

SOIL FEEDBACKS AND ARBUSCULAR MYCORRHIZAL FUNGI OF *LINARIA*
VULGARIS INVASION

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ABSTRACT

Invasive plants reduce native plant diversity both directly through competition and indirectly via changes to soil communities and nutrient dynamics, among other mechanisms. Understanding how invasive plants interact with soil communities and nutrients can provide insight into why these species spread aggressively only in some parts of their range. In this study, I evaluated the effects of *Linaria vulgaris* invasion legacy in Colorado and Illinois soil to determine if soil characteristics could explain why *L. vulgaris* is an aggressive invader in CO and non-aggressive in IL. I also evaluated arbuscular mycorrhizal fungi (AMF) communities and soil nutrients in invaded and uninvaded soil from both states to determine if there is a relationship between invasion, soil nutrients, and AMF community composition. I collected soil from the Rocky Mountain Biological Lab (RMBL) and in the Forest Preserves of Cook County and grew *L. vulgaris* and two native plants in either invaded or uninvaded soil, from CO or IL, and fertilized or control pots in growth-chambers for four months. At the end of the growing period, I injected pots with ^{15}N and measured N uptake rates in leaf tissue using an isotope ratio mass spectrometer. I also harvested root and shoot biomass and measured plant presence across treatments. To evaluate AMF communities, we performed next generation sequencing of soil collected from field sites using AMF targeted primers NS31 and AML2 and tested soil for pH, NO_3 , NH_4 , PO_4 , and soil texture. I found evidence that *L. vulgaris* has a positive soil-feedback in CO soil and a negative soil-feedback in IL soil, which follows patterns of invasion aggressiveness. Native plant root biomass was negatively affected by invasion in CO soil, indicating that *L. vulgaris* invasion negatively impacts native plant success. Nitrogen fertilization did not affect *L. vulgaris* success, and invasion history did not affect nitrogen uptake rates in either *L. vulgaris* or native plants, indicating that nitrogen availability is not responsible for soil legacy effects. AMF communities were not different between invaded and uninvaded soil, but

were different between CO and IL, illustrating that *L. vulgaris* invasion most likely does not change AMF communities, but invasion could still be influenced by already existing AMF community composition. Similarly, PO₄ was different in invaded and uninvaded soil across states, which could either give rise to or be the result of invasion. I hope that uncovering evidence of soil legacy effects in *L. vulgaris* will provide managers better tools to prevent reinvasion and restore native plant communities and encourage researchers to examine soil legacy effects in other invasive plants. Future studies should continue to evaluate the possibility of soil feedbacks and their mechanisms in *L. vulgaris* invasion, including other soil microorganisms and PO₄ availability.

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Chapter 1: Soil Legacy Effects of *Linaria vulgaris* invasion in Illinois and Colorado

Introduction

Invasive species reduce species diversity worldwide because of their ability to dominate natural areas (Mack et al 2000). It has been theorized that invasive species' pervasiveness comes from their lack of predators and pathogens, their ability to colonize disturbed sites, or their novel interactions outside of their native environment, among other mechanisms (Keane & Crawley 2002; Simberloff 2010). However, invasion success can be variable, with only a fraction of species becoming invasive and only in certain areas outside of their endemic range (Mack et al 2000). Environmental factors, such as climate and nutrient availability, as well as the interactions between invasive and native species can all affect the range and severity of a species' invasion (Hayes & Barry 2007; Yang et al 2013).

Invasive species can also play a role in their own biogeography by shaping the environment around them, which can lead to a competitive advantage over native species (Shah et al 2009). Some invasive plant species manipulate their environment both chemically and biologically to reduce other species' abundance or increase their own (Klironomos 2002; Weidenhamer & Callaway 2010; Gibbons et al 2017). Invasive plants can have indirect negative effects on native plant populations if they release chemical compounds into the soil that alter the community of mycorrhizal fungi (Koocheki et al 2012; Muzquiz et al 1994). Mycorrhizal fungi are important mutualists for most plant species. Mycorrhizal associations allow the exchange of critical nutrients between plant roots and the soil, such as N and P, and the uptake of water, while plants supply fungi with sugars (Begum et al 2019). Invaders that disrupt plant-mycorrhizal

relationships can reduce native plants' ability to acquire nutrients and water, negatively impacting plant productivity and survival (Asmelash et al 2016; Lankau 2010). Further, changes to soil communities can be long-lasting, even after the invasive plant has been removed, leaving a legacy effect from its invasion that can alter the plant community for years and which may make areas prone to re-invasion (Lankau 2010, Klironomos 2002).

Some invasive plants directly associate with mycorrhizal fungi, and their spread may be determined by these positive or negative interactions (Afkhani et al 2014; Reinhart & Callaway 2006). Many mycorrhizal fungi are generalists and can associate with a wide suite of hosts, but the effect of the interaction depends on their host plant species (Tedersoo et al 2020). As is true of native plant-mycorrhizal associations, invasive plants can also benefit from mutualistic mycorrhizal relationships in their invaded range (Shah et al 2009). However, some mycorrhizal fungi can become parasitic on their plant hosts, particularly in areas where nutrients and water are widely available, making the cost of the relationship greater than the benefit (Bever 2002; Johnson et al 1997). These parasitic mycorrhizae can suppress plant growth, making invasive plants most successful in regions where they escape these negative mycorrhizal associations or soil pathogens (Maron et al 2013; Bever 2002). Other invasive species use allelochemicals to increase the presence of their mycorrhizal symbionts and to reduce the abundance of their parasites (Shah et al 2009). Invasive plants that either manipulate the mycorrhizal community or escape the range of suppressive soil pathogens gain advantages in nutrient and water availability, enabling them to outcompete native species (Maron et al 2013, Zubek et al 2016). Therefore, invasion legacy in the soil can have negative impacts on plant communities, extending far beyond competition.

In areas with N-limited soil, many plants species utilize mutualisms with mycorrhizal fungi or N-fixing bacteria to acquire N (Asmelash et al, 2016). Where invasive plants have been introduced, some species have been shown to alter N dynamics, either by increasing or decreasing N availability, with subsequent consequences on the native plant community (Evans et al 2001; Corbin & D'Antonio 2004). Invasive plants may be more efficient at absorbing N or are able to increase an environment's rate of N loss, leaving less available for native plants without necessarily disrupting mycorrhizal interactions (Evans et al 2001). An invasive plant's ability to change nutrient dynamics in its environment likely drives its success in a particular region (Lorenzo et al 2013). However, researchers do not have a full understanding of how invasive plants react to different N abundances in different parts of their range.

For example, *Linaria vulgaris* (Plantaginaceae), commonly referred to as 'Yellow Toadflax' or 'Butter and Eggs', is a forb native to Southeastern Europe that is invasive throughout North America (Sing et al 2016). It reproduces both sexually from seed and asexually from small sections of root tissue, making it extremely difficult to eradicate (Sing et al 2016). *Linaria vulgaris* is widespread throughout the U.S. but is particularly successful in Western states throughout the Rocky Mountain region (USDA, NRCS 2021). Outside of its clonal reproduction, little is known about why *L. vulgaris* is such a tenacious invader in the Western U.S. Ambient soil N is historically lower in the Rocky Mountain region, where *Linaria* aggressively proliferates, relative to soil N in the Midwestern U.S., where *Linaria* is non-aggressive (Bowman et al 2012; Jelinski & Kucharik 2009). This suggests that *L. vulgaris* alters the microbial community or outcompetes native plants for N in the Rocky Mountain region to invade more aggressively. Alternatively, *L. vulgaris* could be suppressed by soil biota in the Midwestern U.S., making it an ineffective invader in that region.

This study addresses the biogeography of *Linaria vulgaris* invasion by asking why this species dominates Western subalpine meadows and not Midwestern prairies. Specifically, it aims to uncover whether 1) *Linaria vulgaris* invasion leaves legacy effects that influence native plants and its own success, 2) if soil from Colorado and Illinois influence *L. vulgaris* success, or 3) if N-availability explains the range of aggressive *L. vulgaris* invasion. I examined plant biomass accumulation and N uptake in *L. vulgaris* and two native plants grown in previously invaded and uninvaded soil collected from IL and CO, and in the presence or absence of N-fertilization. Using soil samples collected from *L. vulgaris* populations at the Rocky Mountain Biological Lab (RMBL) in Gothic, CO (aggressive) and Cook County, IL (non-aggressive), I grew plants in all possible treatment combinations in growth-chambers for 120 days. I then supplied ^{15}N -labeled tracer (NH_4NO_3) and analyzed the plant tissue for isotope abundance to detect plant N uptake capacity. I also tested whether native seed germination differed across previously invaded and uninvaded soil from CO and IL sites. The findings of this study will support ongoing research of *L. vulgaris* impacts and may present insight into invasion biology more broadly, particularly in how invasive plants use legacy effects to regulate competition with native plants, and how regional soil biota and N availability influence invasion biogeography.

Methods

Study Sites

I performed fieldwork at the Rocky Mountain Biological Lab in Gothic, CO and in the Forest Preserves of Cook County in Cook County, IL. The Rocky Mountain Biological Lab (RMBL) is a research station located in the former mining town of Gothic Colorado, within Gunnison National Forest. The Gothic area has a mean annual rainfall of 579.6mm, a maximum

daily temperature of 31.6°C and a minimum daily temperature of -19.4°C (NOAA 2022). I sampled in subalpine meadows (~2895m above sea level), which are characteristically made up of diverse grasses, forbs, and shrubs (Price and Waser 1998). *Linaria vulgaris* is an aggressive invader at the Rocky Mountain Biological Lab, and many populations are sprayed with herbicide (Glyphosate, RoundUp) to control the spread. I collected soil samples from populations that had no history of herbicide use. The Forest Preserves of Cook County are semi-urban natural areas in the Chicagoland area and are made up of prairies, woodlands, and wetlands (FPDCC 2020). The Chicagoland area (178m a.s.l.) has a mean annual rainfall of 987.0mm, a maximum daily temperature of 39.4°C and a minimum daily temperature of -23.3°C (USGS 2022; National Weather Service 2022). Here, I conducted sampling in highly disturbed areas adjacent to trails, roads, or railroads as this is where *L. vulgaris* was found. Herbicide history was not possible to obtain, but *L. vulgaris* is not a target species for managers in Illinois, and I avoided areas that were visibly treated with herbicide.

Field Soil and Rhizome Collection

I identified plots containing *L. vulgaris* from research records in Gothic and using iNaturalist in Cook County, IL. In the summer of 2020, I collected soil samples from *L. vulgaris* invaded and adjacent uninvaded areas for growth-chamber experiments. I demarcated plots using two measuring tapes in N-S and E-W directions and calculated the areas of invaded plots by multiplying N-S and E-W lengths. In plots greater than 100 m², I only sampled the 5 m² area surrounding the center point of plots (Fig. 1). To locate paired uninvaded plots, I selected a random intercardinal direction from the invaded plot and established an uninvaded plot of the same size as the invaded plot at 5-15 m from the invaded plot (Fig. 1). To ensure random

sampling throughout plots, I used a random number generator to select points on the tape measures to collect each sample (Fig. 1). In both IL and CO, I collected 160 soil cores (101.6 mm, 152.4 mm; total n=320): eight samples from each of 10 invaded plots and 10 adjacent uninvaded plots. At the same time, I collected 32 2.4cm rhizomes of *L. vulgaris* from each invaded plot, for a total of 640 rhizomes. The soil and rhizomes were used in growth-chamber experiments and soil nutrient analyses at the Chicago Botanic Garden. I collected soil and *L. vulgaris* rhizomes from CO in July of 2020 and from IL in August of 2020. Soil and rhizomes were stored at 20°C for 4 weeks prior to planting meaning CO and IL treatments were staggered.

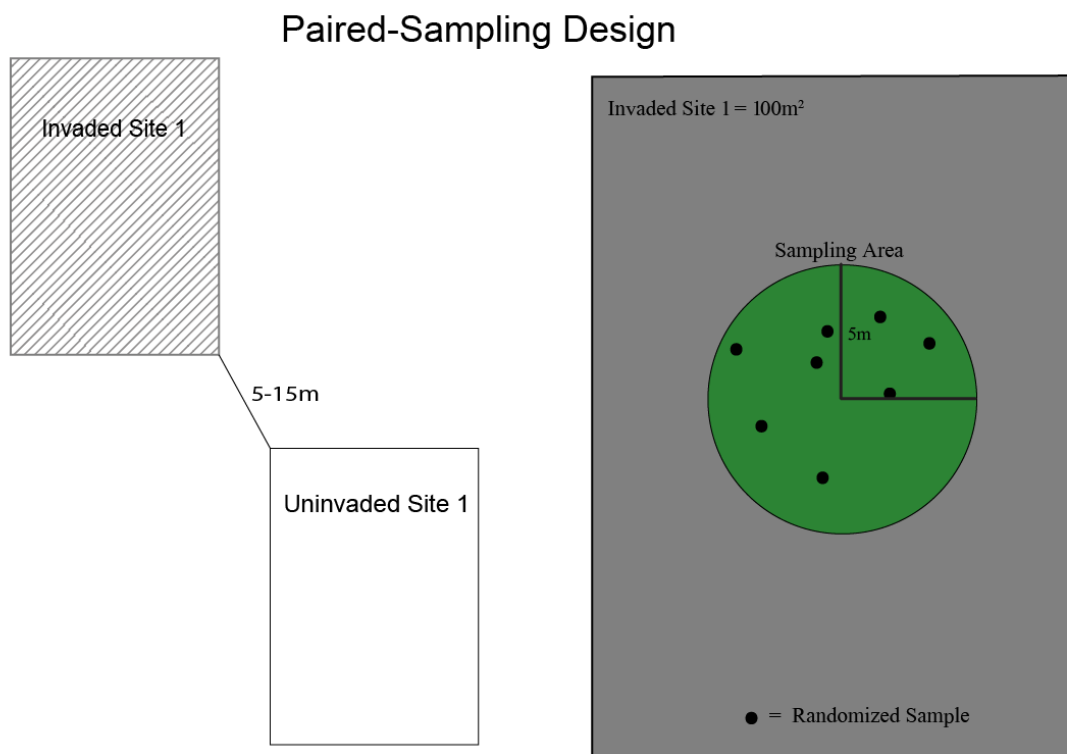


Figure 1. Paired-sampling design with invaded and uninvaded sampling areas and randomized soil samples within sampling areas.

Growth-Chamber Experimental design

This experiment consisted of a complete factorial design (2 x 2 x 2) in which I measured the growth and N uptake in *Linaria* and two native plant species (*Rudbeckia hirta*, *Leptoloma cognata*) in response to combinations of three factors: (i) soil location, either CO or IL ; (ii) invasion history, uninvaded (control) or *Linaria*-invaded soil, and (iii) two levels of N, control or N-fertilized (N kg ha⁻¹ as NH₄NO₃). To prepare the soil for the experiment, fresh soil from each location and invasion type was gently mixed 1:1 (v/v) with coarse sterile sand (autoclaved) by hand. This mix resulted in IL soils with 5 µg NO₃ g⁻¹, 2 µg NH₄ g⁻¹, and 10 µg P g⁻¹, and CO soils with 7 µg NO₃ g⁻¹, 3 µg NH₄ g⁻¹, and 9 µg P g⁻¹. Invaded and uninvaded soil in CO and IL showed similar N and P levels (Appendix I). Pots (10.1 cm³) were filled with prepared soil and planted with either *L. vulgaris* alone; *L. vulgaris* with native plants, or native plants only. Pots were planted with either two *L. vulgaris* rhizomes, three seeds per each native species (6 total), or two rhizomes and three seeds per native species.

I grew *Linaria vulgaris* with two species native to IL, *Rudbeckia hirta* (Asteraceae) and *Leptoloma cognata* (Poaceae), as opposed to Colorado native plants, as IL seed was readily available at the time of soil collection. *Rudbeckia hirta* and *Leptoloma cognata* are also commonly found in disturbed sites, making them more likely to coexist with *L. vulgaris* than plant species that only grow in undisturbed sites. Additionally, *R. hirta* is a forb, whereas *L. cognata* is a grass, which can respond differently to soil legacy effects; by growing both I hoped to capture a larger picture of how *L. vulgaris* invasion influences native species success.

Linaria vulgaris was grown from rhizomes (~2.5cm long), as seed viability is < 25% (Parker and Gassmann 2021). Seed of native species was donated by the Dixon National Tallgrass Prairie Seed Bank at the Chicago Botanic Garden. Seeds of *R. hirta* and *L. cognata* were dry stratified for 30 days at 5°C and then seeds and rhizomes were washed in a bleach

solution (10%) before planting. Pots were planted with two *L. vulgaris* rhizomes (*Linaria* alone), three seeds each of native species (*Rudbeckia hirta*, *Leptoloma cognata*), or two *L. vulgaris* rhizomes and three seeds of each native species. Rhizomes were collected from both CO and IL and were planted in their soil of origin. There were 10 replicate pots per treatment for a total of 240 pots (Table 1). Plants were grown in growth chambers using 12:12 hr photoperiod (day: night), 25/15°C (day/ night) and 75% relative humidity for four months. Plants were watered to field capacity twice a week, and N-fertilized every 30 days at rates of 8.9 kg N ha yr⁻¹ (6.1 kg N ha⁻¹ yr as NO₃, 2.8 kg N ha yr⁻¹ as NH₄) or were unfertilized (control). The level of N fertilization represents double the average N input from anthropogenic N deposition in Gothic, CO (4.05 kg N ha⁻¹ year, 3:1 NO₃:NH₄, 20 yr-average; NADP 2022), which allowed me to study responses to high N availability (Yoshida & Allen 2004). At the end of the growth period, I recorded the presence or absence of plants in all pots and treatments and measured plant root and shoot biomass.

Table 1. Layout of treatments in growth-chamber experiments (n = 240 pots).

Plants Grown	State	Invasion History	Fertilizer
<i>Linaria vulgaris</i>	IL	Invaded	+
<i>Linaria vulgaris</i>	IL	Invaded	-
<i>Linaria vulgaris</i>	IL	Uninvaded	+
<i>Linaria vulgaris</i>	IL	Uninvaded	-
<i>Linaria vulgaris</i>	CO	Invaded	+
<i>Linaria vulgaris</i>	CO	Invaded	-
<i>Linaria vulgaris</i>	CO	Uninvaded	+
<i>Linaria vulgaris</i>	CO	Uninvaded	-
Native plants	IL	Invaded	+
Native plants	IL	Invaded	-
Native plants	IL	Uninvaded	+
Native plants	IL	Uninvaded	-
Native plants	CO	Invaded	+
Native plants	CO	Invaded	-
Native plants	CO	Uninvaded	+
Native plants	CO	Uninvaded	-
Native plants and <i>L. vulgaris</i>	IL	Invaded	+
Native plants and <i>L. vulgaris</i>	IL	Invaded	-
Native plants and <i>L. vulgaris</i>	IL	Uninvaded	+
Native plants and <i>L. vulgaris</i>	IL	Uninvaded	-
Native plants and <i>L. vulgaris</i>	CO	Invaded	+
Native plants and <i>L. vulgaris</i>	CO	Invaded	-
Native plants and <i>L. vulgaris</i>	CO	Uninvaded	+
Native plants and <i>L. vulgaris</i>	CO	Uninvaded	-

Stable Isotope and Biomass Analysis

At the end of the growing period, 5 ml of 14.37 mM $^{15}\text{NH}_4\text{-}^{15}\text{NO}_3$ solution (> 98.9 at % ^{15}N ; Cambridge Stable Isotopes, Tewksbury, MA, USA) was injected into the root zone of each pot using 100 mm long 11-gauge biopsy needles (Fisher Scientific, Waltham, MA, USA; Yoshida & Allen 2004; Dresboll & Thorup-Kristensen 2012), leading to a total of 2.012 mg N added per pot. After 24 hours, plants were destructively harvested with each plant separated into root and shoot fractions, dried in biomass ovens (72 hours, 80°C), and weighed. After weighing, leaf samples ($n = 68$) were collected from all treatments in which there was sufficient leaf material for analysis. Each leaf sample was ground to a fine powder using ceramic beads in

Fastprep-24 MG with sub-samples (1-1.5mg), packed into tin capsules, and analyzed for ^{15}N atom% and N concentrations in a Costech 4010 ECS (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled to an ion ratio mass spectrometer (IRMS; Delta V Plus, Thermo Scientific) at the Northwestern University Stable Isotope Laboratory.

I used the ^{15}N data to calculate the rate of N uptake per day (objective 3; as $\text{NO}_3 + \text{NH}_4$). The ^{15}N atom percent excess (APE) was calculated as the difference in atom% ^{15}N between labelled and non-labeled plants from literature (typically atom% $^{15}\text{N} < 0.1$). The net uptake rate (NUR, $\mu\text{g N g}^{-1}$ shoot per day $^{-1}$) of N was calculated following McKane et al. (2002) as:

$$\text{NUR} = [\text{Plant N content } (\mu\text{g.g}^{-1}) \times (\text{APE}/100)] / ([\text{time (days)} \times (\text{atom\% } ^{15}\text{N}/ 100) \times \text{shoot mass (g)}].$$

The percentage of ^{15}N recovered in plants (^{15}N recovery %) was calculated as:

$$^{15}\text{N recovery (\%)} = (^{15}\text{N uptake} / ^{15}\text{N supplied}) * 100.$$

The ^{15}N uptake ($\mu\text{g N plant}^{-1}$) was defined as the amount of ^{15}N recovered from N pool (shoots) and calculated by multiplying APE (%) with the moles of N in the plant and ^{15}N supplied refers to the ^{15}N mass added to the soil per pot.

For our calculations of N uptake, I assumed that the shoot ^{15}N levels in each plant species were proportional to the amount of ^{15}N label taken up from the soil. While this approach simplifies differences in plant N allocation, our experiment was conducted when plant growth (native plants) and reproductive demands (*Linaria*, in flower) were high, so that N was more likely allocated to above- rather than below-ground structures. I also determined bulk %N from the same leaf samples used for IRMS to compare the effect of plant species and soil treatments on plant total N content.

Germination Experiment

The germination experiment consisted of a factorial design (2 x 2) in which I measured the effects of soil collection location (CO and IL) and invasion history on native seed germination. Seeds of *Rudbeckia hirta* and *Leptoloma cognata* were stratified and surface sterilized as described previously. I planted five seeds of each species together in 20 pots per treatment, including previously invaded or uninvaded soil from IL and CO, for a total of four treatments and 80 pots. I allowed seeds to germinate continuously in growth chambers over 30 days, watering pots to field capacity twice a week and counting the number of seedlings present at each time.

Soil Nutrients

Soil that was not used in growth-chamber and germination experiments was analyzed for NO₃, NH₄, P, and pH to determine whether invasion history or state of collection correlated to nutrient availability. Soil extractions were created for 320 soil samples collected from CO and IL (160 each). To create extractions, five grams of soil was mixed in 45 mL of deionized water by shaking for 30 mins and centrifuging. Extractions were tested for pH using a pH probe (Cite Company). Extractions and reagents were added to 96-well plates and analyzed for NO₃, NH₄, and P using colorimetry on an Epoch Microplate Spectrophotometer (BioTek Instruments, Winooski, VT, USA). Absorbance was reported in Gen5 software (BioTek Instruments, Winooski, VT, USA) and µg was calculated across all samples.

Statistical Analysis

I performed model selection across all datasets to determine the effects of state, invasion, and treatment as well as their interactions (Appendix 1). I used a mixture of linear models and generalized linear models (GLMs) with either quassipoisson, binomial, or Gamma distributions to account for non-normally distributed data. Quassipoisson and Gamma distributions were used where data were numeric, and decimal-degree differences would be ignored using an integer-based approach. I compared additive and interactive models with all predictors for all datasets. Model fit was verified using histograms and the qqPlot function in the car package (Fox & Weisberg 2019). For all model selection, models with the lowest AICc value were considered the best fit. The exception to this were quassipoisson models where I used pseudo- R^2 to determine the models of best fit, as AICc values are not available for quassipoisson distributions. I used the car package to extrapolate p-values from models, and statistical significance was designated by a p-value less than or equal to 0.05 (Fox & Weisberg 2019). For all linear models, I performed Tukey post-hoc tests to determine which treatments were significantly affected by predictors. Results were then visualized using ggplot2 (Wickham 2016).

The pots planted with both native plants and *L. vulgaris* often only had one or the other visibly growing in pots. Therefore, all pots planted with both native plants and *L. vulgaris* were removed from analyses, as there were very few pots in which the data accurately reflected the planting treatment. This reduced the number of pots in biomass and presence/absence analyses to 160. Isotope samples collected from pots with both native plants and *L. vulgaris* were also removed from analyses. Thus, our species treatment refers to pots that either contain *L. vulgaris* only or native plants only.

Plant Presence and Biomass

To determine whether *L. vulgaris* leaves legacy effects that influence plant success (objective 1), I analyzed the effects of invasion history on plant presence and root and shoot biomass. I analyzed our data separated by the state in which the soil and rhizomes were collected (IL or CO; objective 2) because preliminary analysis showed large differences across states. To compare presence/absence among treatments, I used generalized linear models (GLM) with binomial distributions across all possible combinations of variables (species, fertilizer, and invasion history). The species predictor had two factor levels (native species and *L. vulgaris*). Presence and absence of plants was designated with a 1 or 0, respectively, across all pots by treating all pots with shoot biomass greater than 0 as a 1 and all other pots as a 0. I performed model selection to determine if species, fertilizer, or invasion history influenced plant presence. I then calculated mean plant presence across treatments. Because of varying sample sizes, states and species were analyzed individually for biomass. This allowed me to compare the effects of invasion and fertilizer on biomass, but not cross-compare species that have physiological differences. I used the package emmeans to calculate p-values and confidence intervals for pairwise-comparisons (Lenth 2021). I also separated root and shoot biomass into separate datasets. I removed pots without any biomass as these were evaluated in presence/absence analyses. I used generalized linear models with gamma distributions and identity links to compare root and shoot biomass across fertilizer and invasion treatments in all datasets.

Stable Isotope Analyses

I compared the N uptake rates of *L. vulgaris* and native plants across all treatments to determine if N uptake rates were affected by invasion and fertilizer treatments (objective 3). Data was separated by state and species grown to account for different sample sizes. I used

generalized linear models with quasipoisson distributions to compare plant N uptake rates by invasion history and fertilizer treatments.

Germination Experiment

To measure average seedling germination, I calculated the mean number of seedlings across all days per pot. I then compared mean seedling germination across treatments. To account for repeated sampling of seedlings, I removed any days with greater than 5 seedlings as each pot was planted with 5 seeds. For samples grown in CO soil, I used generalized linear models with gamma distributions and identity links to compare mean seedling germination across all treatments. To account for pots with zero germination, 0.001 was added to all sample means. For samples grown in IL soil, I used linear models to compare mean seedling germination across treatments. Different models were used according to data normality for each dataset.

Soil Nutrients

Lastly, I compared soil nutrient availability across invaded and uninvaded soil from CO and IL. I calculated mean NO_3 , NH_4 , P, and pH across all four treatments and used either GLMs with quasipoisson or Gamma distributions with an identity link or linear models to determine whether state, invasion history, or their interaction significantly influenced soil nutrients prior to fertilization (Table 1).

Results

Plant Presence and Biomass

Overall, in CO soil, plant presence varied significantly by the interaction of invasion history and species (Appendix1, Fig. 2). In CO soil, *L. vulgaris* presence was significantly greater in invaded soil than uninvaded soil, irrespective of N fertilization treatment. In contrast, native plant presence in CO soil was not affected by invasion history. Overall, in IL soil, plant presence was significantly affected by the interaction between invasion history and species, (Appendix 1, Fig. 3). In IL soil, *L. vulgaris* was only present in uninvaded control pots, so I could not compare presence across treatments or calculate confidence intervals. Native plant presence was not affected by invasion history.

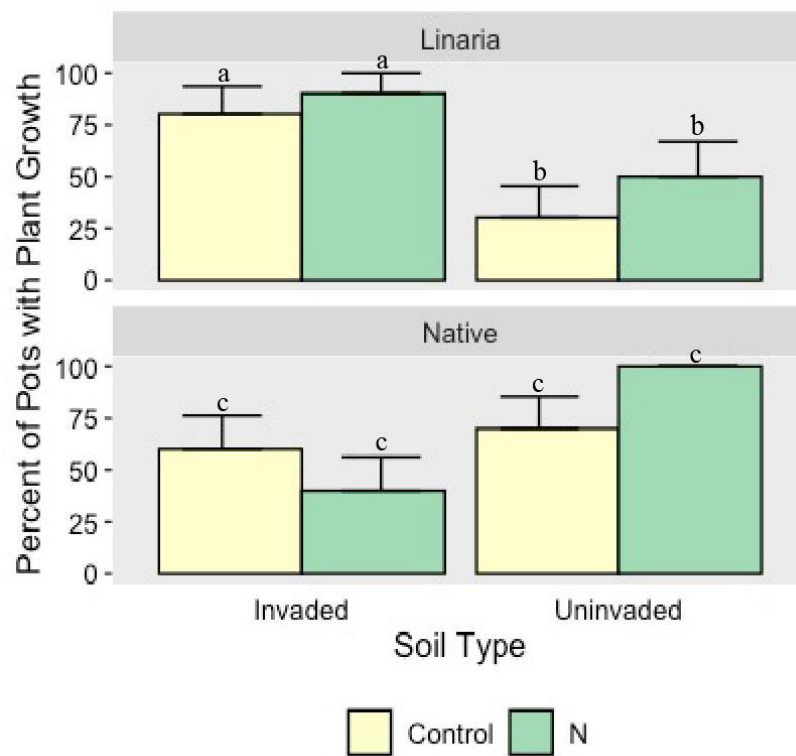


Figure 2. Percent of pots with plant growth representing plant presence/absence (1, 0) in pots containing CO soil (n = 80). Plant presence/absence differs significantly by invasion and species. *Linaria vulgaris* presence is significantly greater in invaded soil than uninvaded soil. Error bars represent mean plus standard error.

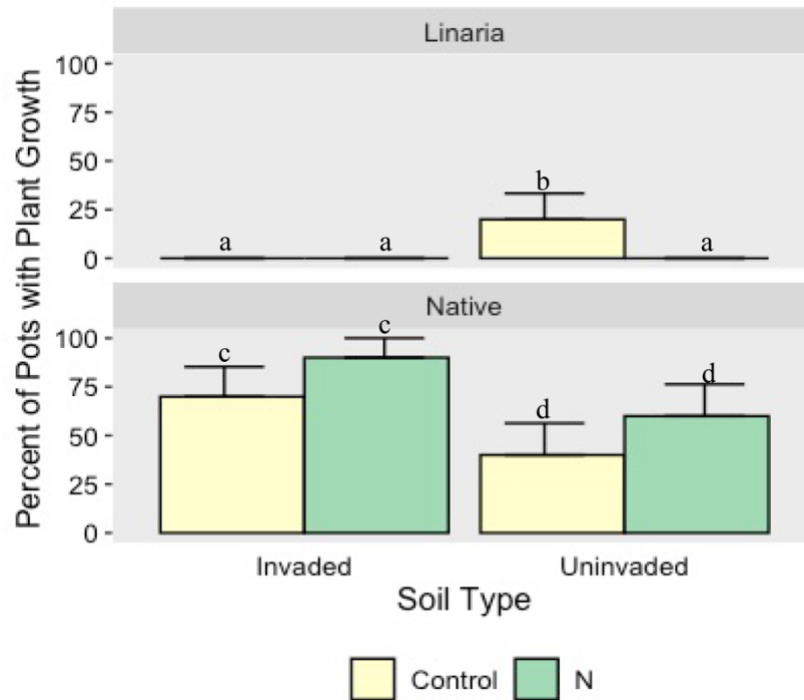


Figure 3. Percent of pots with plant growth representing plant presence/absence (1, 0) in pots containing IL soil (n = 80). Plant presence/absence differs significantly by invasion and species grown. Missing bars signify treatments without any plant presence. Error bars represent mean plus standard error.

In both CO (n = 47) and IL (n = 25) soils and among both *L. vulgaris* and native plants, shoot biomass was not influenced by invasion history or fertilizer. However, in CO soil, *L. vulgaris* root biomass was influenced by the interaction of invasion history and fertilizer (Appendix 1, Fig. 4). *Linaria vulgaris* root biomass was significantly higher in invaded soil than uninvaded soil. Within uninvaded soil treatments, *L. vulgaris* root biomass was higher in fertilized than control pots and in invaded soil, *L. vulgaris* root biomass was higher in control than fertilized pots. Native plant root biomass in CO soil was influenced by invasion alone. Native plant root biomass in CO soil was greater in uninvaded soil than invaded soil, but a 95% confidence interval suggests this relationship is not significant. In IL soil, native plant root biomass was influenced by fertilizer alone (Appendix 1, Fig. 5). Native plant root biomass was

greater in N-fertilized than unfertilized (control) IL soils. However, not enough *L. vulgaris* plants sprouted in IL soil to make a comparison across treatments.

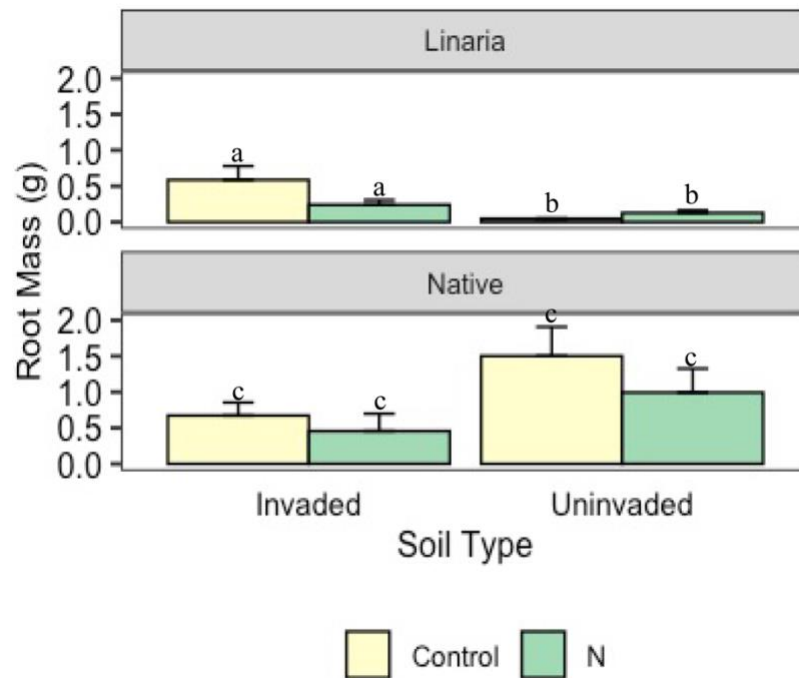


Figure 4. Root biomass for *L. vulgaris* and native plants grown in soil from CO (n = 47). *Linaria vulgaris* root biomass was significantly affected by the interaction between invasion history and fertilizer treatments. Native plant root biomass did not differ significantly by invasion history or fertilizer. Error bars represent mean plus standard error.

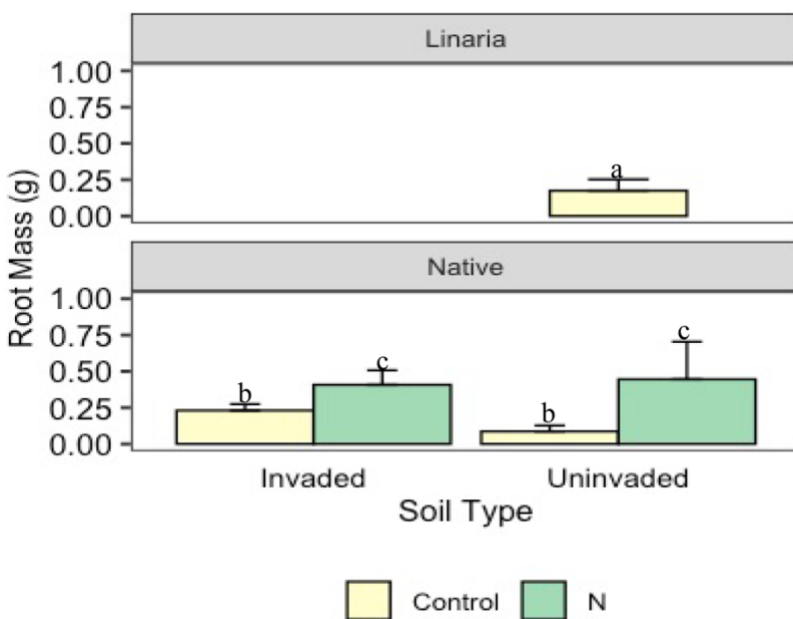


Figure 5. Root biomass for *L. vulgaris* and native plants grown in soil from IL (n = 25). Native plant root biomass was significantly influenced by fertilizer. Missing data in *Linaria vulgaris* pots is the result of few plants growing in IL soil. Error bars represent mean plus standard error.

Stable Isotope Analyses

Nitrogen uptake rates (NUR) for *L. vulgaris* in CO soil (n = 11) were not affected by invasion history or fertilizer treatments (Appendix 1, Fig. 6). N uptake rates for native plants grown in CO soil (n = 12) were not affected by invasion history or fertilizer treatment (Appendix 1, Fig. 6). In IL soil, native plant N uptake rates were not significantly influenced by invasion history or fertilizer treatments (Appendix 1, Fig. 7). Not enough *L. vulgaris* plants sprouted in IL soil to make a comparison across treatments.

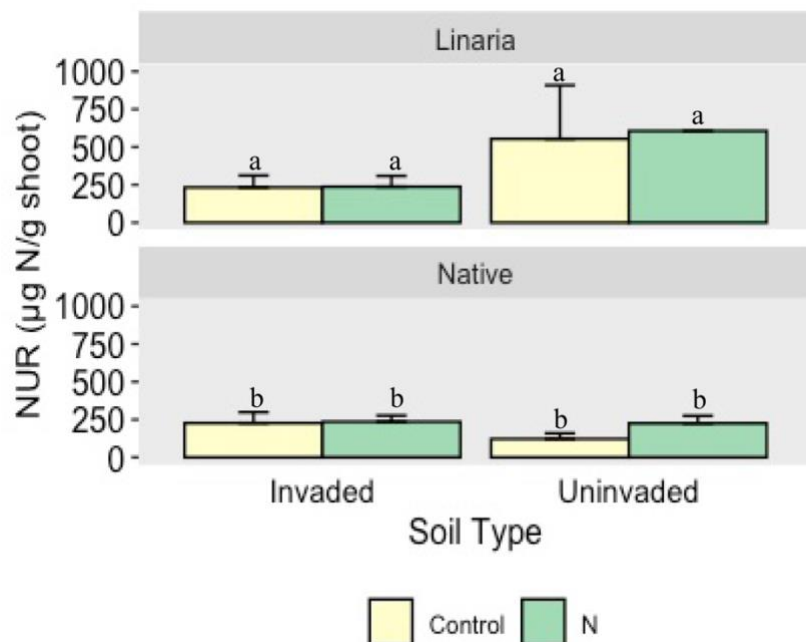


Figure 6. N uptake rates (NUR) in plants grown in CO soil (n = 23). *Linaria vulgaris* NUR did not differ by invasion history or fertilizer. Native plant NUR did not differ by invasion history or fertilizer. Error bars represent mean plus standard error.

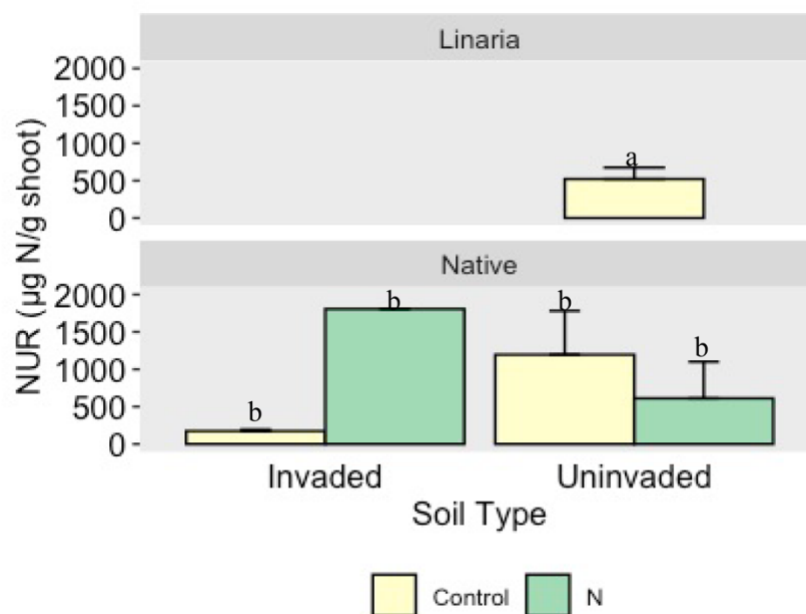


Figure 7. N uptake rates (NUR) in plants grown in IL soil (n = 13). Native plant NUR did not differ significantly by either invasion history or fertilizer treatments. Missing data in *Linaria vulgaris* pots is the result of few plants growing in IL soil. Error bars represent mean plus standard error.

Germination Experiment

Mean seedling germination in CO soil did not vary significantly by invasion history (Appendix 1, Fig. 8). In IL soil, mean seedling germination was also not influenced by invasion history (Appendix 1, Fig. 9).

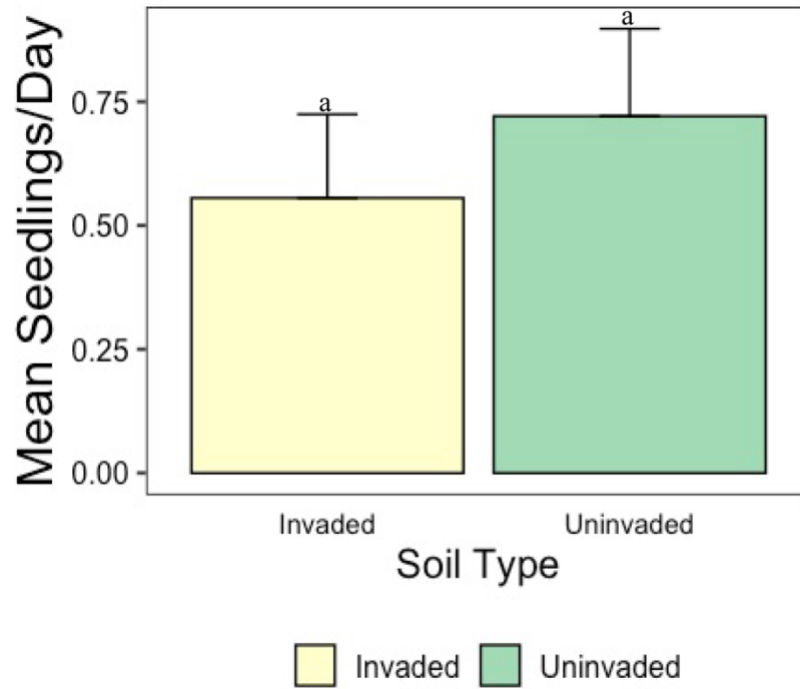


Figure 8. Mean seedling germination across 30 days since planting in *L. vulgaris* invaded and uninvaded soil collected from CO. Seedling germination did not vary significantly by invasion or sampling site.

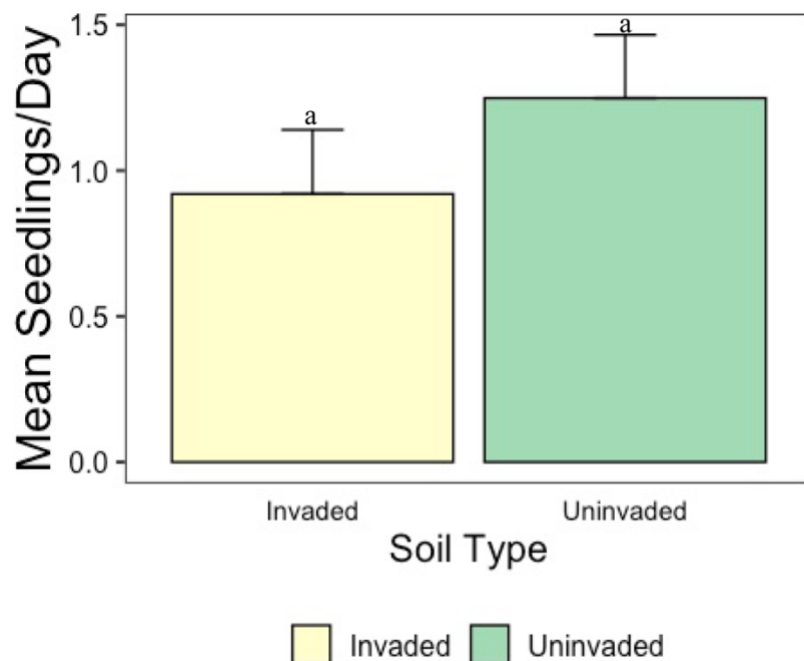


Figure 9. Mean seedling germination across 30 days since planting in *L. vulgaris* invaded and uninvaded soil collected from IL. Seedling germination did not vary significantly by invasion or sampling site.

Soil Nutrients

All soil nutrients and pH varied significantly by state, except for NO_3 and PO_4 (Table 2). Ammonium was significantly higher in CO soil than IL soil. In contrast, pH was significantly higher in IL soil than CO soil. Nitrate, ammonium, and pH were not significantly affected by invasion or the interaction between state and invasion. However, PO_4 was significantly affected by both state and invasion where the effect of invasion depended on state. Soil PO_4 was higher in invaded than uninvaded soil from IL and not different between invaded than uninvaded soil from CO.

Table 2. Summary of soil factors by state of collection and invasion history including means $\pm$ standard error and GLM p-values. Significant p-values are indicated in bold.

CO Soil means $\pm$ 1 SE	IL Soil means $\pm$ 1 SE	p-values
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Soil Factor	Invaded	Uninvaded	Invaded	Uninvaded	State	Invasion	State x Invasion
pH	6.62 ± 0.066	6.68 ± 0.072	7.88 ± 0.121	8.03 ± 0.070	<0.001 X ² = 78 df = 68	0.223 X ² = 71 df = 68	0.588
NO ₃	13.7 ± 1.87	15.3 ± 1.70	9.56 ± 1.58	11.3 ± 2.13	0.105 X ² = 77 df = 73	0.577 X ² = 72 df = 73	0.848
NH ₄	5.46 ± 0.214	5.83 ± 0.212	4.63 ± 0.344	4.80 ± 0.231	<0.001 X ² = 72 df = 69	0.295 X ² = 70 df = 69	0.699
PO ₄	17.1 ± 0.599	18.4 ± 0.811	21.8 ± 3.27	15.9 ± 1.53	0.072 X ² = 76 df = 75	0.592 X ² = 78 df = 75	0.041

Discussion

This study investigated the impacts of *L. vulgaris* invasion legacy on plant success of *L. vulgaris* and two native plants, whether this legacy is different in soil collected from CO and IL, and if N-availability could explain *L. vulgaris*’ success. I found that *L. vulgaris* plant presence differs between previously invaded and uninvaded soil, demonstrating that *L. vulgaris* may leave legacy effects in the soil following invasion. However, the direction of legacy effects for *L. vulgaris* differed between CO and IL soil, with a positive effect on *L. vulgaris* in CO soil and a possible negative effect on *L. vulgaris* in IL; these results indicate that soil characteristics, biological or chemical, influence the direction and magnitude of legacy effects. Lastly, plant N uptake rates were not affected by invasion history or fertilizer in either CO or IL soil. Taken together, these results suggest that *L. vulgaris* invasion results in changes to the soil microbial community or soil chemistry.

Our results suggest that soil characteristics are at least partly responsible for the geographic distribution of aggressive *L. vulgaris* invasion in the U.S. *Linaria vulgaris* is present in more pots with invaded soil compared to pots containing uninvaded soil from CO, suggesting

that invasion results in a positive soil-feedback in CO soil. In contrast, invasion history was not a significant predictor of native plant presence, suggesting that *L. vulgaris* invasion legacy in CO soil does not reduce native plant success. In IL soil, *L. vulgaris* only grew in uninvaded soil, and native plant presence was not affected by invasion history. This result is consistent with the interpretation that invasion results in a negative soil-feedback for *L. vulgaris* in IL soil but does not negatively affect native species.

Evidence of a positive soil-feedback on *L. vulgaris* presence in CO soil shows that *L. vulgaris* shapes soil characteristics in CO, where it is aggressive, to become more successful. I attribute greater plant presence in pots to increased sprouting of rhizomes, which may be the result of soil community changes following invasion. Soil mutualists, including AMF and bacteria, could increase rhizome sprouting by enhancing nutrient and water uptake or increasing pathogen resistance, among other mechanisms (Liu & Ludwig 2019; Quinn & Holt 2008). Alternatively, *L. vulgaris* could be changing soil nutrient and water availability in ways that promote its presence, either directly through its nutrient uptake, or by influencing litter decomposition, leaching rates, and other soil qualities (Evans et al 2001; Corbin and D'Antonio 2004). However, because fertilizer did not influence plant presence, N availability is unlikely to be responsible for legacy effects.

In IL soil, evidence of a negative soil-feedback on *L. vulgaris* presence could be attributed to suppressive microbial interactions. The enemy release hypothesis proposes that species escape negative soil-feedbacks by establishing outside their native range and become invasive without the same biological suppression (Keane and Crawley 2002; Maron et al 2013). In the case of *L. vulgaris*, it has both negative soil-feedbacks in its native range, parts of its non-native range, and positive-feedbacks in its invasive range, which could be the result of AMF

mutualisms (Maron et al 2013; Klironomos 2002). Some invasive plants that benefit from AMF mutualisms in their invasive range can only do so when their pathogens are absent, resulting in strong net positive feedbacks (Klironomos 2002; Reinhart et al 2003). Therefore, in CO soil a lack of inhibitory microbes could enhance a positive soil-feedback. However, I cannot be certain that the lack of *L. vulgaris* plants in invaded soil is due to soil legacy and not site or individual plant differences.

Interestingly, invasion legacy did not influence native plant presence in either CO or IL soil, suggesting that *L. vulgaris* invasion does not negatively affect native plants grown in CO or IL soil. The absence of negative effects on native plants indicates that *L. vulgaris* soil legacy most likely does not inhibit native plant growth in either state. For managers, this means that restoring previously invaded sites should not require soil remediation to reintroduce native plants and management can be centered around *L. vulgaris* removal. However, a caveat of this study is that the native plants I tested are native to IL, so the absence of negative effects on these species may not reflect consequences of *L. vulgaris* invasion on CO native plant communities. While some invasive species can have broad negative effects on native species, such as those that disrupt plant-mycorrhizal mutualisms, other invasive plants have a more targeted approach on their competitors (Zubek et al 2016). In addition, native plant presence is not the only metric for plant success and other mechanisms such as reproductive output, leaf size, among others could also be affected by invasion legacy.

Different responses to invasion legacy in CO and IL soil could correspond to soil nutrient availability. If *L. vulgaris* has soil-feedbacks that depend on nutrient availability, nutrient differences in CO and IL could affect the strength and direction of feedbacks. Soil NH_4 and pH were both significantly different by state, which could change the direction and effect size of

soil-feedbacks. However, the only soil nutrient that varied by state and invasion history (interactive model) was PO_4 , with higher PO_4 in invaded soil than uninvaded soil in IL and no difference between invaded and uninvaded CO soil. It is possible that either *L. vulgaris* can only grow in high PO_4 soil or it directly affects soil PO_4 in IL. However, I only tested nutrients after invasion, so I was unable to determine if invasion directly changes soil PO_4 . Otherwise, soil microbial composition in IL could contribute to PO_4 dynamics, which could reduce *L. vulgaris*' aggressiveness there. Future studies looking at the relationships between invasion, soil microbial communities, and soil PO_4 could uncover the mechanisms behind legacy effects and *L. vulgaris*' aggressive distribution.

While there was a legacy effect of invasion on overall plant presence in both CO and IL soil, the effect size in CO soil was 100x greater than in IL soil. There is evidence of a negative soil-feedback for *L. vulgaris* in IL, but evidence of a positive feedback on *L. vulgaris* presence in CO is a much stronger driver of *L. vulgaris*' aggressive distribution. Aside from soil differences, *L. vulgaris* genotype differences could partially account for feedback direction and magnitude across states. I grew *L. vulgaris* rhizomes from both CO and IL in their soil of origin, meaning we cannot rule out genotypic variation that could influence soil-feedbacks. In some species, different genotypes of the same species can associate with different microbes, influencing plant fitness and nutrient dynamics (Brown et al 2020). Future studies should compare genotypes of *L. vulgaris* across CO and IL populations and whether genotypes influence legacy effects.

Although invasion history affects plant presence in both CO and IL, I did not find evidence that shoot biomass was affected by fertilizer or invasion history in any of our datasets. However, some treatments did affect root biomass. In CO soil, *L. vulgaris* root biomass was greater in invaded than uninvaded soil. This supports our findings that the legacy of *L. vulgaris*

in CO soil increased plant sprouting, as *L. vulgaris* primarily reproduces via rhizomes, and increased root mass could lead to increased clonal reproduction. Plants may be allocating more of their resources into reproduction rather than shoot growth. Fertilizer also affected root biomass but not unilaterally across treatments, making it difficult to assert whether N-availability increases or decreases *L. vulgaris* productivity in CO soil. Additionally, native plants in CO soil had greater root mass in uninvaded soil, contradicting our finding that *L. vulgaris* has no effect on native plant presence. The negative effect of invasion on native plant biomass could be more indicative of native plant productivity and resource allocation than abundance. Native plants in IL soil had more root biomass in fertilized pots compared to control pots irrespective of invasion history. Native plants may be able to absorb more N in their home soil where their mycorrhizal symbionts are, making fertilizer treatments more effective. Because invasion did not significantly affect native plant root biomass in IL soil, *L. vulgaris*' negative effect on native plants is only apparent in CO soil. However, too few *L. vulgaris* plants grew in IL soil to compare across treatments.

Furthering our belief that N availability does not explain *L. vulgaris*' success, in both CO and IL soil, N uptake rates for both *L. vulgaris* and native plants were not different across invasion or fertilizer treatments. If N uptake was a strong determinant of plant success, I would expect to see higher N uptake rates in treatments where sprouting and growth was highest. Because legacy effects from *L. vulgaris* did not impact N uptake rates, this suggests that changes in soil nutrients or microbial communities resulting from invasion do not impact N uptake in *L. vulgaris* or the native plants in this study. Similar N uptake rates in plants from fertilized and unfertilized treatments illustrates that plants were not N-limited, implying that environmental N is not a driver for *L. vulgaris*' success. However, not enough *L. vulgaris* plants sprouted in IL

soil to compare the effect of treatments on N uptake rates, so our conclusions are limited to CO soil. Determining how N-availability impacts *L. vulgaris* in areas outside of CO will require more data, which could reveal more about microbial differences across its range.

Our finding that native plant presence is unaffected by invasion legacy in both states is supported by the results of our germination study. In both CO and IL soil, native seed germination was not affected by invasion history. Based on these results, the soil legacy of *L. vulgaris* invasion does not appear to influence native seed germination. It is possible that plant survival, particularly at the seedling stage, could be affected by invasion, but I did not measure survival in this study. Unfortunately, I could not prevent non-target species from emerging and did not remove seedlings after counting, meaning seedlings could have been counted more than once. Additionally, I only measured native seed germination and thereby cannot make predictions about *L. vulgaris* rhizome sprouting, though increased rhizome sprouting is one possible explanation for greater *L. vulgaris* presence in invaded CO soil. With caution, our results suggest that native plants are not negatively impacted by invasion legacy. Although, I recommend that future studies examine seed germination in a broader suite of native species and *L. vulgaris* rhizome sprouting as possible mechanisms of *L. vulgaris* soil feedbacks.

Disturbance likely contributed to soil characteristics in both states, making it hard to attribute soil properties to soil community differences alone. Specifically, I expected to see lower NO_3 and NH_4 in CO soil than IL soil based on statewide trends, but instead I observed the opposite trend for NH_4 and no difference in NO_3 between states, which could be because disturbances affected N dynamics. In IL, my sampling sites were located along trails, roadsides, and train tracks where soil may be contaminated with chemical runoff or physically disturbed. CO sites had high animal activity, which could have resulted in higher N inputs than anticipated.

While N availability did not appear to influence soil feedbacks, other effects of site disturbance could have influenced soil nutrient data as well as soil feedbacks in one or both states.

Ultimately, our study shows that soil is an important variable shaping *L. vulgaris*' aggressive distribution and its impacts on native plant communities. Differences in genotypes, climate, competition, and other variables may also play a role in *L. vulgaris*' aggressive range. However, reducing soil legacy effects could be a feasible way to manage this species once we understand what microbial and chemical changes result from invasion. In CO, fully eradicating *L. vulgaris* will necessitate repeated removals, as legacy effects will promote re-invasion. Whereas in IL, legacy effects may help control *L. vulgaris* invasion, so removal appears unnecessary. Native plant presence and germination appears unaffected by invasion legacy in both states, but plants appear to be allocating resources differently and other characteristics could be affected by invasion. Furthermore, N availability does not appear to influence *L. vulgaris*' success, but future studies should evaluate how other soil nutrients, including PO₄, and soil microbes influence and are influenced by invasion. My results support previous research in invasive plant soil legacy effects, namely that soil-feedbacks are strong drivers of invasion distributions. I hope that this study will encourage more research in invasive plant impacts on soil communities throughout their range.

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Appendix 1. Model summaries for plant presence, root biomass, and soil nutrient datasets. N/A in the $\Delta AICc$ column is the result of using quasipoisson distributions that lack AIC values. N/A in the Model column means that the best-fit model has only one predictor. The model listed for each dataset is the best-fit model.

Dataset	State	Predictors	Model	$\Delta AICc$	X^2	df	P
Presence	CO	Invasion Species	Interactive	2.8	9.09 5.81	1 1	0.002 0.01
Presence	IL	Invasion Species	Interactive	2.3	2.87 33.8	1 1	0.089 <0.001
<i>Linaria</i> Root Biomass	CO	Invasion Fertilizer	Interactive	1.3	20.6 23.0	21 21	0.005 0.075
Native Root Biomass	CO	Invasion Fertilizer	Additive	3.0	21.5 22.0	22 22	0.046 0.304
Native Root Biomass	IL	Invasion Fertilizer	Interactive	2.5	23.0 23.0	22 22	0.149 0.024
<i>Linaria</i> NUR	CO	Invasion Fertilizer	Interactive	N/A	12.0 12.0	11 11	0.377 0.986
Native NUR	CO	Invasion Fertilizer	Interactive	N/A	12.0 12.0	11 11	0.185 0.926
Native NUR	IL	Invasion Fertilizer	Interactive	N/A	11.0 8.98	9 9	0.179 0.106
Germination	CO	Invasion	N/A	20.2	15.3	17	0.511
Germination	IL	Invasion	N/A	10.2	18.0	16	0.159
pH	Both	State Invasion	Additive	2.0	78.0 71.3	68 68	<0.001 0.233
NO ₃	Both	State Invasion	Interactive	N/A	77.3 72.6	73 73	0.105 0.577
NH ₄	Both	State Invasion	Additive	2.1	72.0 70.0	69 69	<0.001 0.295
PO ₄	Both	State Invasion	Interactive	2.1	76.0 78.0	75 75	0.072 0.592

Chapter 2: The Role of Arbuscular Mycorrhizal Fungi in *Linaria vulgaris* Invasion

Introduction

Mycorrhizal fungi are fundamental to plant diversity on earth (Van der Heijden 2002). Mycorrhizae are plant symbionts that promote plant nutrient and water uptake from the soil and protect plants from pathogens and environmental stressors, such as drought or heavy metal toxicity (Dodd 2000). Arbuscular mycorrhizal fungi (AMF, Glomeromycota) are the most prevalent group of mycorrhizal fungi, forming relationships with upwards of 80% of terrestrial plant families on earth (Dodd 2000). The presence of AMF is necessary to support plant diversity, as removing AMF from soil significantly reduces plant species richness and biomass (Van der Heijden et al 2008). There are currently only around 380 described species of AMF, but the true diversity is expected to be much greater (Lee et al 2013; Maia et al 2020).

The diversity of AMF shape plant communities (Vogelsang et al 2006). Specifically, AMF have extensive functional diversity, with large variation both between and within species of AMF, which varies with environmental conditions and the species of their host plant (Hart and Reader 2002; Munkvold et al 2004). The interactions between AMF and their hosts can vary widely, with species ranging from parasites to mutualists of their host plants (Johnson et al 1997; Lee et al 2013). As mutualists, AMF provide plants with nutrients and water from the soil in exchange for sugar, but this relationship can become parasitic if nutrients and water are in high abundance (Johnson et al 1997). The functional diversity of AMF is one of the reasons why plants are so diverse, as specific mycorrhiza confer different advantages or burdens to plants and as such regulate plant-plant competition (Van der Heijden et al 1998).

Certain species of plants can also change the AMF community in the surrounding soil to encourage their own symbionts, deter their competitors' symbionts, or recruit their competitors' parasites (Van der Putten et al 2007). These plants release secondary compounds that disrupt AMF mutualisms or increase colonization by preferred AMF species (Shah et al 2009). In areas where competition for resources is strong, such as in nutrient- and water-limited environments, plants that manipulate the AMF community can give themselves an advantage against their competitors (Entry et al 2002). Plants that mold the AMF community to their benefit can thereby reduce diversity by outcompeting other species and becoming dominant in an area (Zubek et al 2016). However, research connecting individual mycorrhizal taxa to their functions, particularly across different plant hosts is still in its preliminary stages.

Some invasive plants can modify the AMF community because they are not dependent on the same species of mycorrhizae as native plants, or they are non-mycorrhizal (Lankau 2010; Bunn et al 2015). Plants that change the AMF community outside of their native range are more likely to become successful invaders, which can result in a larger distribution or denser patch of the invader (Shah et al 2009). Several studies have shown that invasive plants have negative soil feedbacks in their native range and positive feedbacks in their introduced range, suggesting that pathogen escape and/or new associations with AMF may explain plants' invasion success (Callaway et al 2004; Dostálek et al 2016; Reinhart and Callaway 2006). These positive AMF associations may confer specific nutrient benefits to invaders, allowing them to outcompete native species when nutrients are already limited (Hawkes et al 2005). Understanding the relationships between invasive plants and mycorrhizas could help us unlock tools to reduce their spread.

This study addresses the biogeography of an invasive plant, *Linaria vulgaris*, and its relationship to AMF community composition in two regions where it is non-native: one with aggressive spread (Colorado, USA) and one with non-aggressive spread (Illinois, USA). Soil in the Rocky Mountain Region has low ambient N, whereas soil in the Midwestern U.S. has relatively high N levels, and *L. vulgaris* is more pervasive in Colorado than in Illinois natural areas (Bowman et al 2012; NADP 2021; USDA, NRCS 2021). Little is known as to why *L. vulgaris* proliferates in N-limited areas within the U.S. when it is found throughout much of North America. This suggests that *L. vulgaris* invasion changes the AMF community in Colorado to outcompete native plants for N, where native plants may be more reliant on AMF for N absorption but does not manipulate the AMF community in Illinois where N is in greater abundance. I ask: 1) Does AMF community composition differ surrounding *L. vulgaris* in CO and in IL; 2) Does *L. vulgaris* cultivate specific AMF communities in comparison to adjacent uninvaded areas; and 3) To what extent do soil nutrients modulate these processes? I collected soil from CO and IL and used Next Generation Sequencing to determine AMF community composition. In addition, I tested soil for plant-available nitrate, ammonium, phosphate, and for pH and soil texture. In total, this study aims to broaden our understanding of plant invasion effects on AMF communities and the relationship between soil characteristics and invasion biogeography.

Methods

Study Sites

I performed fieldwork at the Rocky Mountain Biological Lab in Gothic, CO and in the Forest Preserves of Cook County in Cook County, IL. The Rocky Mountain Biological Lab

(RMBL) is a research station located in the former mining town of Gothic Colorado, within Gunnison National Forest. The Gothic area has a mean annual rainfall of 579.6mm, a maximum daily temperature of 31.6°C and a minimum daily temperature of -19.4°C (NOAA 2022). I sampled in subalpine meadows (~2895m above sea level, a.s.l.), which are characteristically made up of diverse grasses, forbs, and shrubs (Price and Waser 1998). *Linaria vulgaris* is an aggressive invader at the Rocky Mountain Biological Lab, and many populations are sprayed with herbicide (Glyphosate, RoundUp) to control the spread. I collected soil samples from populations that had no history of herbicide use. The Forest Preserves of Cook County are semi-urban natural areas in the Chicagoland area and are made up of prairies, woodlands, and wetlands (FPDCC 2020). The Chicagoland area (178m a.s.l.) has a mean annual rainfall of 987.0mm, a maximum daily temperature of 39.4°C and a minimum daily temperature of -23.3°C (National Weather Service 2022). Here, I conducted sampling in highly disturbed areas adjacent to trails, roads, or railroads as this is where *L. vulgaris* was found (USGS 2022). Herbicide history was not possible to obtain but *L. vulgaris* is not a target species for managers in Illinois and I avoided areas that were visibly treated with herbicide.

Study Species

Linaria vulgaris (Plantaginaceae) is a forb native to Europe and Asia that has spread throughout North America since the 1600s (Sing et al 2016; Parker and Gassmann 2021). While found throughout the U.S., it is considered noxious in much of the Western U.S. and Alaska (Sing et al 2016). It's able to reproduce both from seed and clonally from rhizomes, but the latter is believed to be much more prevalent as its seed viability is only 25% or less (Parker and Gassmann 2021). Reproducing clonally allows *L. vulgaris* to grow in dense patches and

subsequently exclude native species (Sing et al 2016). *Linaria vulgaris* is an undesirable species for cattle and invades both rangeland and agriculture lands (Parker and Gassmann 2021). While several insect controls have been proposed, current management typically consists in applying herbicide (Sing et al 2016; Parker and Gassmann 2021).

Field Sampling

I identified plots containing *L. vulgaris* from research records in Gothic, CO and using iNaturalist in Cook County, IL. In the summer of 2020, I collected soil samples from *L. vulgaris* invaded and adjacent uninvaded areas for growth-chamber experiments. I demarcated plots using two measuring tapes in N-S and E-W directions and calculated the areas of invaded plots by multiplying N-S and E-W lengths. In plots greater than 100m², I only sampled the 5m² area surrounding the center point of to capture soil with the longest invasion history (Fig. 1). To locate paired uninvaded plots, I selected a random cardinal direction from the invaded plot and established an uninvaded plot of the same size as the invaded plot at 5-15m from the invaded plot (Fig. 1). To ensure random sampling throughout plots, I used a random number generator to select points on the tape measures to collect each sample (Fig. 1). In both IL and CO, I collected 160 soil cores (101.6 mm, 152.4 mm; total n=320): eight samples from each of 10 invaded plots and 10 adjacent uninvaded plots. I collected soil and *L. vulgaris* rhizomes from CO in July of 2020 and from IL in August of 2020.

Soil Nutrient Analysis

Soil that was not used in Next Generation Sequencing was analyzed for NO₃, NH₄, PO₄, and pH to determine whether nutrient availability varied with invasion history or state of

collection. Soil extractions were created for all 320 soil samples collected from CO and IL (160 each). To create extractions, five grams of soil was mixed in 45 mL of deionized water by shaking for 30 mins and centrifuging. Soil extracts were tested for pH using a pH probe (Fisher Scientific, Waltham, MA, USA). Extracts were filtered and analyzed colorimetrically on a microplate spectrophotometer (Biotek Epoch, Winooski, VT) using the vanadium reduction method for NO_3 (Doane & Horwath 2003), phenol- hypochlorite method for NH_4 (Weatherburn 1967), and the malachite green method for PO_4 (Baykov et al 1988). Absorbance was reported in Gen5 software (BioTek Instruments, Winooski, VT, USA) and nutrient levels ($\mu\text{g/g}$ soil) were calculated across all samples using standard curves for each nutrient.

Soil Texture Analysis

Texture was analyzed using the Bouyoucos method (Bouyoucos 1962). Soil samples were pooled per sampling site and by invasion, creating four treatments with 10 samples in each (IL invaded, IL uninvaded, CO invaded, CO uninvaded). For each sample, 50-grams of soil was mixed with 50mL of sodium hexametaphosphate solution and allowed to sit for 10 minutes. The solution was then mechanically ground in a blender and added to a graduated cylinder with water filled to the 1000mL mark. The solution was then mixed with a plunger and 40 seconds after mixing, a hydrometer was used to measure the density and a thermometer was used to measure the temperature. Cylinders were left to sit overnight and a second temperature and hydrometer reading was taken 24 hours after the initial reading. The percentage of clay, sand, and silt were calculated using the two readings.

AMF Community Sampling

I extracted genomic DNA from 0.25g of soil for 80 samples using the DNeasy PowerSoil Kit (QIAGEN-MO BIO, Carlsbad, California, USA). Extractions were cleaned using Cytiva Sera-Mag Select beads prior to amplification (Global Life Sciences Solutions, Marlborough, MA, USA). A 550 bp section of small-subunit ribosomal DNA (18S rRNA) was amplified using AML2 and NS31 primers (Lee et al. 2008). The forward primer, NS31, sequence is TTGGAGGGCAAGTCTGGTGCC. The reverse primer, AML2, sequence is GAACCCAAACACTTTGGTTTCC. I created PCR mixtures containing 12ul DreamTaq Hot Start PCR Master Mix, 1ul DNA, 0.5 ul of each primer, 1ul bovine serum albumin (BSA), and 9ul nuclease-free water for a total of 24ul. The first PCR was performed once using EppendorfTM MastercyclerTM pro PCR Systems with primers lacking adaptors and then a second PCR was performed using the products of the first PCR and primers with adaptors attached. PCR reactions entailed 2 minutes at 94°C, 30 cycles of 30 seconds each at 94°C, 1 minute at 59°C, 2 minutes at 72°C, and 10 minutes at 72°C.

DNA amplification was verified by gel electrophoresis on a 1.5% agarose gel, which was then visualized on a Kodak Electrophoresis Documentation and Analysis System 290 (EDAS 290, Eastman Kodak Company, Molecular Imaging Systems, Rochester, NY, USA). Amplified DNA was cleaned again using Cytiva XP beads. Sample concentrations were then quantified in Qubit using a dsDNA HS assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Samples were dried down on a SpeedVac Vacuum Concentrator and then rehydrated with Tris HCL 10mM to 1ng/ul (Thermo Fisher Scientific, Waltham, MA, USA).

Samples were indexed using a Nextera XT Index Kit and reagents from the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA). Sample concentrations were quantified again in Qubit using a dsDNA HS assay Kit and were normalized to 1ng/ul. Aliquots of 1µl of each

sample were pooled and sequenced at Northwestern on Illumina MiSeq version 3, 300-bp MiSeq Reagent Kit for paired-end reads (Illumina, San Diego, CA, USA). The first run yielded low-complexity sequences and as a result, samples were re-sequenced using Illumina MiSeq version 3, 150 bp MiSeq Reagent Kit for paired-end reads (Illumina, San Diego, CA, USA). Fragment size was measured using an Agilent 2100 Bioanalyzer System (Agilent Technologies, Waldbronn, Germany).

Analysis

Samples were demultiplexed in Illumina BaseSpace and processed in QIIME2 (Bolyen et al, 2019). I used DADA2 to denoise demultiplexed samples and truncated reads at 120 basepairs, where quality dropped below a q value of 20 (Callahan et al 2016). Only forward sequences were included in further analyses, which follows research demonstrating that forward sequences are sufficient to capture AMF diversity (Davison et al 2012), totaling 7,092,099 reads. Sequences were included if they were reported at least 10 times and in 3 or more samples. OTUs were clustered at 97% (96 OTUs) using vsearch and chimeras and borderline chimeras were removed. Taxonomy was assigned using the q2-feature-classifier (Bokulich et al 2018) naïve bayes classifier with MaarjAm reference sequences. OTUs with less than 80% alignment to MaarjAm reference sequences were removed. OTUs were blasted against the MaarjAm Database (Öpik et al, 2010) and GenBank (Benson et al., 2012) to determine AMF diversity.

Sequences that could not be identified to AMF family level were aligned in CLUSTAL Omega using default parameters, and the percent identities among sequences determined. Pairwise sequences or multiple-sequence alignments with >97% similarity were classified as the same OTU. This resulted in 47 unique sequences for phylogenetic analyses. Unique sequences

were then concatenated with a reference set of SSU sequences (Krüger et al. 2012) for major AMF genera (*Scutellospora*, *Gigaspora*, *Acaulospora*) as well as *Glomus* species and allied taxa (*Archaeospora*, *Claroideoglossum*, *Ambispora*, *Funneliformis*, *Paraglossum*, *Rhizophagus*). Alignments were trimmed and a maximum likelihood phylogenetic tree was inferred for the concatenated data set using RAxML with default options. Eighteen-sequences could be placed with the *Glomus* clade, while the remaining 29 sequences were consistent with Glomeraceae but showed no specific phylogenetic placement. Clustal Omega and RAxML analyses were undertaken on the CIPRES gateway (v3.3) at the San Diego Supercomputer Center (www.phylo.org).

All other statistics were performed in R (R Core Team, 2019). I performed permutational multivariate ANOVAs (PERMANOVAs) using the `adonis2` function in the ‘vegan’ package to test whether AMF communities were influenced by invasion history, state, and individual soil factors (Oksanen et al. 2020). I also calculated indicator species analyses using the `multipatt` function in the `indicspecies` package (Table 2,3; Cáceres et al 2010). Additionally, I separated relative abundances by families and performed Kruskal-Wallis tests to determine if family abundances differed by state or invasion. Relative abundances of AMF OTUs across treatments were visualized on Nonmetric Multidimensional Scaling (NMDS) plots using the `metaMDS` function in the ‘vegan’ package (Oksanen et al. 2020). Note that the NMDS is used for visualization only. Analysis of community composition was performed using PERMANOVAs. I also performed two-way ANOVAs to test differences in soil factors, OTU richness, and family richness across states and invasion treatments using both additive and interactive models.

Results

Soil Nutrients

All soil nutrients and pH varied significantly by state with the exception being PO₄ and NO₃ (Table 2). Ammonium (NH₄) was significantly higher in CO soil than IL soil, whereas pH was significantly higher in IL soil than CO soil (Table 2). Nitrate, ammonium, and pH were not significantly affected by invasion or the interaction between state and invasion. However, PO₄ was significantly affected by invasion, though this effect depended on state. Soil PO₄ was higher in invaded than uninvaded IL soil and not affected by invasion in CO soil.

Table 1. Summary of soil factors by state of collection and invasion history including means $\pm$ standard error and two-way ANOVA p-values. Significant p-values are denoted in bold.

Soil Factor	CO Soil		IL Soil		Statistical Comparisons		
	Invaded	Uninvaded	Invaded	Uninvaded	State	Invasion	State* Invasion
pH	6.66 $\pm$ 0.1	6.73 $\pm$ 0.1	7.88 $\pm$ 0.1	8.03 $\pm$ 0.1	<0.001 X ² = 78 df = 68	0.196 X ² = 71 df = 68	0.672
NO ₃ (μ /g)	13.6 $\pm$ 2.0	15.3 $\pm$ 1.7	7.72 $\pm$ 1.0	11.3 $\pm$ 2.1	0.105 X ² = 77 df = 73	0.577 X ² = 72 df = 73	0.848
NH ₄ (μ g/g)	5.47 $\pm$ 0.2	5.82 $\pm$ 0.2	4.89 $\pm$ 0.3	4.80 $\pm$ 0.2	0.003 X ² = 72 df = 69	0.686 X ² = 70 df = 69	0.394
PO ₄ (μ /g)	17.3 $\pm$ 0.6	18.6 $\pm$ 0.8	22.6 $\pm$ 3.6	15.9 $\pm$ 1.5	0.063 X ² = 76 df = 75	0.583 X ² = 78 df = 75	0.033
Sand (%)	60.9 $\pm$ 3.9	59.6 $\pm$ 4.6	70.0 $\pm$ 4.3	67.9 $\pm$ 4.0	0.044 X ² = 78 df = 38	0.700 X ² = 78 df = 38	0.926
Silt (%)	28.4 $\pm$ 3.8	29.0 $\pm$ 4.2	17.9 $\pm$ 3.7	19.0 $\pm$ 3.4	0.008 X ² = 78 df = 38	0.823 X ² = 78 df = 38	0.941
Clay (%)	10.7 $\pm$ 0.5	11.4 $\pm$ 0.7	12.2 $\pm$ 0.88	13.1 $\pm$ 1.0	0.056 X ² = 64 df = 32	0.325 X ² = 63 df = 32	0.885

AMF Diversity

Sequencing resolved 61 OTUs. Of these 61 OTUs, 29 were not identified to family level, but most likely belong to Glomeraceae. The remaining 32 OTUs belonged to 7 genera and 6 families. Average family abundances per sample in order from highest to lowest are: Glomeraceae (23 OTUs, 50.0% of total reads), Clarideoglomeraceae (2 OTUs, 20.6% of total reads), Paraglomeraceae (3 OTUs, 12.2% of total reads), Diversisporaceae (1 OTU, 4.5% of total reads), Archaeosporaceae (2 OTUs, 1.8% of total reads), and Ambisporaceae (1 OTU, <1% of total reads). The unidentified OTUs make up the remaining 10.7% of total reads.

AMF Community Composition

The PERMANOVA revealed that there was a significant difference in AMF OTU composition between soil collected from CO and IL (Figure 1, $p=0.001$, $F=10.5$). However, AMF OTU composition was not significantly different between invaded and uninvaded sites from both states ($p=0.857$, $F=0.622$). In CO soil alone, AMF OTU composition was not significantly different between invaded and uninvaded sites ($p=0.792$, $F=0.651$). Similarly, in IL soil alone, AMF OTU composition was not significantly different between invaded and uninvaded sites ($p=0.623$, $F=0.807$). When comparing relative abundances of families by state, only Diversisporaceae ($p<0.001$, $X^2 = 35.2$, $df = 1$) and Archaeosporaceae ($p<0.001$, $X^2 = 10.8$, $df = 1$) were different between CO and IL. In contrast, in invaded and uninvaded soil, only Glomeraceae ($p=0.031$, $X^2 = 4.64$, $df=1$) was significantly different by invasion history.

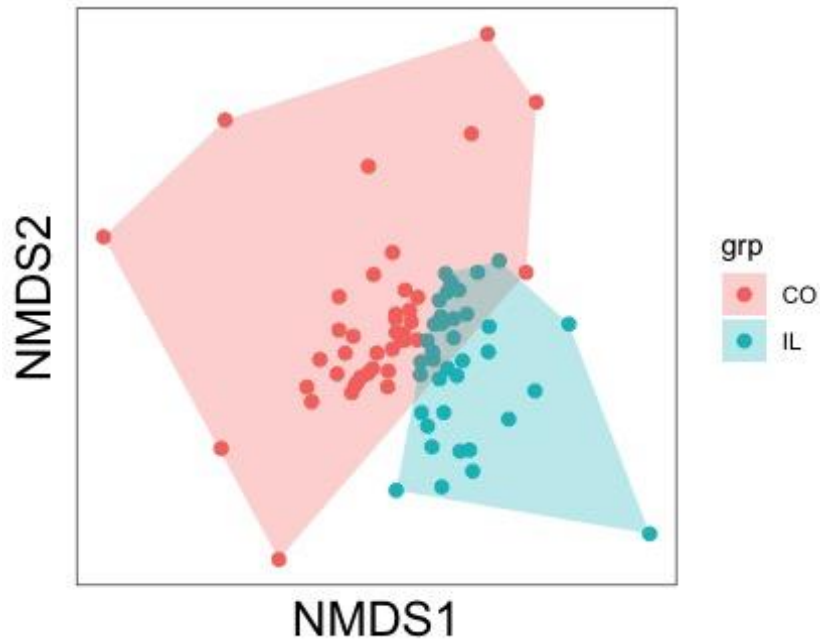


Figure 1. Non-metric multidimensional scaling plot of AMF OTUs in CO and IL soil.

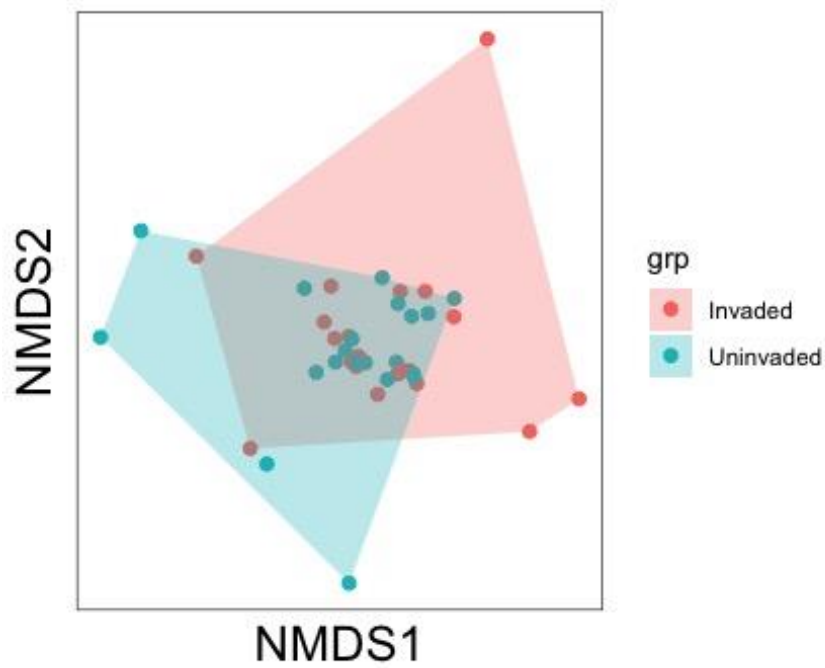


Figure 2. Non-metric multidimensional scaling plot of AMF OTUs in *L. vulgaris* invaded and uninvaded CO soil.

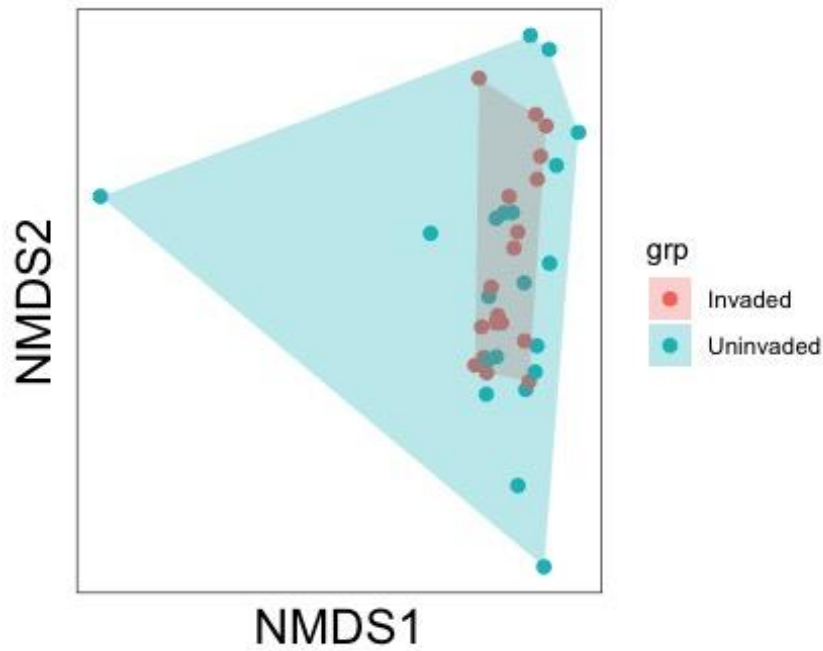


Figure 3. Non-metric multidimensional scaling plot of AMF OTUs in *L. vulgaris* invaded and uninvaded IL soil.

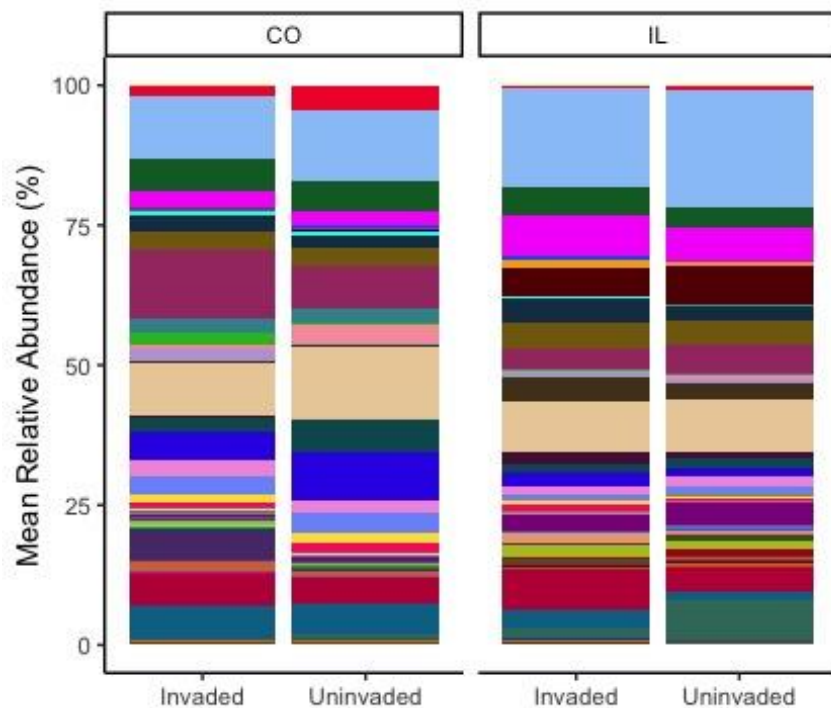


Figure 4. Taxa plot of mean OTU relative abundances (%) by state and invasion history.

OTU richness was not significantly different between CO (58 OTUs) and IL soil (56 OTUs; $p=0.180$, $CI=-0.118, 0.118$), by invasion history ($p = 0.565$, $CI=-0.086, 0.151$), or by the interaction between state and invasion history ($p = 0.180$, $CI = -0.282, 0.053$). Individual OTU relative abundances were significantly different between IL and CO, in which CO soil had 20 unique indicator OTUs and IL had 19 unique indicator OTUs (Table 2). In CO soil alone, there was one indicator OTU in both invaded and uninvaded soil (Table 3). Whereas in IL soil alone, there were seven indicator OTUs in invaded soil and three indicator OTUs in uninvaded soil from IL.

Table 2. Indicator OTUs with mean relative abundances (%) $\pm$ standard error in CO and IL and p-values of OTUs that differ significantly by state.

CO			IL		
OTU	Mean Relative Abundance (%)	P-Value	OTU	Mean Relative Abundance (%)	P- value
Glomus7	3.6 $\pm$ 0.3	0.001	Diversispora1	6.4 $\pm$ 0.4	0.001
Paraglomus2	5.5 $\pm$ 0.7	0.001	Glomus3	1.7 $\pm$ 0.2	0.001
OTU11	0.3 $\pm$ 0.07	0.001	Glomus1	6.3 $\pm$ 1.1	0.001
OTU13	0.3 $\pm$ 0.08	0.002	OTU15	3.8 $\pm$ 0.7	0.001
OTU8	0.1 $\pm$ 0.03	0.001	Funneliformis2	1.1 $\pm$ 0.2	0.001
Glomus13	9.7 $\pm$ 1.5	0.002	OTU12	0.2 $\pm$ 0.06	0.002
Glomus5	6.7 $\pm$ 1.5	0.001	Glomus18	3.7 $\pm$ 1.3	0.001
Glomus9	1.4 $\pm$ 0.2	0.001	OTU22	1.8 $\pm$ 0.3	0.002
OTU9	2.2 $\pm$ 0.07	0.002	OTU4	0.3 $\pm$ 0.09	0.002
OTU25	0.3 $\pm$ 0.1	0.001	Clarideoglomus1	18.8 $\pm$ 1.8	0.002
OTU14	0.1 $\pm$ 0.04	0.005	Glomus8	0.05 $\pm$ 0.02	0.006
Archaeospora2	3.1 $\pm$ 1.0	0.001	OTU3	0.5 $\pm$ 0.2	0.001
Clarideoglomus2	5.6 $\pm$ 0.5	0.008	OTU6	0.1 $\pm$ 0.05	0.010
Glomus10	0.4 $\pm$ 0.07	0.017	Paraglomus3	4.9 $\pm$ 2.3	0.001
OTU28	0.06 $\pm$ 0.02	0.029	OTU20	0.1 $\pm$ 0.09	0.028
Glomus14	2.7 $\pm$ 1.3	0.001	OTU19	1.1 $\pm$ 0.6	0.004
Glomus4	4.2 $\pm$ 1.0	0.002	OTU17	0.3 $\pm$ 0.1	0.037
OTU26	0.03 $\pm$ 0.01	0.030	OTU24	1.0 $\pm$ 0.6	0.005
OTU2	0.1 $\pm$ 0.05	0.009	OTU21	0.6 $\pm$ 0.4	0.012

OTU27	3.0±2.1	0.012	
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Table 3. Indicator OTUs for invaded and uninvaded soil in CO and IL of OTUs with mean relative abundances (%) ± standard error and p-values of OTUs that differ significantly by invasion.

OTU	STATE	INVASION	MEAN	P-VALUE
Glomus3	CO	Invaded	0.2±0.06	0.003
Glomus16	CO	Uninvaded	3.4±2.0	0.038
Paraglomus2	IL	Invaded	3.1±0.3	0.001
OTU1	IL	Invaded	1.3±0.1	0.002
Paraglomus1	IL	Invaded	7.3±0.9	0.001
OTU27	IL	Invaded	0.2±0.08	0.002
Glomus15	IL	Invaded	0.3±0.1	0.001
Rhizophagus2	IL	Invaded	0.4±0.1	0.001
OTU11	IL	Invaded	0.1±0.05	0.002
Glomus16	IL	Uninvaded	0.7±0.2	0.035
Glomus14	IL	Uninvaded	0.5±0.2	0.046
OTU29	IL	Uninvaded	0.2±0.1	0.046
OTU10	IL	Uninvaded	0.08±0.02	0.046

Relative abundance of OTUs across both states were significantly affected by NO₃, sand, and silt (Table 4). Relative abundances of OTUs affected by NH₄, sand, and clay depended on state. Relative abundances of OTUs were not affected by soil factors depending on invasion history or the interaction between state and invasion history.

Table 4. Summary of effects of soil factors on AMF community composition and their interactions with state, invasion, and state and invasion together. Significant p-values are denoted in bold.

	Soil Factor	*State	*Invasion	*State*Invasion
pH	0.379, R ² = 0.01	0.772, R ² = 0.01	0.830, R ² = 0.01	0.373, R ² = 0.01
NO₃	0.015 , R ² = 0.02	0.155, R ² = 0.02	0.571, R ² = 0.01	0.164, R ² = 0.02
NH₄	0.101, R ² = 0.02	0.017 , R ² = 0.02	0.401, R ² = 0.01	0.161, R ² = 0.02
PO₄	0.169, R ² = 0.02	0.148, R ² = 0.02	0.283, R ² = 0.01	0.467, R ² = 0.01
Sand	0.006 , R ² = 0.03	0.050 , R ² = 0.02	0.923, R ² = 0.01	0.630, R ² = 0.01
Silt	0.014 , R ² = 0.03	0.113, R ² = 0.02	0.913, R ² = 0.01	0.677, R ² = 0.01
Clay	0.070, R ² = 0.02	0.040 , R ² = 0.02	0.756, R ² = 0.01	0.471, R ² = 0.01

Discussion

This study evaluated arbuscular mycorrhizal fungi diversity in soil collected from CO and IL in either *L. vulgaris* invaded or uninvaded sites. AMF communities were significantly different between states (objective 1) but were not different between invaded and uninvaded soil in both states together and each individually (objective 2). Similarly, overall species richness was not different between states or invasion history. However, all soil treatments (CO invaded, CO uninvaded, IL invaded, IL uninvaded) had at least one indicator OTU, suggesting that AMF communities are somewhat unique across treatments. Soil factors including sand, clay, and NH_4 influenced community differences between CO and IL soil (objective 3). However, community differences between invaded and uninvaded soil in either or both states were not influenced by soil factors. These results suggest that AMF communities are different between CO and IL, at least in part due to soil abiotic differences, but *L. vulgaris* invasion does not drive changes in AMF diversity.

I expected to see AMF community differences between CO and IL, as previous research has shown high correlation between geographic distance and AMF community composition (Kivlin et al 2011). AMF community differences could explain *L. vulgaris*' aggressive distribution, but future studies need to directly test the effects of AMF species on *L. vulgaris* performance to establish this as a possible cause. I also expected to see variation in diversity between states, since CO and IL climate and plant communities are widely different. Because IL is lower elevation than CO, I expected to find greater OTU richness and taxonomic diversity in IL (Swaty et al 2016). Not only was OTU richness about the same between sites, but each state had almost the same number of indicator OTUs, meaning that states had different communities, but neither had higher community specificity. Similarly, both states had the same number of

AMF families, so differences in community composition are only found at the OTU level. Soil with high AMF diversity is typically more resistant to disturbances like pathogens, moisture stress, and others (Van der Heijden et al 1998). However, my sampling sites were already disturbed in both CO and IL and highly disturbed in IL, which could have resulted in a loss of AMF diversity, particularly in IL soil (Entry et al 2002; Sánchez et al 2012).

Conversely, when I compared invaded and uninvaded soil from both states, as well as each state individually, AMF community composition was not different, suggesting that *L. vulgaris* does not change the AMF community following invasion. Invasion history also did not affect OTU or family richness in both states together and each state alone, revealing that AMF diversity was unaffected by invasion. However, there were state-specific differences in indicator OTUs between invaded and uninvaded soil, with the same number of indicator OTUs between invaded and uninvaded CO soil and slightly more indicator OTUs in invaded than uninvaded IL soil. Differences in indicator OTUs reveal that sites had slightly different communities, but not enough to illustrate any effect of *L. vulgaris* invasion. Because OTU richness was unaffected by invasion, indicator OTUs in invaded and uninvaded sites are most likely the result of site differences unrelated to invasion.

States also had strong differences in soil factors, with higher pH and sand in IL soil and higher silt, NO₃, and NH₄ in CO soil. I was surprised that N availability was higher in CO soil than IL soil, contrary to previously documented state-wide trends, but I attribute this difference to site disturbance. Sites in IL where I was able to locate *L. vulgaris* were adjacent to roads, railroads, or in other highly disturbed areas, making them highly susceptible to leaching and less likely to harbor N-fixing bacteria (Perakis et al 2015). Whereas in CO, deer, moose, and other wildlife were often found inhabiting sites and most likely contributed N inputs to the soil.

Interestingly, PO₄ was affected by both state and invasion, with higher PO₄ in invaded than uninvaded IL soil, but no effect of invasion in CO. It seems likely that PO₄ availability is driven by microbial community differences, as microbes are essential to plant P uptake and disturbance has less of an effect on soil P (Richardson et al 2011; Redel et al 2013). While between-state AMF differences could be partly responsible for PO₄ changes, AMF communities were not very different between invaded and uninvaded sites in CO and IL. Bacteria, saprotrophic fungi, and other microorganisms could influence PO₄ differences between invaded and uninvaded sites, but I only addressed AMF community differences in this study.

When I tested the effects of soil factors on AMF community composition, not all soil factors different by state resulted in community differences. Nitrate, sand, silt, (and clay marginally) influenced AMF communities across both states. Sand, clay, and ammonium influenced AMF community differences between states. I hypothesize that because NO₃ and NH₄ were abnormally low in IL sites and abnormally high in CO sites, N availability had stronger effects on AMF communities than expected. Sand, clay, and silt ratios can affect soil nutrient and moisture retention and because CO has a dry climate, I hypothesize that moisture is at least partly responsible for AMF composition there (Kivlin et al 2011). While pH was higher in IL than CO soil, pH did not drive AMF community composition in both or either state. For all soil factors, the R² values were very low (0.01-0.03), meaning that the AMF communities I observed may be correlated with soil factors, but the variation I saw was not explained by the soil factors I tested.

Soil factors didn't drive AMF community differences between invaded and uninvaded soil in both states together and separately. Despite the differences in soil PO₄ by state and invasion history, PO₄ did not affect AMF community composition across state and invasion

treatments, which could be because other microorganisms are more important for plant P acquisition. If AMF were responsible for plant PO_4 uptake, I would expect to see strong effects on AMF communities, since PO_4 availability would drive the direction of the plant-mycorrhizal symbioses (Johnson et al 1997). While invaded and uninvaded soil AMF communities were not different overall, unique indicator OTUs in invaded and uninvaded soil may be unimportant to plant nutrient acquisition or otherwise not functionally diverse (Lee et al 2013). The NMDS revealed that AMF communities in invaded IL soil were a subset of uninvaded IL soil, despite having more indicator OTUs. This could illustrate that within-family taxonomic diversity was greater in uninvaded than invaded IL soil, but this does not appear to correspond to greater functional diversity.

Unfortunately, the strength of my data was limited, as I had to remove many low-quality base pairs that are important in species-level identification. My library preparation did not yield the quantity or quality of reads I would have liked for strong AMF community analysis. While I am confident in my identification of OTUs to either family or genus, I likely did not completely capture AMF diversity in my samples. Furthermore, I only sequenced soil that had either been invaded by *L. vulgaris* or not but did not do any experimental manipulation to determine whether growing *L. vulgaris* changes the AMF community. While I used paired plots to reduce site variation, I can't be sure that invasion and not site differences contributed to my observed AMF community differences. In future studies, I suggest that researchers grow *L. vulgaris* in previously uninvaded soil prior to sequencing in addition to performing more broad-scale sequencing of AMF, bacteria, and saprotrophic fungi to better capture microbial diversity.

My sequencing revealed that AMF communities are distinct between CO and IL soil, but not between *L. vulgaris* invaded and uninvaded soil in both locations. This leads us to believe

that state differences may contribute to *L. vulgaris*' invasion, but that *L. vulgaris* does not directly cause changes to the AMF community. I compared AMF diversity and found that both OTU and family richness was not affected by either state or invasion, furthering my belief that *L. vulgaris* invasion does not impact AMF community composition. I also established that soil factors including NO₃, sand, silt, and clay contributed to AMF compositional differences between states. Whereas PO₄ was different between invaded and uninvaded sites in IL soil, PO₄ had no effect on AMF community differences between invaded and uninvaded soil. There is no strong linkage between *L. vulgaris* invasion, soil nutrients, and AMF community composition, but I hypothesize that other microbial differences between sites are responsible for nutrient dynamics and subsequently impact *L. vulgaris*' invasion.

While AMF do not appear to be directly impacted by *L. vulgaris* invasion, other soil characteristics seem to be important in shaping *L. vulgaris*' aggressive distribution. Specifically, soil PO₄ and microbial communities apart from AMF could either influence or be influenced by *L. vulgaris* invasion. Understanding the relationships between soil and invasive plant success could help managers better restore previously invaded sites and prevent future invasions. I hope that this study draws more attention to studying the relationships between invasive plants, nutrient availability, and soil microbial communities to uncover additional restoration solutions.

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