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# A widespread nitrogen-fixing invader experiences negative soil feedbacks despite enhancing the abundance of beneficial soil microbes

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## Abstract

**Background and aims** Plant-soil feedbacks may determine the long-term success of introduced species. Here we examined plant-soil feedbacks of a globally invasive shrub, *Cytisus scoparius* (hereafter *Cytisus*), which associates with multiple guilds of microbial mutualists and dominates harvested Douglas-fir forests in the Pacific Northwest.

**Methods** We studied *Cytisus* root nodulation, mycorrhizal colonization, and growth in two greenhouse experiments. First, we compared invaded to uninvaded field soils. Then we did a soil conditioning experiment with *Cytisus* (in both invaded and uninvaded field soil) compared to soil conditioned by Douglas-fir.

**Results** *Cytisus* grown in invaded soils had 67% more root nodules and 72% more AMF colonization than uninvaded soil. Conditioning uninvaded soil with *Cytisus* increased root nodules by 14% and mycorrhizal colonization by 55%, compared to Douglas-fir conditioned soil. Despite the increased abundance of mutualists, *Cytisus* grown in *Cytisus*-conditioned soils were 41% smaller than in uninvaded soil.

**Conclusions** *Cytisus* increased the abundance of its microbial mutualists in soils, but overall plant-soil feedback was still negative, likely driven by soilborne pathogens, nutrient depletion, and/ or reduced benefits of mutualists. Our results do not support the idea that the high densities reached by *Cytisus* in its invaded range are caused by positive plant-soil feedbacks.

**Keywords** *Cytisus scoparius* · Scotch broom · *Pseudotsuga menziesii* · Plant-soil feedback · Soil conditioning · Invasion · AMF

## Introduction

Soil microbes are increasingly recognized as playing an important role in the plant invasions. Escape from soilborne pathogens can promote invasions (Reinhart and Callaway 2006; Inderjit and Cahill 2015), but the loss of specialized mutualists and presence of generalist pathogens can constrain invasions (Levine et al. 2004; Nuñez et al. 2009). Invasive plants can have large impacts on native plant communities and ecosystems when they reach high abundance (Parker et al. 1999; Sofaer et al. 2018). A key question is whether plant-soil feedbacks may promote the greater abundance of high-impact invaders.

Plant-soil feedbacks occur when a plant or a plant population alters abiotic or biotic soil properties which then in turn influence the growth of a conspecific individual or population, leading to neutral, negative or positive effects on plant performance (Bever et al.

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1997; Kulmatiski and Kardol 2008; van der Putten et al. 2013). If a plant changes soil properties such that the chances of conspecific success (survival, growth, reproduction, germination, etc.) are improved, a positive plant-soil feedback is said to occur; conversely if the changes to the soil reduce the likelihood of a conspecific individual's success, this is considered a negative plant-soil feedback (Ehrenfeld et al. 2005; van der Putten et al. 2013). Negative plant-soil feedbacks are a widespread mechanism of negative density dependence (Bever 2003; Lekberg et al. 2018). Negative plant-soil feedbacks may reflect the accumulation of specialist soilborne pathogens (van der Putten et al. 1993; Mills and Bever 1998; Nijjer et al. 2007; van der Putten et al. 2013) or changes in essential nutrient availability (Gustafson and Casper 2004; Ehrenfeld et al. 2005; Manning et al. 2008). Plant-soil feedbacks can also be positive, if beneficial soil microbes are promoted (Reinhart and Callaway 2004; Zhang et al. 2010) or nutrients accumulate (Vitousek et al. 1987; Stark and Norton 2015).

Whether plant-soil feedbacks are positive or negative may determine their role in invasion success (Reinhart and Callaway 2006; Suding et al. 2013; Inderjit and Cahill 2015). When invasive species leave behind their specialist enemies in their native range, they may have a competitive advantage (Elton 2000; Keane and Crawley 2002; Mitchell and Power 2003; Reinhart et al. 2010; Lucero et al. 2019). A lack of specialist soilborne pathogens should lessen negative plant-soil feedbacks in an invader's new range (Callaway et al. 2004; Reinhart and Callaway 2004). However, introduced species may also encounter generalist soilborne enemies in their introduced range or accumulate specialist soilborne pathogens over time resulting in similarly negative plant-soil feedbacks (Beckstead and Parker 2003; Diez et al. 2010). The availability of beneficial soilborne organisms may also influence invasions. If mutualistic soil microbes enhance plant performance, the absence of specialist mutualists in uninvaded sites could reduce positive plant-soil feedbacks (Mitchell et al. 2006; Nuñez et al. 2009; Pringle et al. 2009). However, as populations of mutualist soil microbes build up, plant performance should increase, contributing to a positive plant-soil feedback.

A newly introduced plant species should be able to associate with the symbionts of some native hosts but not others, and the abundance and distribution of these hosts could influence invasion success (Parker et al.

2006). The widely recognized symbionts arbuscular mycorrhizal fungi (AMF) form relationships with roughly 200,000 plant species (Brundrett 2009) and are generally thought to have low host specificity (Smith and Read 2010; Davison et al. 2011). A 2015 meta-analysis found that AMF are as likely to form mutualistic relationships with invasive plants as with native plants (Bunn et al. 2015). Similar to the role AMF play in the invasion of nonnative plant species, the symbiotic relationship between rhizobacteria and legume plants (Fabaceae) has contributed to the success of N-fixing invasive species globally (Richardson et al. 2011). Specialized mutualisms between legumes and strains of rhizobium have been found at the species level (Thrall et al. 2000; Barrett et al. 2012), but specialist legumes are less likely than generalists to be widespread invaders (Harrison et al. 2018). Lack of host specificity of AMF and widespread success of generalist legumes leads to particular predictions about plant-soil feedbacks in invasive plants. If pathogens tend to be more specialized than mutualists, then introduced plants may be more likely to escape negative interactions than to lose positive ones, leading to overall positive, or less negative, plant-soil feedbacks than native species. Indeed, as shown by Meisner et al. 2014, positive or neutral feedbacks have been seen more frequently in invasive species while native species more frequently have negative or neutral feedbacks.

We explored plant-soil feedbacks and the role of mutualistic soil microbes in the non-native legume *Cytisus scoparius* (hereafter *Cytisus*). *Cytisus*, a globally widespread invader originating from Europe, quickly reaches remarkably high abundance in sites in the Pacific Northwest of the United States (Peterson and Prasad 1998; Haubensak et al. 2020). *Cytisus* forms symbiotic relationships with N-fixing bacteria in the genus *Bradyrhizobium* (Sajnaga et al. 2001; Lafay and Burdon 2006; Horn et al. 2014) and generally increases soil N availability (Haubensak and Parker 2004; Caldwell 2006; Grove et al. 2015; but see Shaben and Myers 2010). Inoculation with *Bradyrhizobium* increases *Cytisus* growth in the field (Parker et al. 2006). In its native range in Europe, *Cytisus* is known to associate with AMF (Harley and Harley 1987). Interestingly, no one has tested for positive plant-soil feedbacks in this global invader, and little is known about the importance of AMF or rhizobia in *Cytisus* invasion success. We used greenhouse experiments with field soils to study interactions between *Cytisus* and soil

microbes. We first compared *Cytisus*-invaded and uninvaded field soils for the availability of AMF and rhizobia, with the expectation that *Cytisus* would be less mutualist-limited in invaded soils. Next, we implemented a plant-soil feedback experiment with the expectation that mutualists would increase in soils conditioned by *Cytisus*. We also expected that *Cytisus* growth would increase along with nodulation and mycorrhizal colonization, both in invaded field soils and in *Cytisus*-conditioned soils.

## Methods

We collected soil on January 17, 2017 from two adjacent sites in Western Washington (47° 28' 35" N, 122° 50' 15" W). The uninvaded site was an intact forest dominated by Douglas-fir (*Pseudotsuga menziesii*). The invaded site was an adjacent Douglas-fir forest that was clearcut and heavily invaded by *Cytisus*. The soils were shipped to the University of California, Santa Cruz and stored for 15 days in a 4 °C refrigerator. Large rocks and roots were removed from soils by hand. We used SC7 cylindrical plastic containers, hereafter conetainers (Stuewe and Sons, Tangent, Oregon), 14 cm deep × 3.8 cm diameter (107 mL) that contained approximately 15 mL of perlite at the bottom to improve drainage and 85 mL of field soil.

Experiment one: comparison of invaded and uninvaded soil

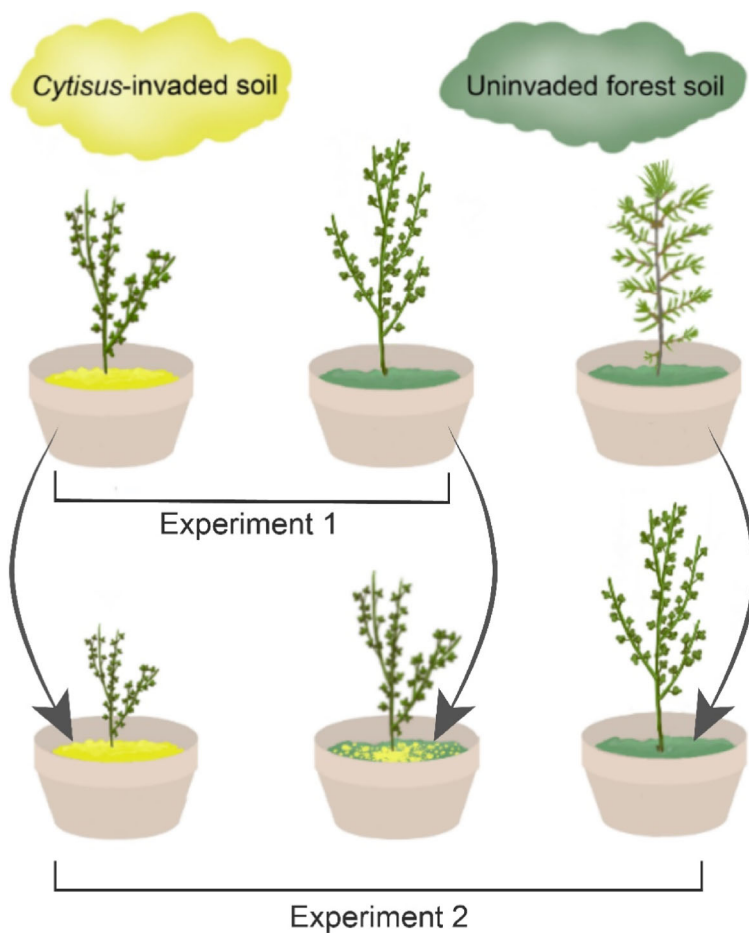
On February 1, 2017, we planted two scarified *Cytisus* seeds into uninvaded forest soil and invaded forest soil ( $N=90$  per treatment). Individual pots were scored as having successful germination and establishment if at least one seedling was present, where two were present they were thinned to one individual. While we did not track individual seeds, our analysis of seedling success is conservative (failures are cases where neither of two seeds established; intermediate cases are scored as successes). Replicates that did not germinate were replanted with seeds germinated on filter paper on February 25; replicates that were still lacking germination were again replanted on May 8. We analyzed plant growth as a function of these three different planting dates, and we found no difference in aboveground biomass for seedlings planted on day 0, day 24, or day 96 ( $F_{2,164}=2.467$ ,  $P=0.09$ ). On February 25, we planted Douglas-fir seeds

in uninvaded forest soil as conditioning in preparation for Experiment Two ( $N=90$ ; Fig. 1; details below). We used a randomized block design of 6 blocks with 45 plants per block and an even number of plants from each treatment in each block. To prevent cross contamination from one container to another we watered from the top and we left an empty space between each conetainer to reduce splash. Plants grew in the UC Santa Cruz Greenhouse for 34 weeks, long enough for the *Cytisus* roots to grow to the bottom of the conetainer but before the plants became root bound. Weeds were removed by hand weekly.

We measured above- and belowground biomass, percent mycorrhizal colonization, and *Bradyrhizobium* nodulation. We harvested the *Cytisus* plants one block at a time and in random order from October 3, 2017 to December 22, 2017. Aboveground biomass of all surviving *Cytisus* was dried at 61 °C for 48 h and weighed ( $N=173$  because 7 individuals were lost to mortality). We washed the roots and counted nodules on the whole root mass. We collected a subset of the nodules to weigh ( $N=16$ ); we confirmed that nodule count was directly related to nodule weight (nodule weight (g) =  $0.013 + 0.00011 \times \text{number of nodules}$ ,  $R^2=0.79$ ,  $P<0.0001$ ). Based on this analysis, we chose to focus on nodule counts alone for the rest of the analyses, rather than counts and nodule biomass. We assigned plants to be assessed for either belowground biomass ( $N=86$ ) or AMF colonization ( $N=69$ ) by alternating as each individual was harvested. The assessment of AMF colonization requires destructive sampling, so plants were either assessed or weighed.

We evaluated AMF colonization of stained root segments on 36 plants from uninvaded soil and 33 from invaded soil. We removed the primary root stalk and cut the secondary and tertiary roots into 0.5–1.5 cm long segments, then cleared and stained the fragments using methods adapted from Phillips and Hayman (1970). In brief, roots were submerged in 10% KOH solution and kept in a 55 °C hot water bath for 12 h. The cleared roots were then rinsed with deionized water and neutralized with 1% HCl for 5 min at room temperature. We soaked root fragments in a staining solution of 0.05% w/v trypan blue in lactoglycerol in a 55 °C water bath. After staining for 30 min, the roots were strained and placed in a vial containing lactoglycerol. To quantify AMF abundance, we placed root fragments in a 1-cm gridded petri dish, then selected 72 root fragments at line intersection points. We then noted hyphae, arbuscules, and vesicles

**Fig. 1** Schematic showing the design of Experiments 1 and 2. Field soils from *Cytisus*-invaded areas and uninvaded Douglas-fir forest were compared in Experiment 1. For Experiment 2, we used the soils conditioned by *Cytisus* in Experiment 1, together with forest soils conditioned by Douglas-fir



on each root fragment under a stereoscope microscope at 60X magnification.

All analyses were performed in R version 3.5.0 (R core team 2018), using the lme4 package (Bates et al. 2015). All models included soil type (*Cytisus*-invaded vs. uninvaded soils) as a fixed effect with greenhouse block as a random effect. We evaluated above- and belowground *Cytisus* biomass using a linear mixed effects model (LMM); biomass variables met assumptions of homoscedasticity and had normally-distributed residuals. We evaluated the number of root nodules on *Cytisus* plants in invaded and uninvaded soil using a generalized linear mixed effects model (GLMM) with the Poisson family and log link function. We compared the proportion of *Cytisus* roots in invaded and uninvaded soils that contained hyphae, or arbuscules and vesicles combined, using a GLMM with the binomial distribution and the logit link function. We also evaluated germination of *Cytisus* seeds that were

planted on February 1, 2017 in invaded and uninvaded soils using a GLMM with a binomial distribution and the logit link function. We used a parametric bootstrap test (pbkrtest, Halekoh and Højsgaard 2014) to calculate  $p$  values. We calculated marginal  $R^2$  values as an indicator of the proportion of the variance explained by the fixed effects, and conditional  $R^2$  values as an indicator of the variance explained by the fixed and random effects combined (Nakagawa and Schielzeth 2013). For GLMM results, we also present AIC and BIC values for full and reduced models for reference (Table 1). Figures were produced with ggplot2 (Wickham 2016).

#### Experiment two: plant-soil feedback

We employed a plant-soil feedback study to determine whether soils conditioned by *Cytisus* support increased AMF colonization and rhizobia, together with increased *Cytisus* growth. We used the first experiment as the

**Table 1** Model results for Experiment 1, comparing *Cytisus*-invaded to uninvaded soils collected from the field, with greenhouse block as a random effect. We present GLMM results for nodule counts, hyphae and vesicles/arbuscules on *Cytisus* roots, and germination/seedling establishment, including marginal and conditional  $R^2$ ,  $p$ -values from a parametric bootstrap model

Response variable	Marginal $R^2$	Conditional $R^2$	P-value	F-value (DF)	AIC	BIC
Nodule count	0.69	0.96	0.01		8396.2 [11,378.6]	8405.8 [11,385.0]
Hyphae	0.31	0.32	0.02		924.9 [2172.4]	931.6 [2166.9]
Vesicles and arbuscules	0.38	0.39	0.02		1058.6 [2994.5]	1065.3 [2999.0]
Germination / seedling establishment	0.10	0.12	0.004		234.6 [247.8]	244.1 [254.1]
Aboveground biomass	0.25	0.29	<0.001	59.3 (1163)		
Belowground biomass	0.18	0.33	<0.001	23.0 (1,78)		

comparison approach, and AIC and BIC for the full model and for the model including only the random effect [in brackets]. For aboveground and belowground biomass, we present LMM results, including marginal and conditional  $R^2$ , F-values with degrees of freedom, and  $P$ -values

*Cytisus* conditioning phase for uninvaded and invaded forest soils. At the same time, we conditioned “control” (our uninvaded forest) soils with Douglas-fir, which is a dominant tree species at our site and across Western Washington forests. We stratified Douglas-fir seeds for 30 days at 4° C and on February 25, 2017 we planted 2 seeds into each of 90 conetainers with uninvaded soils which were weeded and thinned to 1 seed. We randomized these with the other two treatments in the first phase of the experiment, and they were treated as described above. Following harvest, the soil from each invaded and uninvaded replicate was collected in a sterilized container and then immediately placed back into its conetainer with additional perlite (approximately 15 mL) as needed to prevent soil loss. The soils were then stored in its respective conetainer at 4 °C for 37 to 59 days variable across blocks and later used in the second experiment.

We scarified *Cytisus* seeds and germinated them on filter paper. Between January 19 and January 29, 2018, we planted two germinated seeds into conetainers containing the three soil treatments and re-arranged them into randomized blocks. Three weeks after the initial planting we replanted the replicates where *Cytisus* seedlings had failed to establish. Conetainers were thinned to one *Cytisus* individual in the following two weeks and were weeded weekly. We grew the plants for 26 weeks. We harvested *Cytisus* plants one block at a time over a three-week period in July 2018. We measured above-

and belowground biomass as described above. As in experiment one, we removed and cleaned *Cytisus* roots, counted root nodules, and randomly assigned plants to be assessed for either AMF colonization ( $N=67$ ) or belowground biomass ( $N=148$ ).

In Experiment 2, we compared three soil conditioning treatments (uninvaded forest soil conditioned with Douglas-fir (control), uninvaded forest soil conditioned with *Cytisus*, and *Cytisus*-invaded soil conditioned with *Cytisus*). All analyses were performed in R version 3.5.0 (R core team 2018), using the lme4 package (Bates et al. 2015). All models included the soil conditioning treatment as a fixed factor and greenhouse block as a random factor. To test for differences in above- and belowground biomass across soil conditions, we used LMM with Tukey’s post hoc comparisons. Aboveground biomass met the assumptions of homoscedasticity and normally-distributed residuals, and belowground biomass was ln-transformed to meet those assumptions. To evaluate differences in root nodule counts, AMF hyphal colonization, and presence of arbuscules and vesicles, we used GLMM that included the Poisson family and logit link function. We compared the proportion of *Cytisus* roots that contained hyphae, or arbuscules and vesicles combined with GLMMs with the binomial distribution and the logit link function. We used a parametric bootstrap test (pbkrtest, Halekoh and Højsgaard 2014) to calculate  $p$  values. We calculated marginal  $R^2$  values as an indicator of the proportion of

the variance explained by the fixed effects, and conditional  $R^2$  values as an indicator of the variance explained by the fixed and random effects combined (Nakagawa and Schielzeth 2013). To assess the significance of differences between soil conditioning treatments, we used the contrast function in the emmeans package (Lenth 2018). For GLMM results, we also present AIC and BIC values for full and reduced models for reference (Table 2), with all pairwise comparisons presented in Supplementary Table I. Figures were produced with ggplot2 (Wickham 2016).

## Results

### Experiment 1: comparison of invaded and uninvaded soil

*Cytisus* had nearly three times more root nodules when grown in *Cytisus*-invaded field soil than in uninvaded soil (Fig. 2a,  $P = 0.01$ , Table 1). The proportion of *Cytisus* roots colonized by fungal hyphae was 45% greater (Fig. 2b,  $P = 0.02$ , Table 1), and the proportion of roots with arbuscules and/or vesicles was 60% greater in roots grown in *Cytisus*-invaded soils than uninvaded soils (Fig. 2c,  $P = 0.02$ , Table 1).

*Cytisus* seedlings were half as likely to establish in *Cytisus*-invaded soil as they were when sown into uninvaded soil (Fig. 3a,  $P = 0.004$ , Table 1). Despite increased nodulation and AMF colonization in

*Cytisus*-invaded soils, *Cytisus* plants accumulated 27% less aboveground biomass when grown in invaded soil than in uninvaded soil (Fig. 3b,  $P < 0.001$ , Table 1). Likewise, *Cytisus* had 11% less belowground biomass when grown in *Cytisus*-invaded soil than in uninvaded soil (Fig. 3c,  $P < 0.001$ , Table 1).

### Experiment two: plant-soil feedback

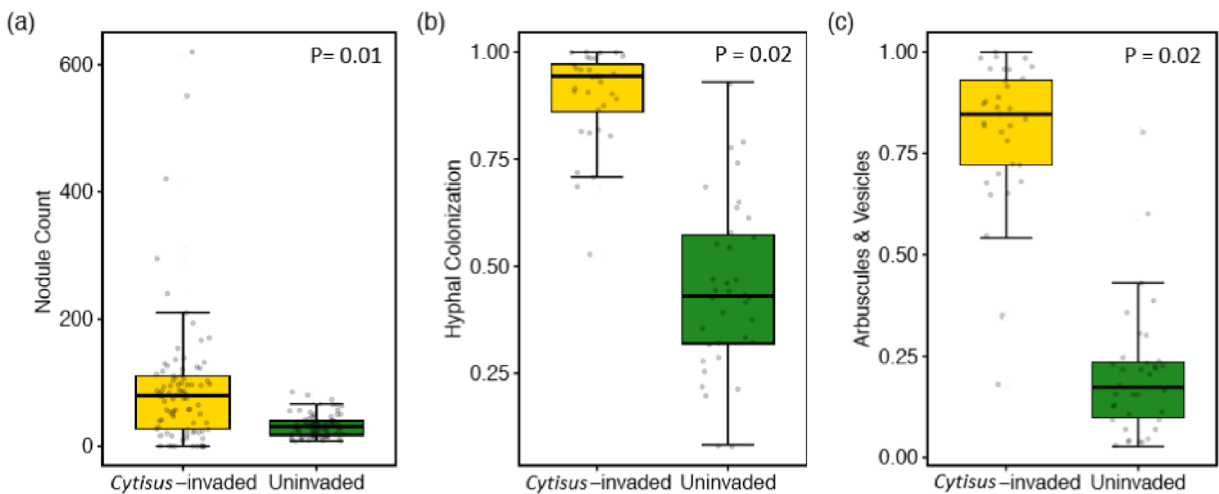
As in Experiment 1, the number of *Cytisus* root nodules responded significantly to soil type ( $P = 0.01$ , Table 2). Soil conditioning for 26 weeks by *Cytisus* resulted in plants with 14% more nodules compared to plants grown in the same soil conditioned by Douglas-fir (Fig. 4a). Plants grown in *Cytisus*-invaded field soils further conditioned with *Cytisus* also had significantly more nodules than those grown in Douglas-fir conditioned soil, but although they had a 14% further increase in root nodule count over plants grown in *Cytisus*-conditioned soil that was originally uninvaded (Fig. 4a, Supplemental Table I), this difference was not significant.

The effect of soil conditioning treatment was also significant for both hyphae ( $P = 0.002$ , Table 2) and arbuscules and vesicles ( $P = 0.002$ , Table 2). Conditioning uninvaded forest soil with *Cytisus* led to a 41% increase in fungal hyphae (Fig. 4b) and a 55% increase in arbuscules and vesicles (Fig. 4c) relative to the same uninvaded field soils that were conditioned with Douglas-fir. Plants grown in invaded soil further conditioned

**Table 2** Model results for Experiment 2, comparing *Cytisus* grown in (1) uninvaded soil conditioned by Douglas-fir, (2) uninvaded soil conditioned by *Cytisus*, and (3) invaded soil conditioned by *Cytisus*, with greenhouse block as a random effect. We present GLMM results for nodule counts, hyphae and vesicles/arbuscules on *Cytisus* roots, including marginal and conditional  $R^2$ , p-values from a parametric bootstrap model comparison

Response variable	Marginal $R^2$	Conditional $R^2$	P-value	F-value (DF)	AIC	BIC
Nodule Count	0.30	0.50	0.01		3211.6 [3365.0]	3225.8 [3372.2]
Hyphae	0.283	0.285	0.002		723.2 [1730.5]	732.0 [1734.9]
Vesicles and Arbuscules	0.171	0.174	0.002		1063.8 [1766.5]	1072.7 [1770.9]
Aboveground Biomass	0.30	0.32	<0.001	58.3 (2254)		
Belowground Biomass (log-transformed)	0.23	0.48	<0.001	332.8 (2141)		

approach, and AIC and BIC calculated for the full model and for the model including only the random effect [in brackets]. See Supplemental Table 1 for AIC and BIC for all pairwise comparisons. For aboveground and belowground biomass, we present LMM results, including marginal and conditional  $R^2$ , F-values with degrees of freedom, and  $P$ -values



**Fig. 2** Box plot of (a) the number of root nodules; (b) the proportion of roots with hyphal infection and; (c) the proportion of *Cytisus* roots with vesicles and arbuscules on *Cytisus* roots grown in *Cytisus*-invaded (yellow) and uninvaded (green) forest soils. Mid-line represents the median, the bottom of the box

represents the 25th percentile, the top of the box represents the 75th percentile, the whiskers represent the minimum and maximum values, and data points are shown as grey dots. *P* values were generated with a parametric bootstrap test (Table 1)

by *Cytisus* had the most fungal hyphae and arbuscules/vesicles (Fig. 4b, c).

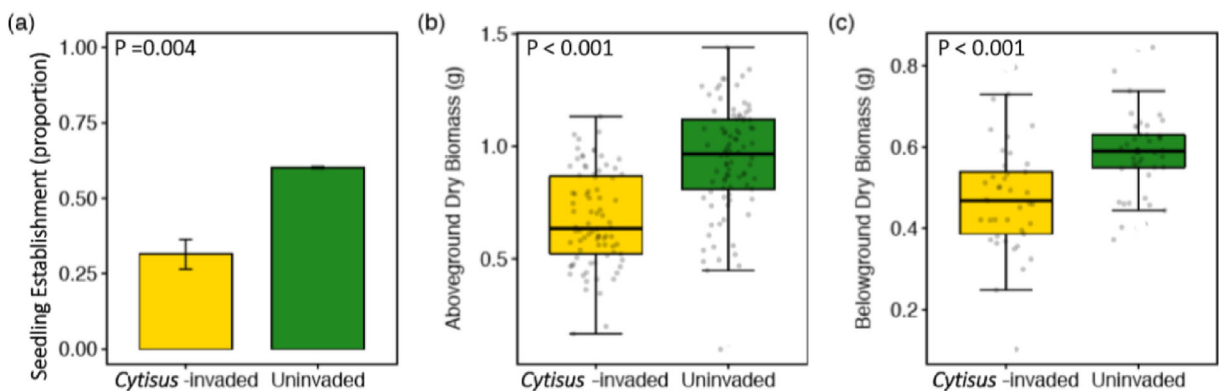
Soil conditioning treatment had a significant effect on both aboveground biomass ( $P < 0.001$ , Table 2) and (log-transformed) belowground biomass ( $P < 0.001$ , Table 2). In a pattern consistent with the first experiment, *Cytisus* grown in *Cytisus*-conditioned soil had 28% less aboveground biomass than plants grown in “control” soil conditioned by Douglas-fir (Fig. 5a). Similarly, belowground biomass was about 25% reduced in plants grown in soil conditioned by *Cytisus* (Fig. 5b). For the soils conditioned by *Cytisus*, neither aboveground nor belowground biomass was different between

soil that was originally invaded and soil that was originally uninvaded (Fig. 5a, b).

## Discussion

### Limitation of microbial mutualists in uninvaded soil

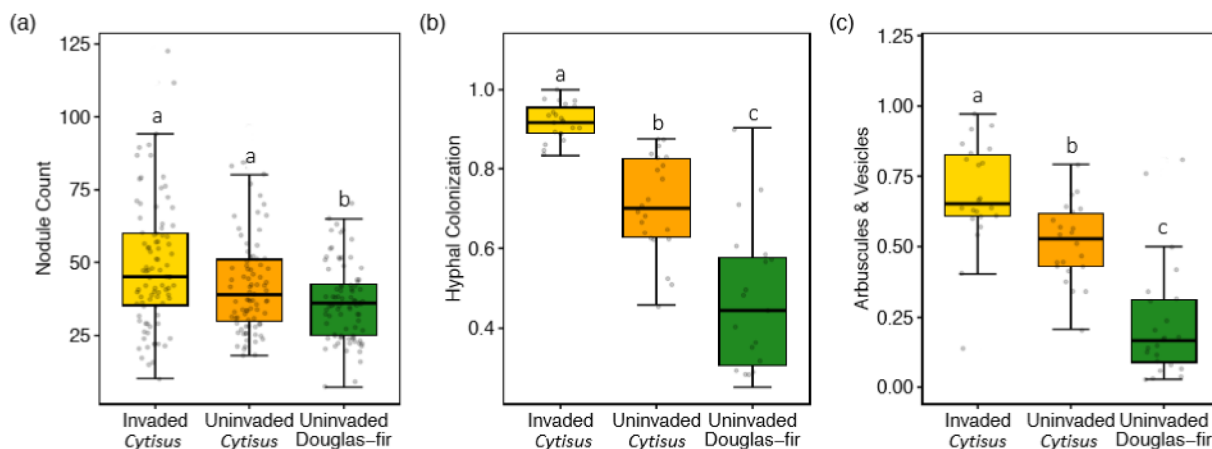
Our two experiments provide consistent and complementary evidence that beneficial microbial mutualists associated with *Cytisus* were limited in uninvaded soils. *Cytisus* plants grown in uninvaded soils had less mycorrhizal colonization and less nodulation than plants



**Fig. 3** (a) Germination and establishment success of *Cytisus* seeds in *Cytisus*-invaded (yellow) and uninvaded (green) forest soils 25 days following initial planting. Error bars are  $\pm 1$  standard deviation, estimated as  $\sqrt{p(1-p)/n}$ . (b) Aboveground and (c)

Belowground *Cytisus* dry biomass (g) when grown in *Cytisus*-invaded (yellow) and uninvaded (green) forest soils. See Fig. 2 for box plot description





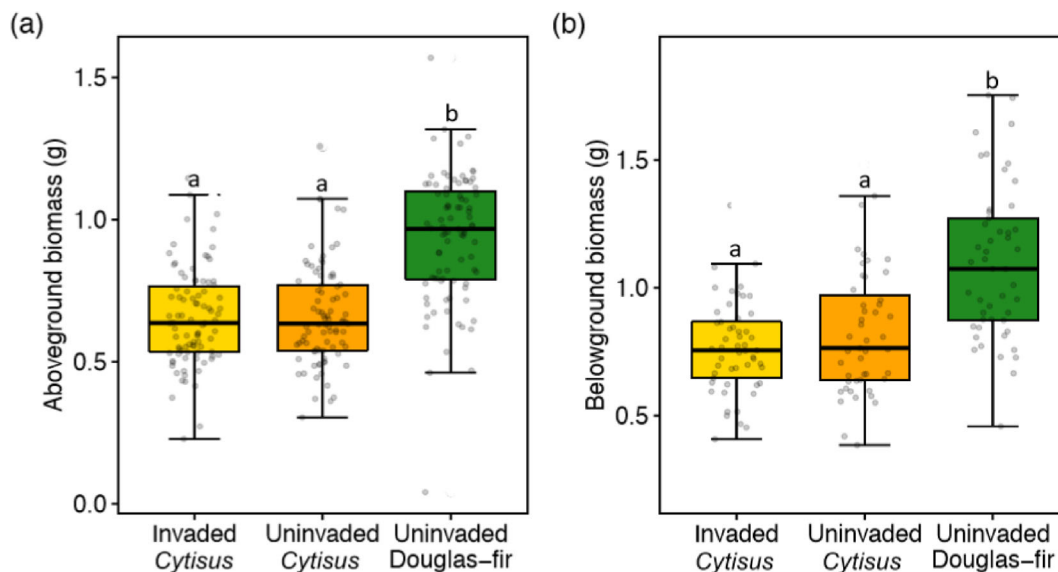
**Fig. 4** Comparison of *Cytisus*-conditioned invaded field soil (yellow), *Cytisus*-conditioned uninvaded forest soil (orange), and Douglas-fir-conditioned uninvaded forest soil (green) for (a) the number of root nodules, (b) the proportion of roots with hyphal

infection, and (c) the proportion of *Cytisus* roots with vesicles and arbuscules. See Fig. 2 for box plot description. Boxes with the same letter are not significantly different at  $P < 0.05$

grown in soils invaded by conspecifics, and we further found that conditioning uninvaded soil with *Cytisus* increased the abundance of its microbial mutualists. These findings suggest that populations of the AMF and rhizobia that associate with *Cytisus* are limited by their access to host individuals. Studies of microbe community composition have found that both spatial heterogeneity and host specificity influence the distribution of root-associated pathogens and mutualists (Liang et al. 2015; Schroeder et al. 2019). Rhizobia and

mycorrhizal mutualists of invaders may be particularly limited because specialists may be left behind in their native range. On the east coast of North America, inoculation with compatible *Bradyrhizobium* increased *Cytisus* growth by up to 5-fold (Parker et al. 2006); others have also shown that plant invasions can be limited by spatial variation in soilborne symbionts (Nuñez et al. 2009).

Rhizobia limitation in *Cytisus* is consistent with an analysis of introduced legumes worldwide by Simonsen



**Fig. 5** Comparison of *Cytisus*-conditioned invaded field soil (yellow), *Cytisus*-conditioned uninvaded forest soil (orange), and Douglas-fir-conditioned uninvaded forest soil (green) for (a)

aboveground and (b) belowground dry biomass (g) of *Cytisus*. See Fig. 2 for box plot description. Boxes with the same letter are not significantly different at  $P < 0.05$

et al. (2017), which found that legumes are often limited by their symbiotic rhizobia in introduced regions. Legume-invaded soils had more rhizobia than uninvaded soils in a number of studies (Slabbert et al. 2014; Birnbaum et al. 2016; LeRoux et al. 2018). For example, in a large greenhouse study, LeRoux et al. (2018) found that 5 different invasive *Acacia* species in South Africa had more root nodulation when grown in invaded soil compared to uninvaded soil. Similarly, terHorst et al. (2018) found that *Medicago polymorpha* had increased nodulation when planted with inoculum from *Medicago*-invaded soils compared to inoculum from uninvaded soils. Lau and Suwa (2016) also found that the invasive legume *Vicia villosa* had more root nodulation when grown in invaded soils. Our results are consistent with these studies and provide further evidence that rhizobia that associate with invaders are frequently limited in their introduced range.

Although rhizobia were less abundant in uninvaded soils, they were in fact initially present, which raises the question of where the inoculum originated. Symbionts of an introduced host can either be introduced along with the host or be acquired from a resident host (Weir et al. 2004; Leary et al. 2006; Parker et al. 2006; Rodríguez-Echeverría et al. 2007; Ndlovu et al. 2013). It is not uncommon for N-fixing legumes to successfully invade novel environments where native legumes are sparse or absent (Vitousek et al. 1987; Paynter et al. 2003), which suggests that invaders may commonly bring symbionts with them. In a study of 270 bacterial isolates from across 15 populations on both coasts of North America, Horn et al. (2014) demonstrated that *Cytisus*-associated *Bradyrhizobium* included some strains from the native range. Because there are no native North American species in the Genistae tribe of Fabaceae, the *Bradyrhizobium* associating with native legumes must cross a fairly wide phylogenetic gap to switch hosts onto *Cytisus scoparius*. Yet, Horn et al. (2014) also found some strains from *Cytisus* that were acquired from native legumes. Intriguingly, the remaining *Bradyrhizobium* strains showed a hybrid origin, suggesting horizontal gene transfer of European symbiosis island genes into a genetic background of native *Bradyrhizobium* lineages. Any of these three scenarios may have contributed to the nodulation of *Cytisus* in our study.

Similar to rhizobia, AMF colonization of *Cytisus* roots was lower in uninvaded soils compared to invaded soils. This was surprising because in Pacific

Northwest forests, many of the dominant understory plant species are AMF-associated (e.g. *Gaultheria shallon*, *Mahonia aquifolium*, and *Polystichum munitum* (Moora et al. 2011; Davison et al. 2015)), so we expected high availability of *Cytisus*-compatible fungal inoculum even in the uninvaded soils. However, mycorrhizal fungi do show a phylogenetically structured host range (Anacker et al. 2014; Chen et al. 2017), and because plants in the Fabaceae were rare or absent in the forest community at our site, phylogenetic isolation may have contributed to the lack of compatible AMF in the uninvaded soils. Further, successful invasive species may rely less on mycorrhizal fungi than their native counterparts (Pringle et al. 2009; Seifert et al. 2009). Although we did not explicitly study the dependency of *Cytisus* survival or growth on AMF, mutualism limitation could be a factor contributing to variation in the rate of population growth and spread in *Cytisus*.

Our data suggests that rhizobia and AMF are initially limiting in uninvaded soils, but that mutualist limitation to invasion may be rapidly released following *Cytisus* establishment. In the second experiment, both rhizobia and AMF increased over a period of 26 weeks in the presence of the invader, providing evidence that *Cytisus* alters the soil microbial community in a way that should influence its own performance. This nearly immediate impact is consistent with other studies in which we observed rapid changes to soil fungal abundance and community composition following *Cytisus* invasion (Grove et al. 2017). Many studies, including our own, have shown that plant invasions alter the abundance, diversity, and community composition of mycorrhizal fungi (Shah et al. 2009; Grove et al. 2012, 2019; Zhang et al. 2019). Although we did not observe a concurrent improvement in plant growth in our experiment (discussed below), it is possible that over longer time frames and under field conditions such changes in soil microbial communities could benefit *Cytisus* establishment, survival, biomass accumulation, or reproductive output. We know for example that AMF can provide benefits that were not captured in our measure of plant growth, such as promoting resistance to drought stress (Rapparini and Peñuelas 2014) and pathogen protection (Veresoglou and Rillig 2012). Under natural conditions, an increase in AMF colonization could in fact contribute positively to *Cytisus* invasion success during times of nutrient limitation or drought.

## Negative plant-soil feedback in an aggressive invader

Despite the higher abundance of its soilborne microbial mutualists, *Cytisus* growth was reduced in invaded soil, and reduced in uninvaded soil conditioned for 6 months with *Cytisus*. That is, *Cytisus* showed a negative plant-soil feedback. In addition, germinating seeds of *Cytisus* were suppressed in *Cytisus*-invaded soils. Review papers have demonstrated that negative plant-soil feedbacks are common, and more common than neutral or positive feedbacks (Bever 2003; Kulmatiski et al. 2008; Petermann et al. 2008; Lekberg et al. 2018). Perhaps it is surprising that we did see the development of such clear negative feedback in plant growth given the small size of our pots. We did monitor the size of the plants throughout the experiment and started the harvest before the roots began to be pot-bound. We estimate that the ratio of mean root dry mass to soil in the pot was 0.0069. Methodological variation in the measurement of plant-soil feedback is a general problem in this field, which highlights the particular strength of our study. We quantified plant-soil feedback for *Cytisus* using two independent methodologies: comparing field-collected soils (conspecific vs. away) and using a multi-generational greenhouse experiment. The results of the two experiments were highly congruent both qualitatively and quantitatively: *Cytisus* showed negative feedbacks consistently with both methodologies.

There are several possible mechanisms for the negative feedback that we document here, although unfortunately our experimental design does not allow us to distinguish among these mechanisms. Negative plant-soil feedbacks can have abiotic and/or biotic origins. One possible abiotic mechanism is the species-specific depletion of soil nutrients (Gustafson and Casper 2004; Ehrenfeld et al. 2005; Manning et al. 2008). For example, Gustafson and Casper (2004) found that the signature of a negative plant-soil feedback in *Sorghastrum nutans* was eliminated when NPK fertilizer was added. As a nitrogen-fixer, *Cytisus* tends to increase nitrogen availability and fertility of soils (Haubensak and Parker 2004; Caldwell 2006; Grove et al. 2015), and so nutrient depletion seems an unlikely explanation for the negative plant-soil feedback we found. However, nitrogen-fixation is also a phosphorus-demanding process (Schulze 2004), and there is evidence that some nitrogen-fixers can deplete phosphorus pools relative to other functional groups (e.g., Hooper and Vitousek 1998). Without a nutrient addition experiment, we

cannot eliminate the possibility that nutrient depletion contributed to the negative plant-soil feedback seen in our experiments. In a recent review, Crawford et al. (2019) argued that changes in abiotic soil properties are not as important as soil microbes in driving negative plant-soil feedbacks. However, nutrient depletion can also interact with biotic factors (Bennett and Klironomos 2019); for example, nutrient-limited plants could be unable to provide sufficient carbon to mycorrhizal fungi. Under these conditions, the functional role of mycorrhizal fungi may switch from mutualistic to parasitic (Johnson et al. 1997; Westover and Bever 2001) and contribute to negative plant-soil feedbacks (Bever 2002). This could have contributed to the patterns we found. However, under this scenario we might expect to see an overall decrease in mycorrhizal colonization in *Cytisus* plants grown in their own soil, but we saw the opposite.

The most common biotic driver of negative plant-soil feedbacks is thought to be pathogen accumulation in the rhizosphere (Mills and Bever 1998; Schnitzer et al. 2011; Liang et al. 2016; Crawford et al. 2019). Soilborne pathogens include widespread taxa that have a cosmopolitan origin, and many fungi, oomycetes, and nematodes are broad host generalists. Therefore, in addition to bringing pathogens with them, invaders also acquire pathogens in their introduced range (Beckstead and Parker 2003; Reinhart and Callaway 2006; Parker and Gilbert 2007; Flory and Clay 2013; Goss et al. 2020). While we did not explicitly quantify the abundance or impact of pathogens associated with *Cytisus* in this study, in previous work at nearby sites Grove et al. (2019) used a high throughput (Illumina) sequencing approach and established that *Cytisus*-invaded soils include fungal genera that are commonly plant pathogens, such as *Fusarium*, *Alternaria*, *Plectosphaerella*, and *Cladosporium*. Of particular note, Grove et al. (2019) identified *Pleiochaeta setosa* in *Cytisus*-invaded soil, which is known as an important plant pathogen of legumes (Sweetingham 1984). Other pathogen genera, such as *Pythium* and *Rhizoctonia*, have been observed on other *Cytisus* species in North America (Farr et al. 1989). Our observation that germinating *Cytisus* seeds were heavily suppressed in *Cytisus*-invaded soils also points to a role for soilborne pathogens, which are the cause of damping off diseases of seeds and seedlings. Plant-soil feedbacks are less frequently studied at the seed

germination stage (Miller et al. 2019), but they could be important drivers of selective seedling recruitment.

Ultimately, invaders will encounter both enemies and mutualists among their novel microbial associates. The net effect of negative and positive interactions will determine whether invasion success is enhanced or limited by the novel microbial community (Mitchell et al. 2006; Reinhart and Callaway 2006). The argument that AMF show less host-specificity than pathogens led to the prediction that enemy escape would be more common than mutualism limitation in invasive plants, and this would lead to positive plant-soil feedbacks in invaders (Klironomos 2002; van der Putten et al. 2013). For example, *Centaurea maculosa* showed positive feedbacks in its invaded range, compared to negative feedbacks in its native range (Callaway et al. 2004). *Cytisus* invasions reach extremely high abundance, and it was reasonable to consider that this might reflect positive plant-soil feedback in its introduced range. Positive plant-soil feedback could generate an Allee effect, in which *Cytisus* plants perform better and better as populations grow, with consequences for patterns of spread (Cappuccino 2004; Taylor and Hastings 2005). However, our results are not consistent with this prediction.

In terms of community dynamics, it is the relative strength of plant-soil feedbacks across species that determines the influence of plant-soil feedbacks on the outcome of competitive interactions (Bever 2003; Agrawal et al. 2005; Kulmatiski et al. 2008). Therefore, invasion could be promoted by plant-soil feedbacks if native species experience stronger negative effects than introduced species (Suding et al. 2013). The presence of AMF has also been shown to contribute to competitive dominance of an invader over a native through indirect effects (Marler et al. 1999). In our previous work, we demonstrated that ectomycorrhizal fungi associated with native Douglas-fir trees declined following *Cytisus* invasion (Grove et al. 2012, 2019). *Cytisus* stems and leaves produce the alkaloid sparteine, which strongly depressed the growth of some fungi in a bioassay but *Wilcoxina mikolae*, an AMF species commonly found in *Cytisus*-invaded areas, was tolerant of sparteine (J. Thompson, S. Grove, G.S. Gilbert, and I.M. Parker, unpublished data). Shifts in the mycorrhizal community caused by *Cytisus* invasion may have important impacts

on the ability of native plants to compete with *Cytisus* (Richardson et al. 2000; Inderjit and van der Putten 2010; Nuñez and Dickie 2014). Therefore, a progressive increase of AMF colonization and rhizobia nodulation with invasion may still contribute to ecological dominance of the invader even in the absence of positive conspecific plant-soil feedbacks.

#### Implications for invasion dynamics

We found evidence for mutualism limitation of *Cytisus* growth in the first experiment, suggesting that mutualist limitation may initially depress the rate of *Cytisus* invasion into novel habitats. Seedling establishment is the most important life stage in incipient and fast-growing populations of *Cytisus* (Parker 2000), and is the stage at which rhizobia and AMF are most likely to affect plant survival and growth (Wandrag et al. 2013; van der Heijden et al. 2016). We also found, however, that soilborne mutualists of *Cytisus* quickly increase in abundance, suggesting that mutualism limitation of *Cytisus* growth following incipient invasion may rapidly disappear. We speculate that soilborne mutualists may influence plant performance under stressful field conditions and may contribute to the ecological dominance of *Cytisus* outside its native range by reducing the magnitude of negative plant-soil feedback relative to native competitors. However, our results consistently showed that *Cytisus* develops negative plant-soil feedbacks that affect germination and plant growth, and which are probably mediated by microbial communities including fungal pathogens. Future work should explore how these altered microbial communities affect both *Cytisus* populations in the field and the native species they displace.

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