

Invasive legume management strategies differentially impact mutualist abundance and benefit to native and invasive hosts

Running head: Invasive legume management strategies

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Abstract

Determining the best management practices for plant invasions is a critical, but often elusive goal. Invasive removals frequently involve complex and poorly understood biotic interactions. For example, invasive species can leave potent legacies that influence the success of native species restoration efforts, and positive plant-microbial feedbacks may promote continued reinvasion by an exotic species following restoration. Removal methods can vary in their effects

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on plant-soil feedbacks, with consequences for restoration of native species. We determined the effects of invasion by a leguminous shrub (French broom; *Genista monspessulana*) on the density and community composition of, and benefit conferred by, its microbial mutualists in its invading range. Densities of soil-dwelling rhizobia were much higher in areas invaded by *G. monspessulana* relative to uninvaded areas, and this increased density of rhizobia fed back to increase seedling growth of both the invader and native legumes. We further compared how three techniques for removing *G. monspessulana* affected the densities of rhizobia relative to areas where *G. monspessulana* was still present. Removal by hand-pulling reduced soil rhizobial densities, but reduced growth of one native legume, while having no effect on the growth of the invader. Overall, our results show that the consequences of restoration techniques, both above- and belowground, could be critical for the successful removal of an invasive legume and restoration of native species.

Keywords

French broom, *Genista monspessulana*, hand-pulling, herbicide, mowing, rhizobia

Conceptual Implications

- Strong feedbacks between plants and soil-borne microbial symbionts can leave biotic legacies that influence the success and durability of restoration projects.
- Positive plant-microbial feedbacks may hamper restoration by promoting reinvasion from the seed bank.
- Some species used in restoration might benefit from the biotic legacy of an invader.

- Removal methods can alter soil microbial communities.
- Best removal practices will manipulate the soil microbial community to deter reinvasion and/or promote the persistence of restored native plant populations, which in some cases may be conflicting goals.

Introduction

Invading plants can fundamentally alter the plant-microbial feedback loops that stabilize native ecosystems (Callaway et al. 2004; Van der Putten et al. 2013; Pringle et al. 2009; Klironomos 2002; Wolfe & Klironomos 2005). Moreover, legacies of invasive species removal can persist for years (Grove et al. 2017). Thus, it is crucially important to consider microbial community dynamics when evaluating methods for managing plant invasions (Corbin & D'Antonio 2012).

Plant-microbial feedbacks might be particularly pertinent to invasive legume management. Legumes comprise some of the world's most noxious invasive species (Daehler 1998; Richardson et al. 2000; Yahara et al. 2013). Their invasions profoundly impact communities directly via competition (Lattera et al. 1997) and indirectly by adding nitrogen to a system (Archer 1995; Vitousek et al. 1997; Rothstein et al. 2004; Maron & Jefferies 1999), a nutrient that often limits terrestrial plant growth. Legume effects on nitrogen dynamics arise from their symbiotic relationship with soil-dwelling bacteria called rhizobia. These bacteria infect legume roots, induce and colonize nodules, and therein endosymbiotically fix atmospheric di-nitrogen (Dilworth et al. 2008). Legumes periodically release rhizobia into the soil, producing free-living rhizobial communities (Sprent et al. 1987); new nodules are formed by colonization of legume roots from this soil pool. Because of their interaction with rhizobia, legumes can exhibit particularly strong positive plant-microbial feedbacks (Ehrenfeld et al. 2005; Cortois et al. 2016), and legume invasion can significantly alter rhizobium communities (Boudiaf et al. 2014; Le Roux et al. 2016). Legume-caused changes in the soil rhizobial community might persist long after plant removal. For example, rhizobia in agricultural settings can maintain high abundances for more than a decade after a field has last been planted with a legume (Sanginga et

al. 1994). A similarly persistent legacy of legume invasion on the soil community could influence restoration success by altering the possibility of future reinvasion and/or establishment of desired native species.

Techniques for managing invasive legumes might differentially affect the soil rhizobial community, with important consequences for subsequent restoration. Some removal techniques directly impact rhizobia in the soil environment (Covington & DeBano 1990; Neary et al. 1999), whereas others could affect plant release of rhizobia into soil. These effects should be considered when designing management plans. If management efforts do not reduce the abundance of rhizobia that are compatible with the invasive legume, these persistent symbionts might facilitate an invader's continual reinvasion. In contrast, a management technique that both removes an invasive legume and reduces the abundance of compatible rhizobia in the soil could reduce the potential for reinvasion and future investment in control. Here, we extend beyond most studies of restoration efforts, which focus primarily on the aboveground effects of invasive species removal (Richardson et al. 2000; Mason & French 2007; Konlechner et al. 2015; Maoela et al. 2016), to also examine belowground effects of invasion and removal of an invasive shrub.

In previous work on French broom (*Genista monspessulana*), and two additional European shrubs, we found that root nodules collected from populations of these leguminous shrubs invading Marin County, California contained rhizobia distinct from those associating with nearby legumes native to California (La Pierre et al. 2018). Moreover, the rhizobia collected from the invading plants were more closely related to those associated with these legumes in their European native ranges than with rhizobia found in the nodules of nearby legumes native to California (La Pierre et al. 2018). Using single-strain inoculations under greenhouse conditions, we found that both the native and invasive legumes could productively associate with a wide

variety of rhizobial strains, which suggests they are fundamentally generalists; however, they act as specialists when presented with a choice among rhizobial partners (La Pierre et al. 2018).

Here, we aim to (1) assess whether the invasion of an area affects the soil rhizobial community; (2) determine how any alterations to the soil rhizobial community affect growth of the invader and native species commonly used in restoration efforts; (3) assess how commonly used restoration techniques affect the soil rhizobial community; and (4) determine the impacts of restoration efforts on the growth of the invader and native legume species. We accomplished these objectives by examining how invasion by the exotic legume *G. monspessulana* affects soil rhizobial densities and community composition, and by evaluating three methods (hand-pulling, herbiciding, and mowing) that have historically been used to manage leguminous shrubs invading California. We also used geo-referenced management records to examine whether management techniques differentially affect the soil rhizobium community developed by *G. monspessulana* (Table 1). We finally used a greenhouse experiment to examine the potential consequences of these removal techniques for reinvasion by *G. monspessulana* versus restoration with three native legumes (*Acmispon glaber*, *Lupinus bicolor*, and *L. nanus*).

Methods

Site selection and soil sampling

Soil samples were collected from six parks from around Marin County in fall 2015 (Table 1; Marin County Open Space District Permit 15-098). Within each park, we sampled sites that had been invaded by *G. monspessulana* and a site that was known to have never been invaded. Sites were chosen during a long drought, during which native legume density was low; the

history of occupancy by native legumes was unknown. Among invaded sites, some sites were currently occupied by *G. monspessulana* whereas at other sites *G. monspessulana* had been removed by either hand-pulling (N=3), herbiciding (N=4), or mowing (N=3). The timing and duration of these restoration treatments differed across sites (Table 1). Each park included at least one *G. monspessulana* removal technique, as well as uninvaded and untreated areas (Table 1). Within each park, the distance among collection sites ranged from 200-1500 m. Distances between parks ranged from 3-16 km, with all parks encompassed within a 50-km² area. All sites have similar Mediterranean climates, with rainy winter months and cool, dry summers. Sites were primarily grasslands, apart from the invading leguminous shrubs. Five soil cores (1.5 cm diameter, 10 cm long) were collected from each site, aggregated by site, sieved, and air-dried within 48 hours of collection.

Soil density and composition of the nodulating rhizobial community

Rhizobial density of the aggregate soil sample from each site was determined using the Most Probable Number (MPN) method (Brockwell 1963; Wooster et al. 1990; Somasegaran & Hoben 2012) with trapping by four species of legumes (*G. monspessulana* and three common native legumes: *A. glaber*, *L. bicolor*, and *L. nanus*). Each aggregate soil sample was serially diluted from 10⁻¹ to 10⁻⁴ and each dilution applied to five replicate plants of each trap species. Dilutions were replicated in temporal blocks such that each dilution for each soil type*plant species combination occurred once within each of the following planting dates: (1) Dec 15, 2015; (2) Dec 21, 2015; (3) Jan 5, 2016; (4) Jan 10, 2016; and (5) Jan 17, 2016. Prior to each planting date, seeds of each legume species were surface sterilized in full-strength commercial bleach (6% sodium hypochlorite) for 30 sec, rinsed five times with sterile water, scarified with

sulfuric acid for either 10 min (*A. glaber* and *L. bicolor*), 30 min (*L. nanus*), or 40 min (*G. monspessulana*), and thoroughly rinsed using a sterile 20% sodium bicarbonate solution and sterile water. Scarified seeds were kept on moist sterile filter paper in petri dishes in the dark. Two to three days following scarification, three scarified seeds were planted at a depth of 1.5 cm into SC10 Ray Leach Conetainers (Steuwe and Sons) filled with sterile sand. Upon emergence, seedlings were thinned to one individual per Conetainer and fertilized once per week with 1 ml of N-free Fahreous solution (Somasegaran & Hoben 2012).

Three weeks following planting, seedlings were inoculated with 1 ml of the appropriate soil dilution applied at the base of the stem. Two uninoculated control pots of each species were planted in each replicate time block; none of the uninoculated control plants formed nodules. Individuals were harvested 7 weeks following planting (4 weeks following inoculation) to identify the number of plants that bore at least one nodule. The MPN of rhizobia in the soil (including 95% confidence intervals) was calculated using the Bacteriological Analytical Manual Most Probable Number calculator for serial dilutions (Blodgett 2010), which accounted for the dilution levels, the number of replicates at each dilution, and the number of plants nodulated at each dilution.

Nodules were collected from individuals of each species at each soil dilution across all planting dates (up to 20 nodules per species by soil by date combination). Briefly, nodules were randomly selected from the roots of each individual by splaying the roots over a numbered grid and excising the nodule closest to a grid cell selected by a random number generator, surface-sterilized by vortexing for 30 seconds in 900 μ l full-strength commercial bleach (6% sodium hydroxide), vortexed in five 30-second rinses of 900 μ l sterile water, and crushed in 100 μ l sterile water. Each nodule suspension was streaked onto a Yeast-Mannitol Agar plate (YMA;

1.5% agar), incubated in the dark at room temperature, and twice re-streaked onto new YMA plates from single-cell initiated colonies. A single-cell initiated colony was picked from each final re-streak plate, inoculated into sterile YM broth, and incubated at 25°C and 120 rpm. Each late-log-phase culture was pelletized and stored at -20°C for PCR.

The relative identities of rhizobial isolates were determined by REP PCR using the BOX A1R primer (Kaschuk et al. 2006). The 25 µl PCR reaction contained: 10x PCR buffer (2.5 µl); 50 mM MgCl₂ (1.5 µl); 2.5 mM of each dNTP (5 µl); BOX A1R primer (1 µl); taq (5U/ µl, 0.2 µl); liquid culture as DNA template (1 µl); and DNA free water to complete the volume. The PCR protocol was: 95°C (7 min); 35 cycles at 94°C (1 min), 53°C (1 min), 65°C (8 min); 65°C (16 min). Amplified fragments were separated on a 1.5% agarose gel run for 8 hours at 120 V and visualized under UV light after staining with ethidium bromide. For each isolate, band lengths were identified from gel images using GelCompar II. A positive control was included in each gel for comparison across gels. Banding patterns for these positive controls were consistent across gels, indicating high repeatability of the REP PCR method for these samples.

Effect of invasion and treatment history on legume growth

The effects of *G. monspessulana* invasion history and removal technique on the growth of two native legumes (*A. glaber* and *L. nanus*) and the invasive legume (*G. monspessulana*) were assessed using a greenhouse experiment. The native legume *L. bicolor* germinated poorly and therefore was not included in this experiment. Sterilized Conetainers were filled with sterile sand and inoculated 2 cm below the sand surface with a 2.4 mL layer of soil from the appropriate aggregate soil. Eight replicate seeds per species and soil sample were scarified, planted, and

grown in these inoculated pots using the methods described above. After 12 weeks, plants were harvested to obtain nodule number and dry weight of above- and belowground tissues. Final replicate numbers were uneven among treatments due to uneven germination success across soil samples.

Statistical Analysis

MPN values for each plant species at each site were natural log transformed as $\ln(\text{MPN} + 1)$. Total biomass was calculated as the sum of above- and belowground biomass for each individual in the whole soil inoculation experiment. Because of the way they are calculated, one MPN value is generated for each for each species at each site. In contrast, multiple values for total biomass exist for each species at each site to represent each replicate plant. Both variables were analyzed in the same way, as described below. For both MPN and total biomass, to examine the effect of *G. monspessulana* invasion, separate mixed-effects models were run for each legume host species (*G. monspessulana* and the three native legume species), with soil invasion status (*i.e.*, uninvaded vs invaded) as a fixed effect and site as a random effect. To examine the overall effect of *G. monspessulana* removal, the proportional difference in \ln MPN and total biomass between invaded and uninvaded areas ($[\text{treated-uninvaded}]/\text{uninvaded}$) was calculated for each host species and site. To compare removal techniques, separate mixed-effects models were run for each legume host species, with removal technique (hand-pulling, herbiciding, and mowing) as a fixed effect and site as a random effect. T-tests were conducted to determine whether each removal technique differed significantly from uninvaded sites (proportions not significantly different from 0 were considered to show no difference from the uninvaded areas). P-values for the t-tests were Bonferroni corrected for multiple comparisons.

The composition of the rhizobial community nodulating each test legume species was compared in two ways. First, uninvaded sites were compared with untreated sites. Second, untreated invaded sites were compared with invaded sites treated by each type of removal technique. These comparisons were performed by calculating similarity values for each pairwise combination of isolates based on band number and length using GelCompar II v. 6.1. The community composition for each soil type was visualized using MDS for each legume species for each comparison. Except where noted, all analyses were performed in R v. 3.4.3. All statistical code is archived at <https://github.com/klapierre/Invasive-Shrub-Marin-Co>.

Results

Effects of G. monspessulana invasion on the soil rhizobial community and plant growth

Invasion by *G. monspessulana* increased the soil density of rhizobia that nodulate each of the test hosts, although the trend was not significant for *L. bicolor* (Figure 1, Table S1). Notably, the density of nodulating rhizobia was near zero for most uninvaded sites. However, the composition of the nodulating rhizobial community did not detectably differ between uninvaded and invaded sites (Figure S1). For two of the native legumes and the invasive legume, total biomass was greater when grown in soils collected from areas invaded by *G. monspessulana* than in soils that had never been invaded (Figure 2, Table S2).

Effects of restoration techniques on the soil rhizobial community and plant growth

The restoration techniques did not differ significantly in their effects on densities of rhizobia associating with two of the native legumes (Figure 3, Table S3). However, removing *G. monspessulana* by hand-pulling and herbiciding reduced the densities of soil-dwelling rhizobia that nodulate *A. glaber* significantly more than mowing (Figure 3; Table S3). Further, removing *G. monspessulana* by hand-pulling reduced the densities of soil-dwelling rhizobia that nodulate *G. monspessulana* significantly more than herbiciding, and both hand-pulling and herbiciding reduced the density of soil-dwelling rhizobia that nodulate *G. monspessulana* significantly more than mowing (Figure 3; Table S3). However, due to high variation in MPN values, the proportional MPN difference between areas where *G. monspessulana* had been removed and areas that had never been invaded were not significantly different from zero for any plant species tested (Figure 3; Table S5). Removal technique did not detectably affect the community composition of the nodulating rhizobial community (Figure S2).

The total biomass of *L. nanus* seedlings was significantly lower when grown in soils collected from areas where *G. monspessulana* had been removed by hand-pulling than in soils where *G. monspessulana* had been removed by either herbicide or mowing (Figure 4, Table S4), but *A. glaber* and *G. monspessulana* exhibited no significant variation in seedling biomass among restoration techniques. Further, total seedling biomass of *G. monspessulana* grown in soil where *G. monspessulana* had been removed by any of the restoration treatments was significantly higher than in areas where *G. monspessulana* had never invaded (Table S6).

Discussion

Positive plant-microbial feedback between the invasive legume *G. monspessulana* and compatible rhizobia could promote *G. monspessulana* invasion and leave a legacy that would facilitate reinvasion following removal. Certain restoration efforts might slow *G. monspessulana* re-establishment by severing this feedback loop. Since *G. monspessulana* invasion enhances rhizobial densities without altering the soil rhizobial community, it could also facilitate restoration by some native legumes. Clearly, soil legacies produced by plant-microbial feedbacks should be explicitly considered when planning and evaluating restoration efforts (Corbin & D'Antonio 2012; Kulmatiski et al. 2008).

Two observations in our study revealed positive plant-microbial feedback. First, relative to uninvaded areas, the soil density of rhizobia that could nodulate with the invasive legume *G. monspessulana* was 90-fold greater in areas currently occupied by *G. monspessulana*. Second, *G. monspessulana* seedlings inoculated with soils collected from areas currently invaded by conspecifics produced twice as much biomass as those inoculated with soils from uninvaded areas. These results corroborate previous literature on biotic legacies of legumes (Parker 2001; Thrall et al. 2000; Richardson et al. 2000; Rovira 1961; Chatel & Parker 1973; Elkins et al. 1976; Sanginga et al. 1994) and suggest that *G. monspessulana* facilitates its own invasion through positive plant-microbial feedbacks.

The legacy of plant invasion can persist long after a plant has disappeared from the landscape (Magnoli et al. 2013; Reynolds & Cooper 2011; Grman & Suding 2010), and abiotic legacies produced by legumes are well-documented (McLauchlan 2006; Nsikani et al. 2017; González-Muñoz et al. 2012; Grman & Suding 2010; Corbin & D'Antonio 2012; Magnoli et al. 2013; Novoa et al. 2013). Our results add to a growing literature that documents similarly important biotic legacies.

The differing effects of invasive removal treatments on this soil legacy might promote different restoration goals. Preventing reinvasion of an invasive species following its removal is one important goal. Hand-pulling *G. monspessulana* could achieve this goal by severing its positive feedback loop with compatible rhizobia, which would reduce its biotic legacy in the soil. Hand-pulling physically removes a plant's shoots and major roots (generally using hand tools or small bulldozing equipment) without prior use of herbicide, mowing, or burning (Bossard et al. 2000). Removing an invader's major root system can provide multiple benefits. For example, invasive *Alliaria petiolata* (garlic mustard) produces compounds that are novel to its invaded range (Barto et al. 2010) and are allelopathic to mycorrhizal fungi (Wolfe et al. 2008). Hand-pulling *Al. petiolata* improved native plant establishment more than did removal by herbicide or clipping, in part because removing the roots reduced its allelopathic legacy (Barto et al. 2010). Removing legume roots could mitigate one component of their biotic legacy by preventing nodule senescence and release of rhizobia into the soil environment. In our study, hand-pulling reduced soil densities of rhizobia that nodulate with *G. monspessulana* nearly 100%, down to densities observed in soils collected from currently uninvaded areas. As expected from such reduced rhizobium density, we found a decrease in the biomass of *G. monspessulana* seedlings grown in soil collected from areas where *G. monspessulana* had been removed by hand-pulling. For a species that generates a large seedbank in areas where it has successfully invaded, which is the case for many legume species (Passos et al. 2017; Bossard et al. 2000), reinvasion from the seedbank is a serious concern. Seedlings could still germinate and initiate a new positive feedback loop, but our results suggest that low initial rhizobium density would likely hamper seedling growth and slow reinvasion.

Although high densities of soil rhizobia that accompany legume invasion may promote subsequent reinvasion, these legacy effects might sometimes be harnessed to restore native legume populations. The magnitude of benefit depends on the rhizobium specificity of the native legumes. Invading legumes often negatively affect native legumes by shifting rhizobium community richness and/or composition (Weir et al. 2004; Crisóstomo et al. 2013; Rodríguez-Echeverría 2009; Busby et al. 2016; Boudiaf et al. 2014; Le Roux et al. 2016), which could preclude restoration with native legumes. However, legumes vary greatly in how dependent they are on specific rhizobia (Barrett et al. 2012; Ehinger et al. 2014). Some legumes are specialized to particular rhizobium genotypes (Heath & Tiffin 2007) or local populations of rhizobia (Mytton 1975; Chanway et al. 1989), whereas other legumes are remarkably unselective (Lewin et al. 1987; Perret et al. 2000; Lima et al. 2009). Two of our observations suggest that the specificity of native legumes in our system would not preclude their use in restoration following *G. monspessulana* removal. Firstly, we found no evidence that *G. monspessulana* affects the nodulated community composition. There was no difference between uninvaded areas and either invaded areas or invaded areas from which *G. monspessulana* had been removed. Moreover, several native legumes in our system can function as rhizobial generalists when they have no choice in rhizobial partners (La Pierre et al 2018). As our previous work predicted, seedling growth of two native legumes common to the area (*A. glaber* and *L. nanus*) was greater in soils collected from areas where *G. monspessulana* had invaded than from uninvaded areas, where the rhizobial isolate composition remained the same, but rhizobium densities were lower. These natives also grew better in soils from areas where *G. monspessulana* had been removed by mowing rather than hand-pulling. Rhizobial densities were much lower in hand-pulled than mown areas. Results from these experiments corroborate our earlier evidence that these native

legumes are rhizobial generalists (La Pierre et al. 2018) and might be good candidates for restoration efforts, particularly in areas where an invasive legume has been removed by mowing. To the extent possible, restoration efforts following removal of an invasive legume should focus on native legumes that can nodulate and benefit from a wide variety of rhizobium types.

Because our study made opportunistic use of previously conducted removal efforts, environmental conditions varied among our study sites and the exact invasive removal technique varied somewhat within treatment types. Our results therefore represent a conservative estimate of the effects of *G. monspessulana* removal on the potential for reinvasion or growth of native legumes, which suggests that they are robust and generalizable across a wide variety of environmental conditions.

To summarize, invasion by a legume can alter the density of its soil-dwelling symbionts in a way that benefits its own success and that of its seedlings. However, restoration efforts that reduce the density of compatible soil rhizobia can slow the growth of future generations of the invader. Moreover, subsequent revegetation efforts should focus on native legumes that can associate with and benefit from a wide diversity of rhizobia. The dynamics of plant-microbial interactions are important to invasive species prevention, management, and restoration. Specifically, for species with known strong plant-microbial feedbacks, such as the invasive legume studied here, preventing initial invasion is crucial because once the positive feedback begins it can be difficult to stop (Reinhart & Callaway 2006; Levine et al. 2006; Callaway et al. 2004). To prevent legacies left by invaders from promoting reestablishment, decisions on how to remove invasive species should consider both above- and belowground components of a system (Reynolds et al. 2017). Finally, these same issues should inform the choice of species for restoration projects. Management models should incorporate both the ecological and economic

costs and benefits of removal and restoration across multiple generations, as it might take many generations for the legacies of invaders to fade.

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Accepted Article

1 Table 1. Soil sampling sites across six Marin County parks and open spaces that had been
 2 invaded by *Genista monspessulana*. UTM Easting, Northing indicates the location of each site.
 3 Soil type provided as USDA NRCS soil classifications for each site obtained from the Web Soil
 4 Survey (<https://websoilsurvey.sc.egov.usda.gov/>). Invasion status indicates the state of *G.*
 5 *monspessulana* at the site at the time of soil sampling: uninvasion=site never invaded;
 6 untreated=site currently invaded and no removal strategies employed; pulled=*G. monspessulana*
 7 removed by pulling out by hand; herbicided=*G. monspessulana* removed by herbiciding;
 8 mowed=*G. monspessulana* removed by mowing. Removal details specifies removal treatments
 9 where applicable, starting in 2009 and running through soil collection in 2015. Herbicide applied
 10 as 2% glyphosate at a rate of 47 ml/m² in all herbicided sites, with the exception of Ring
 11 Mountain, where 2% RoundUp Custom with Liberate surfactant was applied to cut stems.

Sampling Site	UTM Easting, Northing	Soil Type	Invasion Status	Removal Details
Alto Bowl	542015.07, 4196602.81	Los Osos-Urban Land-Bonnydoon complex	uninvaded	n/a
			untreated	n/a
			herbicided	2013-2014
Blithedale Summit	540081.85, 4196482.17	Tocaloma- McMullin complex	uninvaded	n/a
			untreated	n/a
			mowed	2011-2015
Camino Alto	541487.12, 4196482.17	Tocaloma- McMullin complex	uninvaded	n/a
			untreated	n/a
			herbicided	2012-2014
Cascade Canyon	534688.02, 4204002.43	Tocaloma-Saurin complex	mowed	2011-2015
			uninvaded	n/a
			untreated	n/a
Old St. Hilary's	537726.43, 4192587.63	Cronkhite- Barnabe complex	pulled	2009-2012, 2015
			untreated	n/a
			herbicided	2011-2014
Ring Mountain	545093.95, 4196175.35	Henneke stony clay loam	uninvaded	n/a
			untreated	n/a
			pulled	2009-2015
			herbicided	2014

12 **Figure Captions.**

13 **Figure 1.** Soil densities of rhizobia nodulating (a-c) three native legumes and (d) the invasive
14 legume *Genista monspessulana* (measured as $\ln(\text{Most Probable Number})$ per gram soil) in areas
15 either uninvaded by *G. monspessulana* (uninv.) or invaded, but unmanaged (untrt.). Letters
16 denote significant differences between uninvaded and invaded sites based on separate mixed-
17 effects models for each legume host species, with soil invasion status as a fixed effect and site as
18 a random effect. Error bars represent $\pm\text{SE}$.

19 **Figure 2.** Total plant biomass per individual (*i.e.*, root and shoot) of (a-b) two native legumes
20 and (c) the invasive legume *Genista monspessulana* grown in soils collected from areas either
21 uninvaded by *G. monspessulana* (uninv.) or invaded but unmanaged (untrt.). Letters denote
22 significant differences between uninvaded and invaded sites based on separate mixed-effects
23 models for each legume host species, with soil invasion status as a fixed effect and site as a
24 random effect. Error bars represent $\pm\text{SE}$.

25 **Figure 3.** Proportional difference in soil densities (measured as Most Probable Number per gram
26 soil) of rhizobia nodulating (a-c) three native legumes and (d) the invasive legume *Genista*
27 *monspessulana* between uninvaded sites and areas where *G. monspessulana* had been removed
28 by either hand-pulling (pulled), herbiciding (herbic.), or mowing. Greater proportional
29 differences indicate higher soil densities of nodulating rhizobia in sites where *G. monspessulana*
30 has been removed compared to areas that were never invaded. Letters denote significant
31 differences among removal techniques based on separate mixed-effects models for each legume
32 host species, with removal technique as a fixed effect and site as a random effect. Error bars
33 represent $\pm\text{SE}$.

34 **Figure 4.** Proportional difference in total plant biomass per individual (*i.e.*, root and shoot) of (a-
35 b) two native legumes and (c) the invasive legume *Genista monspessulana* between uninvaded
36 sites and areas where *G. monspessulana* had been removed by either hand-pulling (pulled),
37 herbiciding (herbic.), or mowing. Greater proportional differences indicate higher individual
38 biomass for plants grown in soils from sites where *G. monspessulana* has been removed
39 compared to areas that were never invaded. Letters denote significant differences among removal
40 techniques based on based on separate mixed-effects models for each legume host species, with
41 removal technique as a fixed effect and site as a random effect. Asterisks denote Bonferroni-
42 corrected significant differences from zero (*i.e.*, difference from uninvaded sites) based on t-
43 tests. Error bars represent \pm SE.

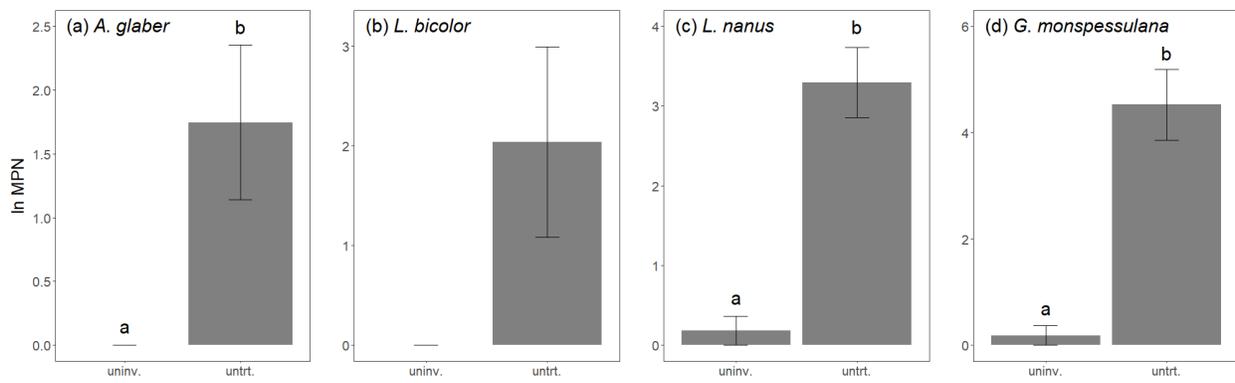


Figure 1.

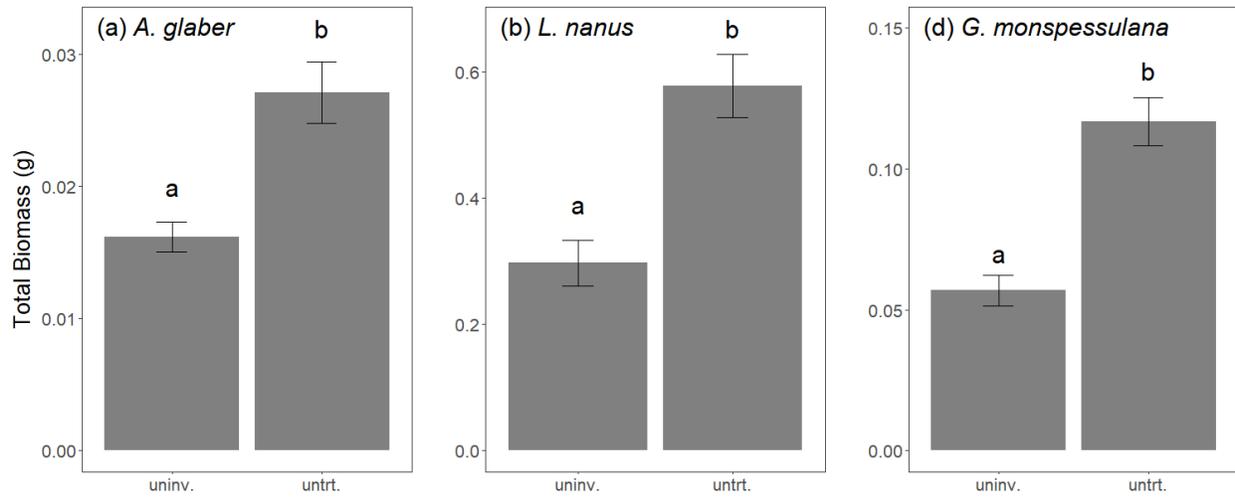
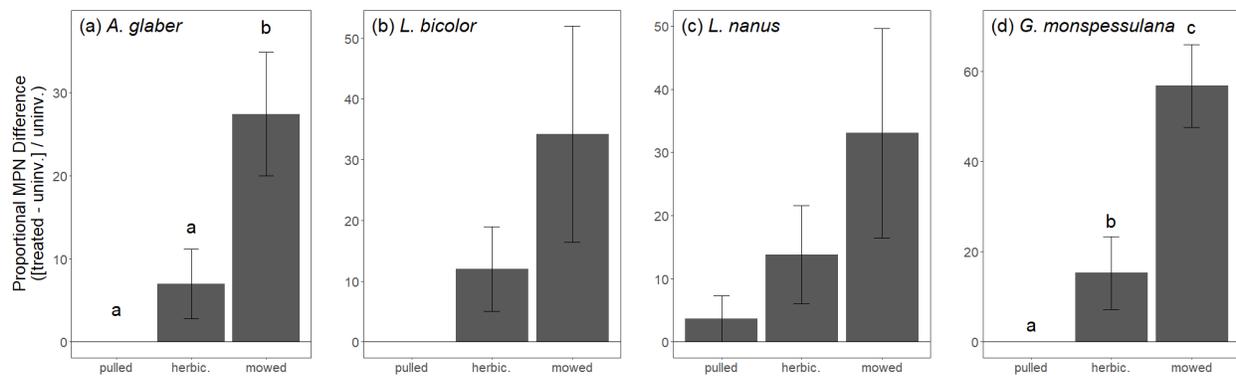
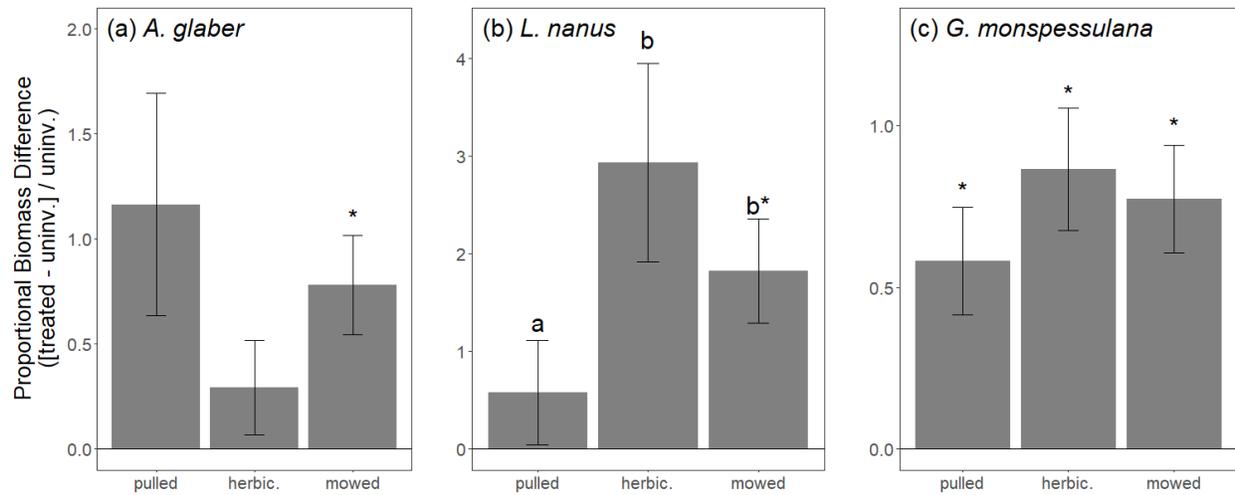


Figure 2.



48
49 Figure 3.



50
51 Figure 4.