. Seeds were buried in soils treated with fungicide (F) or soils with no fungicide (NF) and kept dry, well-watered, or saturated (n=323). Panels A and C display the mean germination rates of each treatment across sites for purple loosestrife (A) and cattail (C). Error bars are not displayed for ease of interpretation. Panels B and D display the mean germination rate of each treatment for each individual site for purple loosestrife (B) and cattail (D). Error bars represent  $\pm$  SE.

germination rates across water treatments. However, fungicide did increase the germination rate of purple loosestrife under well-watered conditions ( $z = 2.912$ ,  $p = 0.0036$ ) (Table 1, Figure 5A,B). Furthermore, the effect of fungicide application in well-watered conditions increased germination significantly more than it did in dry conditions ( $z = -2.117$ ,  $p = 0.0343$ ).

Cattail was more affected by fungicide and water treatments than purple loosestrife. Fungicide application significantly affected germination rate across water treatments for cattail ( $z = 3.846$ ,  $p < 0.001$ ) (Table 1, Figure 5C,D). Dry treatments yielded significantly different germination rates from both well-watered treatments ( $z = 6.065$ ,  $p < 0.001$ ) and saturated treatments ( $z = 6.501$ ,  $p < 0.001$ ). The effects of fungicide application on germination rate in dry

conditions were significantly different from those in well-watered conditions ( $z = -4.427, p < 0.001$ ) and saturated conditions ( $z = -4.040, p < 0.001$ ). In dry conditions, fungicide application reduced germination, whereas fungicide application increased germination in moderate and saturated conditions.

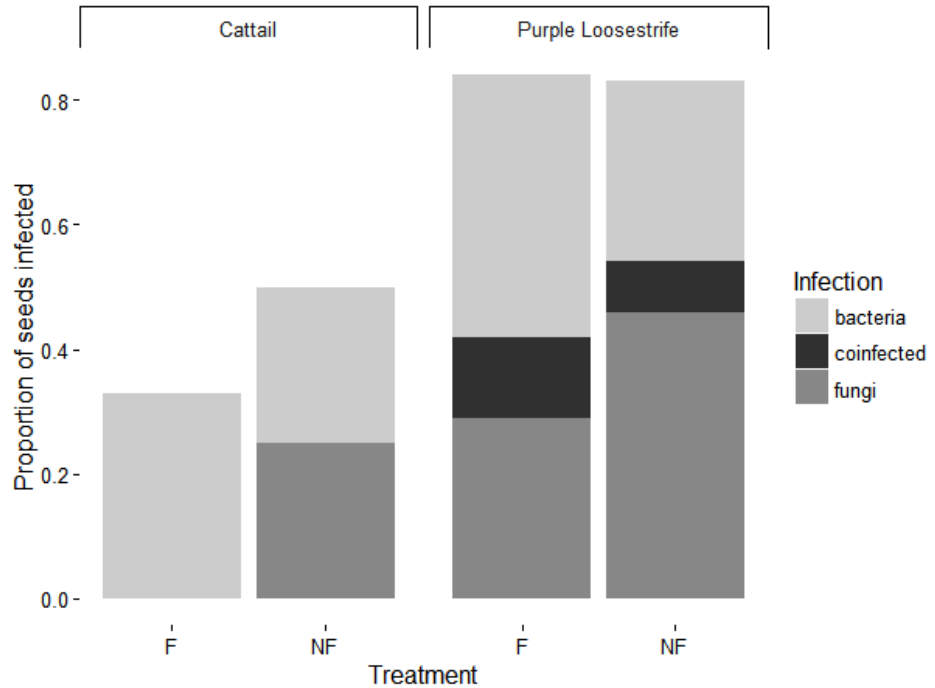
#### Seed microbiome

I found that infection patterns varied between the two species (Figure 6). For example, across fungicide treatments, I found that fungal infection rate was significantly higher in purple loosestrife than cattail ( $n = 24, \chi^2 = 9.645, p = 0.002$ ). Additionally, no cattail seeds were found to be co-infected with bacteria and fungi and the composition of the fungal communities infecting seeds (based on morphotype) varied between species. However, bacterial infection rate was similar between both species ( $n = 24, \chi^2 = 0.33425, p\text{-value} = 0.5632$ ).

I found that the effects of fungicide were neither complete nor universal. Fungicide did not reduce fungal infection rate in purple loosestrife seeds ( $n = 24, \chi^2 = 0.8, p = 0.371$ ) or cattail seeds ( $n = 24, \chi^2 = 2.602, \text{estimated } p = 0.107$ ). Fungicide also did not significantly influence bacterial infection for seeds of purple loosestrife ( $n = 24, \chi^2 = 0.8, p = 0.371$ ) and cattail ( $n = 24, \chi^2 = 0.0502, p = 0.823$ ).

## DISCUSSION

The results of this study show that purple loosestrife forms a dominant seed bank which interacts with fungal and bacterial communities in the soil differently than cattail. In saturated conditions, loosestrife is less affected by fungal pathogens than cattail. As this is the simulated condition most similar to the observed conditions at the field sites, these results indicate that purple loosestrife may have a competitive advantage in the seed bank due to reduced pathogen



**Figure 6.** Infection rates of seeds after burial in field soil. Dark grey represents the proportion of buried seeds that were infected with only bacteria only after burial in soils treated with fungicide (F) and soils left untreated (NF). Light grey represents the proportion of seeds infected with only fungi, and black represents the portion of seeds co-infected with both fungi and bacteria.  $n=24$  for each species\*fungicide treatment.

loads. The confounding factor of bacterial infection of seeds indicates an exciting potential for future study.

#### *Survey of existing seed bank*

The seed bank survey using germination from soil cores indicated that in areas where purple loosestrife is present, it establishes a dominating presence belowground, which is consistent with former literature (Yakimowski et al 2005). Particularly in roadside areas which are repeatedly disturbed due to road maintenance and drainage construction, the abundant seed bank could be more consequential to loosestrife success. Disturbances increase aboveground-belowground similarity (Luzuriaga et al 2005, Roberts et al 2014), which will result in increased purple loosestrife density if ~40% of the seed bank is comprised of purple loosestrife, as this study indicates. Moreover, the positive correlation between aboveground and below ground density indicates that there is a positive feedback loop between aboveground and belowground density. Combined with Wilcox's finding (1989) that highways act as dispersal corridors for purple loosestrife, my findings indicate that it is likely that purple loosestrife will continue to invade at an increased rate, based solely on roadside disturbance increasing above ground density, which in turn increases dispersal.

It is possible that the results of this study may have been exaggerated due to differential timing of seed dispersal. Purple loosestrife releases most of its annual seeds in mid-November (Klips & Peñalosa 2003), whereas cattails generally disperse their seeds gradually throughout the winter (field observations). As soil cores were collected in December, there may have been a bias towards purple loosestrife. However, this likely would not have significantly altered the trends I saw, as both species are known to have persistent seed banks (Rawinski 1982, Leck & Simpson 1987) and estimates of annual cattail seed rain (Leck & Simpson 1987) would not account for the difference between seed bank sizes I observed.

Overall, the finding that purple loosestrife comprises a large portion of the germinable seed bank and that its belowground density is positively related to its aboveground density whereas cattails is not underscores the importance of studying the seedbank survival of these two species.

#### *Accumulation of germinants*

After reaching an asymptote, cattail seed germination did continue to slowly increase. It is possible that, given more time, some of the patterns I observed may have been slightly altered by a continued increase in germination. However, in a competitive natural environment, it is likely that slowing germination may effectively reduce recruitment. This is especially likely to be the case when purple loosestrife is present, due its superior competitive ability to cattail as a seedling (Yakimowski et al 2004). Thus, though the trends I observed may have changed with time, it is likely that in a natural environment these trends may still effectively relate to recruitment of seedlings.

### *Effects of water and fungicide*

My results indicate that the outcomes of plant/fungi interactions are contingent on the environmental conditions of the seed bank. For both species, fungicide appeared to provide a protective effect in well-watered conditions. This finding is consistent with former literature, which suggests that moist, but not saturated, conditions provide the best environment for fungal growth (Schafer & Kotanen 2003, Wagner & Mitschunas 2008).

Interestingly, in saturated conditions, I found that fungicide provided a protective effect for only cattail seeds. This indicates that the fungal community differs between well-watered and saturated soils in a very consequential manner. Namely, saturated soils contain fungal pathogens that target cattail seeds but not purple loosestrife seeds. During field work, I observed that sites were often partially or fully flooded, which indicates that the results of the saturated condition may be most representative of the natural seed bank dynamics. These conditions are especially applicable to large wetlands where purple loosestrife is known to invade (Thompson et al 1987).

In dry conditions, I found that cattail germination decreased with the addition of fungicide. Based on former literature (Shafer 2003, Wagner & Mitschunas 2008), I had predicted that under dry conditions, fungal activity would be reduced. If my hypothesis is true, the reduced germination rate in fungicide treated soils implies that fungicide may harm seeds, especially cattail seeds. If fungicide does have a deleterious effect on seed survival, the positive effects of fungicide application I observed are likely conservative, indicating that fungal pathogens are more influential than the results of this study suggest.

### *Seed microbiome*

I found that purple loosestrife seeds were infected at a significantly higher rate than cattail. This portion of the study was only conducted in well-watered soils, where both cattail and purple loosestrife benefited from fungicide application. Studying the differences in seed microbiomes in saturated soils, where fungicide provided a protective effect for only cattail would possibly yield different results.

The application of fungicide did not significantly affect the proportions of fungal infections in seeds of both species, and fungicide effects were neither complete nor universal. Results based on visual estimates of morphotypes indicate that the fungal and bacterial communities varied between fungicide treatments, suggesting that new morphotypes of infecting fungi arise with the application of fungicide. This may be due to a competitive release: with the growth of fungicide-targeted morphotypes reduced in treated soils, other infecting fungi have more resources available to grow and infect seeds. If these other fungi are also pathogenic, this would again result in conservative estimates of effects of fungal pathogens based on fungicide protective effects.

New morphotypes of infecting bacteria also emerged with fungicide application, which may also be a result of competitive release from fungal morphotypes. The emergence of bacterial infections with fungicide treatment may mean that my estimates of fungal effects are conservative. For example, in purple loosestrife, total infection rate remains almost constant between fungicide and non-fungicide treatments, meaning that it is possible that the reduced

fungus pathogen load was simply replaced by bacterial pathogens. If some of the infecting bacteria are pathogenic, this may have reduced the positive effects of fungicide on seed survival.

It is worth noting that though fungicide did not significantly reduce fungal infection rate of either species, the replication was very small. The trends found highlight the fact that the microbiome of the seed is very complex and more study is necessary to fully understand. These findings also imply that studies that focus solely on effects of fungal pathogens are likely not gaining a comprehensive understanding of the role of soil pathogens in the seed bank, as bacterial pathogens may compensate for removed fungal pathogens.

#### *Conclusions:*

Overall, I found that loosestrife has two major advantages in the seed bank: abundance of seeds and decreased fungal pathogen attack in saturated soils. This indicates that in roadside drainages that are frequently disturbed and saturated, it is likely that purple loosestrife will continue to arise and persist. By persisting in these roadside areas, loosestrife's ability to disperse to new wetlands will also increase. My findings suggest that there are two possible approaches to reducing roadside presence. Firstly, reducing the severity of human disturbances (e.g. total vegetation clearing from drainage trenches) would reduce the similarity of aboveground density to the loosestrife dominant seed bank. Secondly, constructing drainage ditches with well-drained soils may reduce the abundance of fungal pathogens which persist in saturated conditions and harm cattail seeds.

Finally, this study highlighted the complexity of the seed microbiome. The results complemented previous studies (Schafer & Kotanen 2003, Wagner & Mitschunas 2008, Mordecai 2012) which found that seed bank/fungal pathogen interactions are contingent on environmental conditions. Furthermore, I found that bacteria may be equally important actors in the seed bank, which encourages future studies to account for this co-occurring interaction. The patterns of diversity and pathogenicity of soil microbiota in these selected host species under varying moisture conditions are still unknown and may provide exciting avenues for future insights.



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