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Do Conspecific Soil Microorganisms Inhibit Potentilla recta?

By

Faythe Duran

Accepted in Partial Completion
of the Requirements for the Degree of
Master of Science

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MASTER'S THESIS

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Faythe Duran

7 July 2017

Do Conspecific Soil Microorganisms Inhibit Potentilla recta?

A Thesis Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Faythe Duran
July 2017

Abstract

In the U.S., *Potentilla recta* is an invasive, exotic forb. Previous research suggests that the soil microbes of native congeners (relatives within the same genus as *P. recta*) may inhibit *P. recta*, presumably because phylogenetically similar species may culture and be susceptible to similar pathogens. Our study aimed to answer three questions: (1) how do the fungal communities within the roots of *P. recta* compare to the fungal communities within the roots of neighboring congeners *Potentilla gracilis* and *Drymocallis glandulosa* (hereinafter referred to as the congeners) and native forbs, (2) what are the effects of the whole microbial community (microflora, microfauna, and some mesofauna <2 mm), and the small microbial community (microflora, <20 μm) on *P. recta*, (3) is there evidence that conspecific soil microbes mediate the distribution of *P. recta* in the field?

To address question one, we used high-throughput sequencing to compare the fungal communities within the roots of *P. recta*, its native congeners and neighboring forbs. To address our second question, we conducted a greenhouse experiment testing the effects of microbe fraction [none, small (< 20 μm), whole (< 2 mm)] and microbe source plant (congeners, forb, grass, and *P. recta*) on *P. recta*'s biomass. To address our third question, we observed and analyzed the distribution of *P. recta* in relation to its congeners in an intermountain grassland in Western Montana.

The fungal communities within the roots of *P. recta* and its congeners were different from other neighboring forbs, but pathogen abundance did not correspond to *P. recta* biomass. Further, the fungal communities within the roots of *P. recta* were unchanged by neighboring plant identity. In the greenhouse, we found reduced *P. recta* biomass from the whole microbial

community collected from all source plants, but biomass did not differ significantly by source plant. Additionally, the magnitude of this negative effect was correlated with percent of colonization by arbuscular mycorrhizal fungi. We found neutral effects from the small microbe fraction, and no significant differences among source plant. In the field *P. recta* and other common grassland forbs were distributed at equal distances from the native congeners. We found no association between *P. recta* and the congeners co-occurring at the landscape scale. Overall, our results contradict previous findings and suggest that the direct and indirect effects of soil microbes on *P. recta* are nonspecific.

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1 Introduction

Invasive species reduce economic value and the resiliency of ecosystems (Vitousek 1997, Pimentel et al. 2005, Ehrenfel 2010). Plant invasion ecology is the study of which factors influence the spread, establishments, and ecological impacts of non-native plant species. The goal being to quantify, predict, prevent, or reduce the effects of invasion (Elton 1958, Levine and D'Antonio 1999). Ecologists have many hypotheses to explain why some plants species are successful invaders, when others are not. Broadly, these hypotheses consider invaded ecosystems, ecosystem and invader interactions, or only the invader (Jeschke et al. 2012). An area of ongoing research with consideration for the ecosystem and the invader is the interaction between invasive plants and soil microbes (Kulmatiski et al. 2008, Jeschke et al. 2012).

Soil microbes are a diverse group of microorganisms spanning the three domains of life. They can be broadly grouped by size¹ or functional group (i.e. pathogens, mutualists, and saprobes; Wall et al. 2012). One hypothesis particularly applicable to plant-soil-microbe interactions is the Enemy Release Hypothesis. The Enemy Release Hypothesis predicts that natural enemies (pathogens) limit invasive species in their native range, but not in their non-native ranges (Elton 1958, Agrawal et al. 2005). This has been observed for invasive plants by negative effects such as reduced biomass (total or shoot) and reduced relative growth rate when grown with their native soil microbes compared to non-native range soil microbes relative to sterile soils (Reinhart and Callaway 2004, Maron et al. 2014). Additionally, in their non-native

¹ Microfauna (<0.2mm) and mesofauna (0.2mm – 10mm) are protozoa, nematodes microarthropods and arbuscular mycorrhizal fungi (AMF)

ranges, invasive plant species may experience less negative soil feedbacks (smaller reduction of biomass) than native plant species (Klironomos 2002, Agrawal et al. 2005, van der Putten et al. 2007). This suggests that plants are more responsive to their coevolved soil microbes.

Phylogenetically related plants may culture and be susceptible to the same or similar plant pathogens. This has been found for plant species in the tropics where most plants were generally resistant to any given pathogen, but more related plants were susceptible to the same pathogens (Gilbert and Webb 2007). In a California grassland Parker et al. (2015) found, at the community scale, disease pressure (tissue lost to disease) was stronger when the plant communities were more phylogenetically related. However, the phylogenetic relatedness of plants in a community may not precisely predict plant and soil-microbe interactions. This was demonstrated by the meta-analytical findings of Mehrabi and Tuck (2015) that phylogenetic distance did not predict the magnitude of plant-soil feedbacks. Additionally, Ma et al. (2016), found that invasive plants more closely related to native plants tended to be less successful at the local scale (e.g. plant-to-plant interactions), but unaffected by phylogenetic distance of the plant community at the regional scale (e.g. landscape). Therefore, phylogenetic distance between invasive plants and the native plant community may predict pathogen susceptibility but not necessarily the effects of all the soil microbes associated with the invader. Furthermore, research on the effects of soil microbes on invasive plants typically compares plant biomass when grown with all living soil microbes to sterile soils. Lost in this method is the ability to differentiate between the contributions of potential fungal pathogens (small-spored fungi and viruses) from mutualists (e.g. big-spored arbuscular mycorrhizal fungi).

The interactions between plants and arbuscular mycorrhizal fungi (AMF) change with plant functional group and are contingent on environmental factors such as nutrient concentrations, and light availability (Roberts and Paul 2006, Hoeksema et al. 2010, Sun and He 2010, Van der Putten et al. 2013, Bunn et al. 2015). AMF are generally considered plant mutualists that increase uptake of host nutrients such as phosphorus and nitrogen in exchange for plant-fixed carbon (Smith and Read 2008). However, this association is a continuum between mutualism and parasitism (Johnson et al. 1997, 2003). Mutualism is more likely when nutrients such as phosphorus and nitrogen are limiting and the plant benefits from AMF, while parasitism is more likely when nutrients are abundant and the plant derives less benefit from AMF (Johnson et al. 1997, 2003, Hoeksema et al. 2010, Werner et al. 2014). AMF may function to offset the effects of pathogens in some conditions (Newsham et al. 1995, Sikes et al. 2009). Because environmental factors (e.g. phylogenetic distance between invader and native plant community and nutrient concentration) may be confounding we build from past research on specific plant species to compare or control for those environmental factors.

Potentilla recta (sulphur cinquefoil) is a Eurasian forb that is invasive in North America. *P. recta* has been documented in all U.S. states except for Arizona and Utah ("*Potentilla recta* L. sulphur cinquefoil" 2016). In grassland ecosystems of Montana, *P. recta* has been found to increase the biomass of experimental native forb communities and has a large range (occurring in many surveyed plots) and local abundance (high percent cover; Maron and Marler 2008, Pearson et al. 2016). In the same Montana landscape, McLeod et al. (2016), found invaded *P. recta* plots to have a higher nitrification potential (estimate of nitrifier abundance) and ammonia oxidizing bacterial gene abundance compared to paired native plant communities. Yet, they

found *P. recta* did not alter the concentration of nitrate (NO_3^-) in the soil, aboveground net primary productivity, or ammonia oxidizing thaumarchaeal gene abundance in invaded compared to noninvaded plots (McLeod et al. 2016). Thus, the effects of invading *P. recta* on the ecosystem are mixed.

Investigating the interaction between *P. recta* and the native plant community, researchers found that native relatives (congeners) of *P. recta* may provide resistance against *P. recta* invasion (Maron and Marler 2008b, Callaway et al. 2013). Specifically, they found *P. recta* had smaller mean biomass when seeded into experimental monocultures of its native congener, *Potentilla arguta*, compared to experimental monocultures of other native, common, non-congener forbs (Maron and Marler 2008). To test if *P. recta* were inhibited by conspecific soil microbes, Callaway et al. (2013) conducted two greenhouse experiments. First, they compared *P. recta* biomass when grown with soil (whole or sterile) from the experimental monocultures used by Maron and Marler (2008) of the native congener (*P. arguta*), a native grass (*Festuca idahoensis*), or itself. They found *P. recta*'s biomass to be significantly less when grown with whole compared to sterile soils from itself and the congener, but found no significant difference in biomass of *P. recta* in whole versus sterile soils of *F. idahoensis* (Callaway et al. 2013). Second, they used two congeners (*P. arguta* and *P. gracilis*), another relative (*Dasiphora fruticosa*), a grass (*F. idahoensis*), and *P. recta* to culture soil microbes that they used as inocula for a plant-soil feedback experiment. In agreement with their first experiment, they found *P. recta*'s biomass to be significantly less when grown with whole compared to sterile soils cultured by the congeners and the relative, but found no significant difference in biomass of *P. recta* in whole versus sterile soils of *F. idahoensis* (Callaway et al. 2013). Thus, the reduction in biomass

of *P. recta* could be attributed to inhibition by conspecific soil microbes. Unanswered is whether the effects of conspecific soil microbes observed by Callaway et al. (2013) are significant in the field, or if the negative effects of pathogens outweigh any benefit from mutualists such as AMF. Our study aimed to answer three questions: (1) how do the fungal communities within the roots of *P. recta* compare to the fungal communities within the roots of neighboring congeners and forbs, (2) what are the effects of the whole microbial community (microflora, microfauna, and some mesofauna <2 mm), and the small microbial community (microflora, <20 µm) on *P. recta*, (3) is there evidence that conspecific soil microbes mediate the distribution of *P. recta* in the field?

2 Methods

2.1 Site Description

MPG Ranch is in the Bitterroot Valley of Western Montana (46.688N, -113.986W 1375m; Figure 1). Currently, the ranch is a private research institution, but has a history of more than 100 years of raising cattle and crops. The sites we surveyed and collected soil microbial inocula are grasslands with interspersed pine trees and shrubs where *P. recta* and the native congeners *Potentilla gracilis* and *Drymocallis glandulosa* were all known to co-occur (A, B, C, D; Figure 1). Average yearly rainfall is 31.6 cm and average yearly temperature is 7.1 C° (National Centers for Environmental Information 2016).

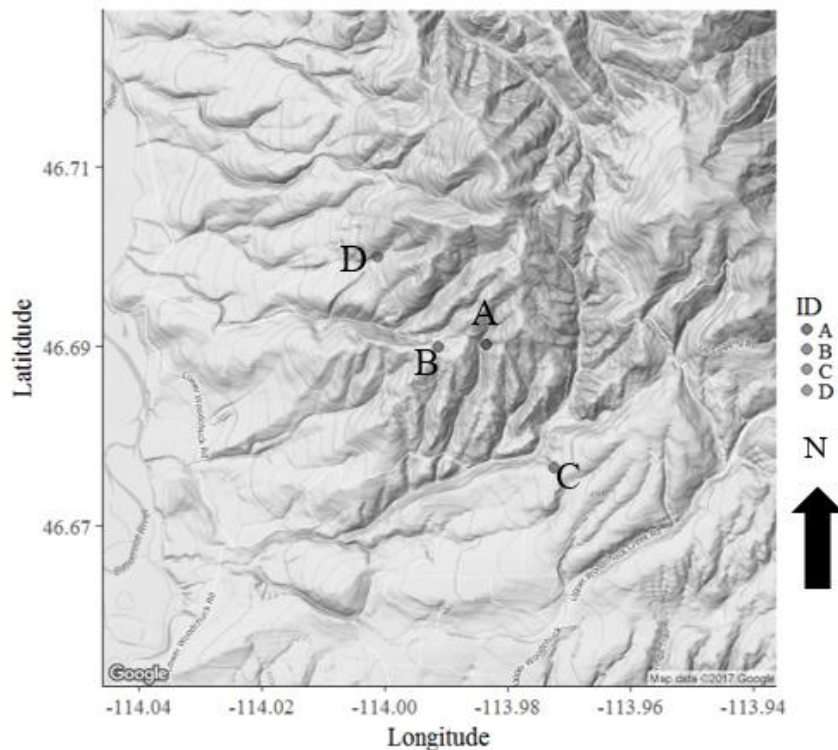


Figure 1. *Potentilla recta* surveys were completed at sites A, B, C, and D on MPG Ranch in Western MT. We collected soil microbe inocula from sites A and B. Site A is also known as Whaley draw.

2.2 *Potentilla recta* description

Potentilla recta is a perennial Eurasian forb, that produces erect stems (30-70 cm), reproduces by achenes, and forms a large woody root (Werner and Soule 1976). The leaves are coarsely serrate oblong and hairy on the top and underside (Figure 2; Werner and Soule 1976). The five petal flowers are sulfur yellow and have five green sepals and five additional bracts (Werner and Soule 1976). In the Blue Mountains of Northeastern Oregon, Tuitele-Lewis (2004) found *P. recta*'s maximum mean relative growth rate to be 1.01 g per gram of dry mass per week. *P. recta* flowers in late May and throughout June, and seeds begin to set in mid-June (Tuitele-Lewis 2004).

2.3 *Native congener species*

Two congeners of *P. recta* native to the grasslands of the intermountain west are *Potentilla gracilis* and *Drymocallis glandulosa* (formally *Potentilla*). *P. recta* has similar leaf shape is more closely related to *P. gracilis* than to *D. glandulosa* (Figure 2). Using chloroplast DNA, Dobes & Paule (2010) found *P. recta* and *P. gracilis* to be in the core *Potentilla* group that diverged 4.6 – 8.1 million years ago, and they diverged from *D. glandulosa* 45.1 – 53.4 million years ago.

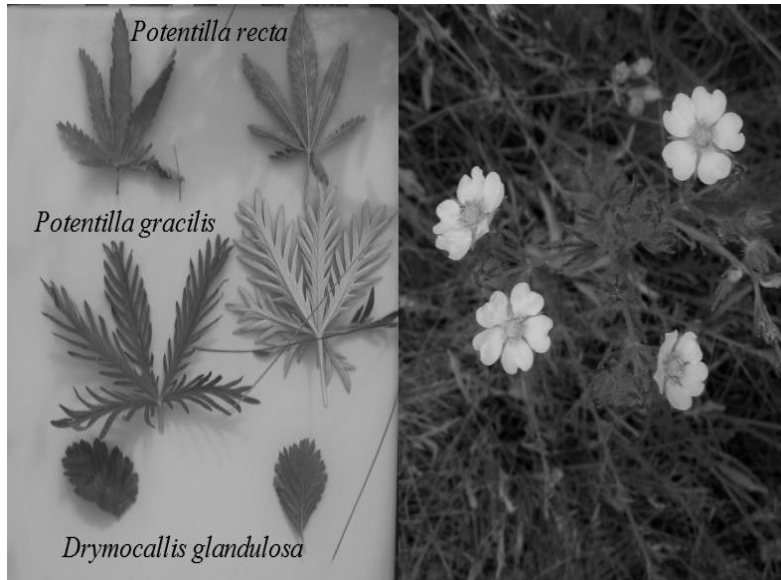


Figure 2. Leaf shape front and back of *P. recta* and its native congeners *P. gracilis*, and *D. glandulosa* (left). *D. glandulosa* leaves are compound and pinnate. Flowering *P. recta* (right).

2.4 *P. recta* 2014 harvest

At the end of June 2014, we harvested *P. recta*, co-occurring *P. gracilis* and *D. glandulosa* hereinafter referred to as “the congeners”, and other co-occurring native forbs. For each co-occurring plant pair, each plant was within 10 cm of the other plant. Plant pairs were at least 2 m apart. For each harvested plant, we took a sample of fine roots to use for molecular analysis of the fungal communities (molecular methods described in section 2.4.1). We washed then dried all harvested plants at 65 °C for 24 h and recorded dry mass (g). We collected rhizosphere soil and quantified the abiotic characteristics and those methods are described in section 2.7.2.

2.4.1 Molecular methods

We extracted DNA from 25-30 mg of lyophilized root tissue using the MoBio PowerPlant[®] Pro-htp DNA isolation kit (MoBio Laboratories, Inc. Solana Beach, CA) following the manufacturer's instructions. We used two-step PCR amplification to prepare for Illumina sequencing. In PCR1 we amplified the ITS region using the general eukaryotic primer ITS4 (White et al. 1990) and a mixture of the fungal specific forward primers fITS7 and ITS7o (Ihrmark et al. 2012, Kohout et al. 2014). We flanked each primer with 22 bp Fluidigm universal tags CS1 or CS2 (Fluidigm Inc. San Francisco, CA, USA). We performed all PCR1 in 12.5 μ L reaction volumes containing 1 μ L of DNA extract as template, 0.05 pmol bovine serum albumin, 20 pmol of each primer in 1X GoTaq[®] Green Master Mix [(Green GoTaq[®] Reaction Buffer, 200 μ M dATP, 200 μ M dGTP, 200 μ M dCTP, 200 μ M dTTP and 1.5mM MgCl₂) Promega, USA]. Each reaction was performed in a Techne TC-4000 thermocycler (Bibby Scientific, Burlington, USA) under the following conditions: initial denaturation at 95 °C for 2 minutes followed by 35 cycles at 95 °C for 1 min, 57 °C for 1 min, and 72 °C for one min, with a final elongation for 10 min at 72 °C. We confirmed the presence of our target amplicon, using a 100 bp ladder (O'GeneRuler DNA Ladder, Thermo Scientific, USA) as a size standard, all reactions were analyzed by 1.5% agarose gel electrophoresis.

We diluted amplicons generated during PCR1 1:10 to use as template in PCR2. PCR2 primer complexes consisted of the same Fluidigm tags (CS1 or CS2) as PCR1 primers, 8 bp Illumina Nextera barcodes (Illumina Inc., San Diego, CA, USA), and Illumina adapters. PCR2 was carried out in 25 μ L reaction volumes containing 1 μ L of template, 20 pmol of each primer in 1X GoTaq[®] Green Master Mix (Promega, USA). Each reaction was performed in a Techne TC-

4000 thermocycler (Bibby Scientific, Burlington, USA) under the following conditions: 95°C for 1 min followed by 10 cycles of 95°C for 30 sec, 60°C for 30 sec, 68°C for 1 min with a final elongation at 68°C for 5 min.

We purified PCR2 amplicons using AMPure XP beads (Beckman Coulter Genomics, USA) and pooled based on band intensity before sequencing. Sequencing was done at the Institute for Bioinformatics and Evolutionary Studies (iBEST) genomics resources core at the University of Idaho (<http://www.ibest.uidaho.edu/>; Moscow, ID, USA). Amplicon libraries were sequenced using 2 x 300 paired-end (PE) reads on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA).

The DNA template were unintendedly held at 23°C for 24 hours. To ensure that the thawed template would not significantly alter the results, we compared the fungal community's beta diversity between pre and post thawed samples. We found no statistical difference between the fungal communities (Monte Carlo $p < 0.001$) and we continued with our analysis.

2.5 *Molecular Bioinformatics*

MPG employee, Alexii Rummel, performed our molecular bioinformatics and they are detailed in Appendix B. Briefly, QIIME (Quantitative Insights Into Microbial Ecology, version 1.9.0; Caporaso et al. 2010) was used to perform all analyses. The fastq-join method, allowing for 10% mismatch, was used to pair trimmed raw forward and reverse PE and ITS reads (Aronesty 2013). After demultiplexing, we used Uclust to cluster operational taxonomic units (OTUs) based on a 97% sequence similarity (Edgar 2010). We used ITS sequences from UNITE as seed clusters and OTU taxonomic assignment (Koljal et al. 2013). Unmatched reads were

clustered *de novo* and we used the BLAST algorithm to compare sequences to Genbank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al. 1990). We retained the reads that matched with fungi for analysis, but they were not assigned taxonomy. We used the FUNGuild reference database to assign functional traits to our OTUs (Nguyen et al. 2016).

2.6 Greenhouse Study

2.6.1 Experimental Design

To determine if and which conspecific soil microbes cause disease in *P. recta* research could follow Koch's postulates, that is to demonstrate a consistent association of a microbe and a disease, isolation and pure culture of the microbe, inoculation of a disease-free host with the cultured microbe, and reisolation of the microbe to pure culture (Stobel and Mathre 1970). Although it is important to identify causal agents of disease, this approach is limited in that it considers microbes in isolation. In the soil, plants are surrounded by a multitude of soil microbes (Fierer et al. 2007). Therefore, it is useful to investigate the net effects of all soil microbes on plant biomass replicating field conditions. However, while some soil fungal microbes may be pathogenic, others are mutualistic but the effects of each functional group have been tested together in previous *P. recta* and soil microbe research (Callaway et al. 2013, Maron et al. 2014). We therefore, simplified our experimental design by investigating the effects of small (<2 μm) versus large (< 2 mm) soil microbes, that generally separates the large-spored mutualists such as AMF from the small-spored potential pathogens (Wall et al. 2012).

Our molecular analysis identified *Coccomyces*, *Gnomonia*, and *Erythriconium* as pathogenic fungal taxa within the roots of *P. recta*. Ascospores of *Coccomyces dentatus* are 55 μm by 3 μm (Sherwood 1980). Ascospores of *Gnomonia fragariae* are 16 μm by 3.5 μm , and ascospores of

Rosaceae associated *Gnomonia radicicola* are 9-15 μm by 1.3-2.5 μm (Noordeloosh et al. 1989, Morocko et al. 2006). Ellipsoid basidiospores of pink disease causing *Erythricium salmonicolor* are 24 μm by 8-12 μm (Akrofi et al. 2014). The thinner profile of these fungal spores would allow these representative fungal taxa to pass through our 20 μm filter, unless they were oriented so that the longest edge was horizontal. Therefore, we are justified in using microbes filtered to 20 μm for a fungal pathogen treatment.

To determine the effects of the whole ($>2\text{mm}$) and small ($<20\mu\text{m}$) fractions of the soil microbial community and if the effects differed by source host plant, we conducted a greenhouse experiment. We grew *P. recta* inoculated with the soil microbes of either the native congeners, *P. recta*, any native forb, or any native grass. To isolate the effects of large versus small microbes, we deconstructed the soil community into three parts: none (sterile soil), small (microbes smaller than 20 μm such as fungal pathogens and bacteria), and whole (all living soil microbes). The soil microbial communities were not pooled and separation of these components was done individually for each replicate. Therefore, we had four microbe source plants, three microbial treatments, and 10 replicates for a total of 120 plants (Figure 3). The effect of small microbes is the ratio of *P. recta* biomass grown with small microbes to biomass grown with no microbes. The effect of the whole microbial community is the ratio of *P. recta* biomass grown with the whole microbe treatment to biomass grown with no microbes. *P. recta* grew in the Western Washington University Biology greenhouse for 12 weeks.

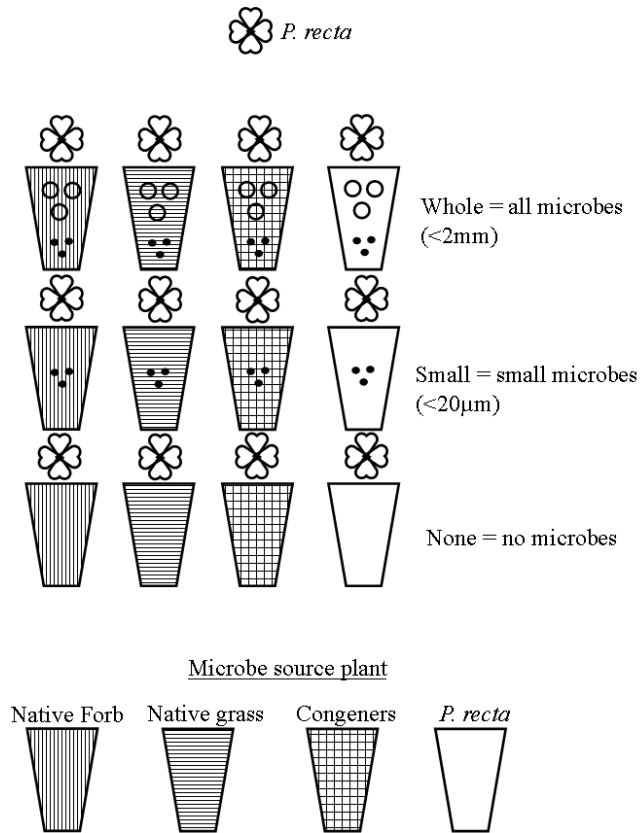


Figure 3. Diagram of the greenhouse experiment, $n = 10$, $N = 120$. Pot shading represents the source of the microbial inocula.

2.6.1.1 Soil inocuum collection

We collected soil used as inocula for the greenhouse experiment between 8 and 10 July 2016. We collected soil from sites A and B (Figure 1). Mean water content, ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and pH of the two collection sites are found in Table 1 (Methods in section 2.7.1). Soil was collected from monocultures (>40% cover of the source plant in a 1 m^2 quadrat) for each source plant treatment (forb, grass, congener, *P. recta*). Each inoculum was made from three trowels of soil 10 cm deep within a 1 m^2 area. There are 10 sets of experimental replicates and inocula for each set were collected from 10 distinct locations, at

least 50 meters apart. The source plant inocula (*P. recta*, congeners, native grass, and native forb) within each set were collected from the same general area, within a 25 meter radius.

2.6.1.2 *Small microbe treatment preparation*

To distinguish between the effects of small versus whole soil microbes, we prepared a soil microbial wash that excluded organisms larger than 20 μm for our small microbe treatment. We prepared each wash separately by set (10) and by source plant (*P. recta*, congeners, native forb, and native grass). Therefore, we made 40 distinct washes. We cleaned equipment in a 5% bleach bath between samples. We made each wash by mixing 90 mL of fresh soil with 135 mL of sterile water ($135 \text{ mL}_{\text{water}} : 90 \text{ mL}_{\text{soil}} = 1.5_{\text{water}} : 1_{\text{soil}}$). The mixture was then allowed to settle for 5 to 10 minutes before it was filtered through a 125 μm mesh metal sieve. We used a vacuum pump apparatus with a Whatman #1 qualitative filter paper to filter the wash down to microbes smaller than 20 μm . We changed the filter paper between samples, and when it became too clogged with soil. To add a concentration equal to 60 mL of soil microbes, we added 90 mL of the final microbial filtrate to the pot that contained the corresponding sterilized inoculum ($1.5_{\text{filtrate}} : 1_{\text{microbes}} = 90 \text{ mL}_{\text{filtrate}} : 60 \text{ mL}_{\text{microbes}}$).

2.6.2 Growing conditions

We use *P. recta* seeds collected from MPG in 2014 in our greenhouse experiment. To surface sterilize *P. recta* seeds we soaked them in 3% hydrogen peroxide for 2 hours, then quadruple rinsed them with distilled water. We sowed sterilized seeds into sterilized vermiculite in sterilized seedling trays in Western Washington University's Environmental Science building (laboratory room number 306) on 22 August 2016 and kept them moist with distilled water until cotyledons sprouted.

On 8 August 2016, we transplanted the 17 day-old seedlings into 120 pots ($v = 656$ mL, the Deepot™, D40H, Stuewe and Sons. Inc. Tangent, Oregon) and added the microbial community treatment inocula. We composed all pots as follows: a base layer of polyester filling to prevent the sand and growing mixture from falling out of the bottom, followed by 100 mL of play sand (grainsize <1 mm), then 400 mL of growing mixture, then the microbial community treatment (none, small, or whole) and a final layer of the growing mixture. The growing mixture was equal parts sterilized sand, turface (PROFILE Products LLC), and sterilized field soil collected near the field survey sites in Montana. Exact Scientific Services, Inc. (Ferndale, WA; www.exactscientific.com) tested the nutrient availability colorimetrically (Bray solution for phosphorus) and pH of the growing mix. In the growing mixture, plant available nitrogen (NH_4^+ , NO_3^-) was 18 mg kg^{-1} , phosphorus was 54 mg kg^{-1} , potassium was 193 mg kg^{-1} , and pH was 6.92. We used 50 mL of sterilized inocula for the no microbe treatment. We added $30 \text{ g} \pm 2 \text{ g}$ of whole soil inocula to the whole soil community treatment. We use mass instead of volume to save time and to prevent contamination between samples because 50 mL of our inocula weighed 30 g on average. The small microbe treatment plants received 50 mL of sterilized soil inocula as well as 90 mL of microbial wash (contained microbes $< 20 \mu\text{m}$). Every pot that was not a small microbe treatment pot received 90 mL of autoclaved water. We applied the microbial wash and sterile water over a two day period to prevent the wash from leaching out from the bottom of the pots.

The transplanted seedlings were kept moist by a misting system (7 minutes three times a day). Misting was reduced slowly until hand watering began on 2 September 2016. We watered the plants to field capacity when the top inch of growing mix was dry, about every three days. To

simulate a 16-hour day, the plants received natural sunlight in addition to 16 hours of supplemental lighting: S51 series luminaries (208W, 2.2A) high pressure sodium lamps and M59 type (208V, 2.3A) metal halide lamps. On average, we either rotated trays of 12 plants or shuffled all plants to new positions every week throughout the growing period. In the greenhouse mean daily temperature was 22.38°C, mean daily relative humidity was 59.33%, mean daily dew point was 13.80 °C, and mean photosynthetic photon flux density during the 16 hour day was 201 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

2.6.2.1 Nutrient addition

Around six weeks, some leaves yellowed indicating nutrient deficiency. We supplemented the plants with 20 mL of a reduced phosphate, 1/4 strength modified Hoagland's nutrient solution on 13 August 2016. Therefore, each pot received 1.05 mg nitrogen and 0.155 mg phosphorus. Six weeks later we observed yellowing again and added 10 mL of a 1/3 strength modified Hoagland's nutrient solution. Each plant received 0.65 mg nitrogen and 0.096 mg phosphorus.

2.6.2.2 Pesticide application

The leaves of some plants displayed signs of insect damage. We concluded that the culprit was within the Thysanoptera order due to distinctive leaf damage (silver scars and small black excrement), however, this was not confirmed by viewing the larval stage of any insects. Fungus gnats were also present in the greenhouse. Spinosad (0.001% mixture of spinosyn A and spinosyn D) was applied weekly to the tops and undersides of every leaf starting 22 September 2016, and lasting throughout October. Doktor doomTM total release fogger (0.40% pyrethrins and 2.00% piperonyl butoxide) was applied, as directed, to the greenhouse on 29 September 2016.

2.6.3 Greenhouse harvest

Destructive harvesting of the greenhouse plants began on November 14, 2016 and concluded on November 18, 2016. Bleach (5%) was used to sterilize the working area and harvesting tools before and between harvesting treatments (none, small, and whole). Each plant was removed from its pot, then its roots were rinsed clear of soil and organic debris using tap water. We separated the roots from the shoots at the crown. Root subsamples (three to five random selections of root 2 cm long) were taken from each plant to be used for determining the colonization by AMF. The wet mass of the root subsamples and remaining roots were weighed and recorded. The remaining roots and shoots were then placed in letter envelopes and dried at 60°C for at least 48 hours before recording dry masses (g).

2.6.4 Root staining and scoring

Root samples from the greenhouse experiment were placed in 2.5% potassium hydroxide (KOH) by volume solution on 18 November 2016 and left to clear for 48 hours at 1.5°C. After 48 hours, the roots had not cleared completely; they were then placed into a 5% KOH solution for an additional 48 hours. Then they were left in distilled water for 5 days before being placed into a 5% KOH solution for 4 days. The roots were still not adequately cleared, so we used a 90°C hot water bath to warm the 5% KOH solution for approximately 6 hours the last two days of clearing. We rinsed the cleared roots with distilled water five to seven times before placing them into a 0.05% Trypan blue solution for 4 days. We kept the stained roots in distilled water for at least 12 hours before they were mounted on microscope slides. We scored an average of 144 root intersections per plant, at 100x magnification, using a Nikon Eclipse 80i compound microscope to quantify the percent of AMF colonization as outlined by McGonigle et al (1990).

We scored root intersections for AMF presence if either (a) blue stained, aseptate, knobby hyphae; (b) blue stained vesicles; (c) blue stained arbuscules; (d) or blue stained vesicles and arbuscules were present (Figure 4). We calculated the proportion of colonization by AMF as the ratio of intersections where any AMF structures were present to total intersections scored. We then multiplied by 100 to calculate the percent colonized. We scored all the roots from the whole microbe treatment, and three of each source plant type for the no and small microbe treatments. We also counted the occurrence of *Olpidium* resting sporangia if they were stained blue, stellate bodies with thick folded walls lacking discharge tubes as detailed in Webster and Weber (2009; Figure 5).

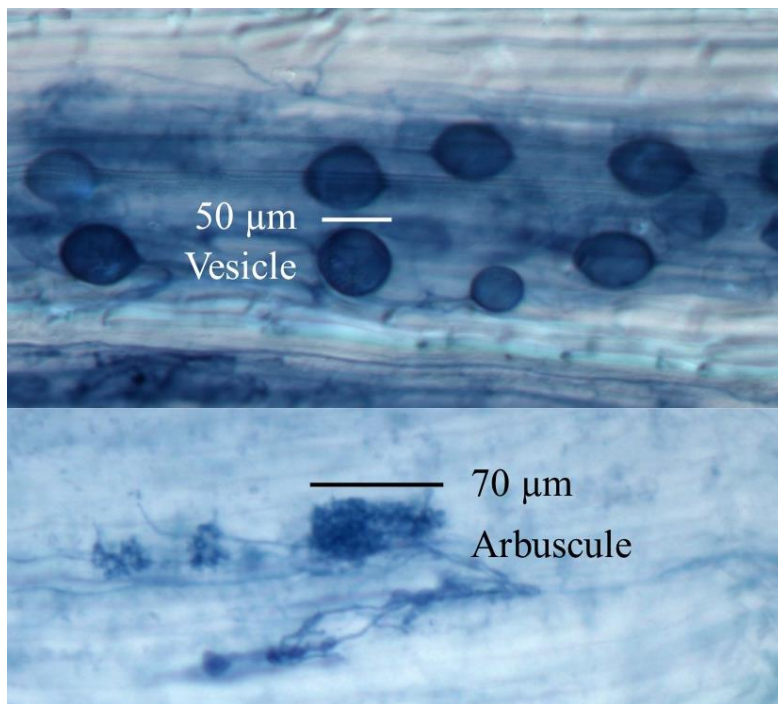


Figure 4. Vesicles (top) and arbuscule (bottom) in roots of greenhouse-grown *P. recta*. Image taken at 100x. Roots were cleared with KOH and stained with trypan blue.

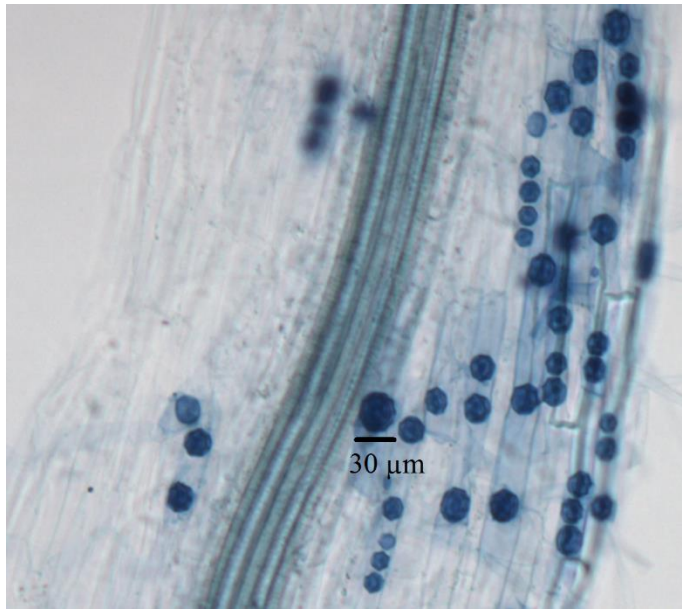


Figure 5. *Olpidium* sp. resting sporangia in the roots of greenhouse-grown *P. recta*. Image taken at 100x. Roots were cleared in KOH and stained with trypan blue.

2.7 Field Studies

2.7.1 Abiotic Soil Characterization

We measured pH, available nitrogen (NH_4^+ and NO_3^-), phosphate (PO_4^{3-}), and water content at sites A and B following Soil Sampling and Methods of Analysis (Carter 1993; Table 1). To measure pH, 10 g of air-dried soil was mixed with 20 mL of distilled water, shaken 30 minutes and left to settle for 1 hour. We measured pH in the liquid part of the solution with an Accumet Dual Channel pH/Ion/Conductivity meter (Pittsburg, PA, USA), without the probe touching the settled soil. We measured available nitrogen by mixing 10 g of fresh soil with 35 mL of 2.0M potassium chloride for two hours, then let them settle at 4°C overnight before they were filtered through a Whatman #1 filter. The filtrates were frozen immediately and available nitrogen was measured colorimetrically using a Synergy 2 Microplate Reader (BioTek, USA). Phosphate was extracted by shaking 2 g of air dried soil and 20 mL of a Bray solution (0.03M

ammonium fluoride and 0.1 M hydrochloric acid). It was shaken for 30 minutes and filtered through a Whatman #1 filter, then frozen immediately until phosphate was measured colorimetrically in a Synergy 2 Microplate Reader. We determined water content of the soil gravimetrically by weighing then drying 10 g of fresh soil in 100°C for 48 hours and recording the mass of the dry soil. Water content of the soil was calculated by dividing the difference between the fresh and dry soil masses by the dry soil mass, therefore: soil water content = (fresh soil mass – dry soil mass) ÷ dry soil mass.

2.7.2 Abiotic characterization of 2014 soil

The soil that was collected from the rhizospheres of the plants harvested from MPG in June 2014, were analyzed by Ward Laboratories (Kearney, NE, USA). A LabFit AS-3010D (Burswood, Australia) was used to measure pH in a 1 soil : 1 water solution. Inductively coupled argon plasma optical emission spectrometry (ICP-OES; iCAP 7400 ICP-OES, Thermo Fisher Scientific, Beverly, Massachusetts, USA) was used to quantify aluminum, iron, manganese, calcium, and potassium (Hou and Jones 2000). Aluminum was extracted with 1 M potassium chloride and filtered with an Advantec (Dublin, CA, USA) #1 qualitative filter. Iron and manganese were extracted with diethylenetriaminepentaacetic acid and calcium and potassium were extracted with ammonium acetate. Nitrate and sulfate were extracted with calcium dihydrogen phosphate and phosphorus was extracted with Mehlich III solution analyzed by flow-injected analysis (QuikChem 8500 Series 2, Lachat Instruments, Loveland, CO, USA; Frank et al. 1998, Gelderman and Beegle 1998). Heratherm and Thermolyne ovens (Thermo Scientific, Beverly, Massachusetts, USA) were used to quantify organic matter by loss on ignition (Combs and Nathan 1998).

Table 1. Abiotic soil characteristics for sites A and B mean \pm standard error (N=40)

Site	Water Content (g g ⁻¹)	NH ₄ ⁺ mg/kg	NO ₃ ⁻ mg/kg	PO ₄ ³⁻ mg/kg	pH
A	0.22 \pm 0.06 ^a	2.04 \pm 0.3 ^a	1.12 \pm 0.5 ^a	17.6 \pm 5 ^a	7.07 \pm 0.2 ^a
B	0.42 \pm 0.04 ^b	3.03 \pm 0.6 ^b	1.19 \pm 0.7 ^a	20.9 \pm 4 ^a	7.22 \pm 0.2 ^a

Different superscript letters indicate significant differences between sites ($p < 0.05$) using ANOVA after meeting assumptions of normality and homogeneous variance. Log_e transform were required for NH₄⁺, NO₃⁻, PO₄³⁻.

2.7.3 Plant-to-plant level (neighbor survey)

To determine if *Potentilla recta* occurs preferentially nearer to or farther from its native congeners we surveyed the distance to the congeners from *P. recta* relativized by other native forbs. The survey occurred between 13 and 16 June 2016 at four sites on MPG Ranch where *P. recta* and the congeners co-occur (Figure 1). At each site, we established five to six transects and each transect was 5 meters long. We established transects where *P. recta*, congeners, *Geum triflorum*, and one other focal native forb (*Geranium viscosissimum*, or *Hieracium scouleri*) co-occurred. The focal forbs were *G. triflorum*, *G. viscosissimum*, or *H. scouleri* and were used as a comparison for *P. recta*'s relative distance to the congeners. *G. triflorum* was chosen because it is also in the Rosaceae family, but not a *Potentilla*. The other focal forbs were selected because they were found near the congeners with relative consistency and are not in the Rosaceae family.

The focal plant surveyed (either *P. recta*, *G. triflorum*, *G. viscosissimum*, or *H. scouleri*) was within a half meter on either side of the transect and 50 cm apart from the last-surveyed plant. All plants surveyed were either flowering or displayed evidence of past flowering (old stems or a woody base larger than 1/2 cm). We measured the distance between *P. recta* and the

nearest native congener and *P. recta* and the nearest native perennial forb. Along the same transects we measured the distance between a focal forb and the nearest native *P. recta* congener and the focal forb and the nearest native perennial forb (Figure 6). For each focal plant (*P. recta*, *G. triflorum*, *G. viscosissimum*, *H. scouleri*), we calculated the relative distance to the congeners as (distance to congener – distance to forb) ÷ distance to forb.

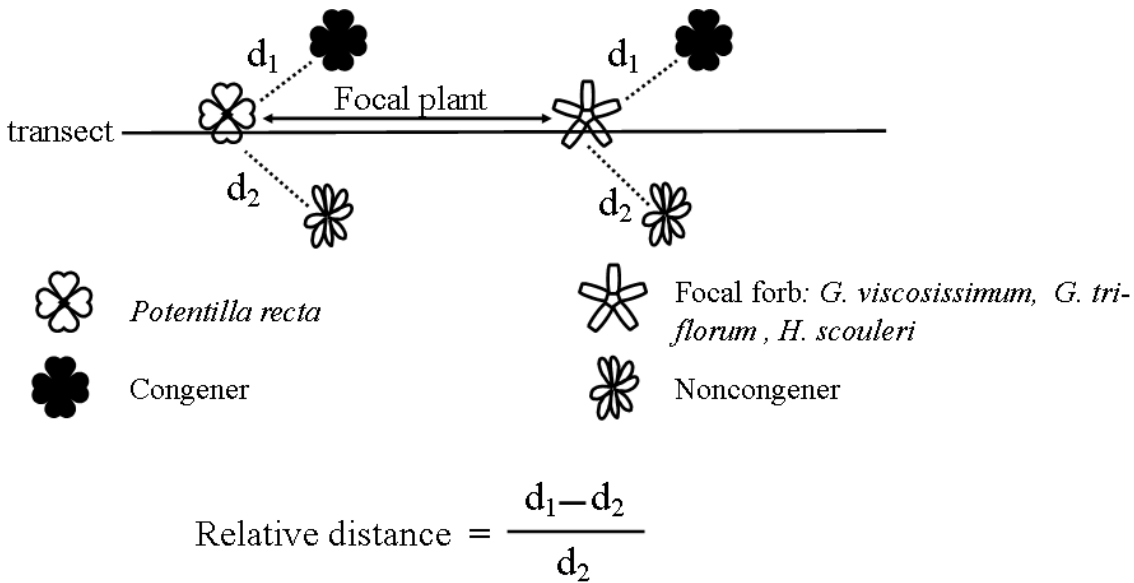


Figure 6. Diagram of the *Potentilla recta* plant-to-plant (neighbor survey) where d_1 = focal plant to nearest congener, d_2 = focal plant to nearest forb. Relative distance = (distance to congener – distance to forb) ÷ distance to forb. $N = 281$; *G. viscosissimum* $n = 48$, *G. triflorum* $n = 76$, *H. scouleri* $n = 30$, *P. recta* $n = 127$.

2.7.4 Community level (abundance survey)

We surveyed the percent cover of the focal plants, congeners, two most common neighbors (*Arnica sororia* and *Lupinus sericeus*), and native grass at the four sites. Within a 1 m x 1 m quadrat (1 m²; Figure 7), we recorded the percent cover of each plant as 0%, 1%, 5%, and increasing in increments of 5% up to 100%. At each site, we established an area of interest that

was representative of the vegetation recorded in the neighbor surveys; we excluded areas where trees and shrubs were dominant. We did this because we wanted to record the relative abundance of *P. recta*, the congeners, and other neighboring forbs recorded in the plant-to-plant, neighbor survey. The 1 m² quadrat locations were selected randomly by using a list of computer-generated numbers as approximate coordinate locations on the grid of our area of interest. At each site, we surveyed approximately 1.67% of our area of interest (except C, the datasheet was mislabeled and one quadrat not recorded). At site A, we surveyed 15 quadrats within a 15 m x 60 m area. At site B, we sampled 20 quadrats within a 20 m x 60 m area. At site C, we survey 10 quadrats within an 11 m x 60 m area. At site D, we surveyed 15 quadrats within an 18 m x 50 m area.



Figure 7. Flowering *P. recta* in a 1 m² quadrat. The grids inside were used as guides to assign percent cover, where each square represents five percent of the 1 m² quadrat.

2.7.5 Landscape level (complete MPG vegetation survey)

MPG Ranch vegetation was surveyed in 428 equally distributed plots between 2011 to 2012 by MPG staff. Percent cover was calculated from 200 points along four perpendicular 15.24 m transects. Every 0.3 m along each transect a vertical stick was placed, and every plant touching the stick was recorded to species. Percent cover for each plant species within a plot was the number of times that species touched the stick divided by 200 and then multiplied by 100. For each plant species, we converted percent data to presence/absence data and then performed a co-occurrence analysis as described in section 2.8.6.

2.8 *Statistical Analysis*

All analyses were performed in R (3.3.2 R Core Team 2017). All data figures were created using the ggplot2 package (Wickham 2009).

2.8.1 Ordination

The vegan package was used to ordinate the fungal OTUs using the “metaMDS” function. Environmental data were fit to the ordination using the “envfit” function in the vegan package. The “adonis2” function was used to calculate differences in community composition by host plant or neighbor.

2.8.2 ANOVA

The function “leveneTest” from the car package was used to test for homogeneous variance (Fox and Weisberg 2011). We used a one-way ANOVA to test for differences in the

proportion of mutualists to pathogens. The proportion of mutualists to pathogens was calculated as:

$$\frac{(\text{AMF} \div 2000)}{(\text{Pathogens} \div 2000)}$$

We used a one-way ANOVA to test for differences between the relative distances to the native congeners between *P. recta* and the three focal forbs, the whole soil effect (whole:none) by microbe source plant, the whole soil effect grouping congeners versus noncongeners, the small microbe effect (small:none), and AMF colonization by source plant ($\alpha = 0.05$). We used a two-way ANOVA to test total biomass for differences between microbe fraction (whole, small, none), source plant (congener, forb, grass, *P. recta*) and the interaction of microbe fraction and source plant. The relative distance data were $\log_{10}(x+1)$ transformed to reduce the skew of the data. The *P. recta* dry mass data (full factorial), whole soil effect, small soil effect, and AMF colonization data met the assumptions of normality and homogenous variance. Effect sizes (partial η^2) were calculated as: $(SS_{\text{factor}}) / (SS_{\text{factor}} + SS_{\text{error}})$.

2.8.3 t-tests

We used a two-sample, two-sided t-test to compare mean relative distances to *P. gracilis* from *P. recta* or the other focal forbs as a group. To test for a small microbe effect (small:none) we used a one-sample, two-sided t-test with a null hypothesis that the true mean equaled one. If the small effect mean is different from one, this suggests that the small microbe treatment affected the biomass of *P. recta*. If not different from one it suggests that the small microbe treatment was not different from the no microbe treatment.

2.8.4 Tukey's HSD

We used Tukey's HSD (honest significant difference) to test for post hoc, pair-wise differences among host plant for the proportion of mutualists to pathogens, differences among microbe fraction of *P. recta* grown in the greenhouse, differences in colonization by AMF by source plant, differences in the small microbe effect (small:none) by source plant, and differences in the whole effect (whole:none) by source plant.

2.8.5 Linear regression

We used a linear regression to test if percent colonization by AMF or *Olpidium* sp. predicted *P. recta* biomass. These data met the assumptions of homogeneous variance. For the molecular data, we used linear regression to predict biomass by pathogen abundance. The pathogen abundance data were transformed (\log_{10}) to meet the assumptions of normality.

2.8.6 Co-occurrence

We used the package *coocurr* (Griffith et al. 2016) to determine if the observed instances of co-occurrence between *P. recta* and the congeners was greater than, less than, or equal to random chance. We define co-occurrences as two plants occurring in the same survey plot (1 m² or along a one of four 15.24 m transects). The “cooccur” function calculates the probability that two species co-occur at exactly j number of sites ($p(lt)$) using:

$$P_j = \frac{\binom{N_1}{j} \times \binom{N - N_1}{N_2 - j}}{\binom{N}{N_2}}$$

N_1 = number of sites where species #1 occurs, N_2 = number of sites where species #2 occurs and N = total number of sites that were surveyed (where both species could occur). The probability that the two species co-occur less than expected by chance is ($p(lt)$), and the probability that the two species co-occur greater than expected by chance is ($p(gt)$) (Griffith et al. 2016).

3 Results

3.1 Fungal Communities

The unidentified fungal OTU denovo875 was the most abundant for every host plant (*P. recta*, *P. gracilis*, *D. glandulosa*, other neighboring forbs. Table 2). The fungal communities within the roots of *P. recta* and its congeners (*P. gracilis* and *D. glandulosa*) were similar to each other and significantly different from the fungal communities within the roots of the other neighboring forbs (Figure 8, Table 3). The fungal communities within *P. recta* were not significantly different by neighbor (*P. gracilis*, *D. glandulosa*, or other neighboring forb; Figure 9, Table 4). *P. recta* and the congeners hosted a significantly lower ratio of AMF to pathogens relativized by the total number of OTUs than the other neighboring forbs (Figure 10). Within *P. recta*, the ratio of AMF to pathogens was not different by neighboring plant identity (Figure 11). *P. recta* biomass was not predicted by pathogen sequence abundance (Figure 12).

Table 2. Top three most frequent OTUs within the roots of each host plant collected in 2014.

Plant	OTU	Percent of all sequences	Taxon	Trophic Mode
<i>P. recta</i>	denovo875	17.6	unidentified	
<i>P. recta</i>	denovo1238	12.2	unidentified	
<i>P. recta</i>	denovo4156	9.8	unidentified	
<i>D. glandulosa</i>	denovo875	15.4	unidentified	
<i>D. glandulosa</i>	denovo1238	13.4	unidentified	
<i>D. glandulosa</i>	denovo4156	6.6	unidentified	
<i>P. gracilis</i>	denovo875	16.1	unidentified	
<i>P. gracilis</i>	denovo1690	13.6	<i>Lachnum</i>	Saprobe
<i>P. gracilis</i>	denovo351	12.5	unidentified	
Other	denovo875	29.0	unidentified	
Other	denovo501	4.6	<i>Alatospora</i>	Saprobe
Other	denovo4052	4.3	unidentified	

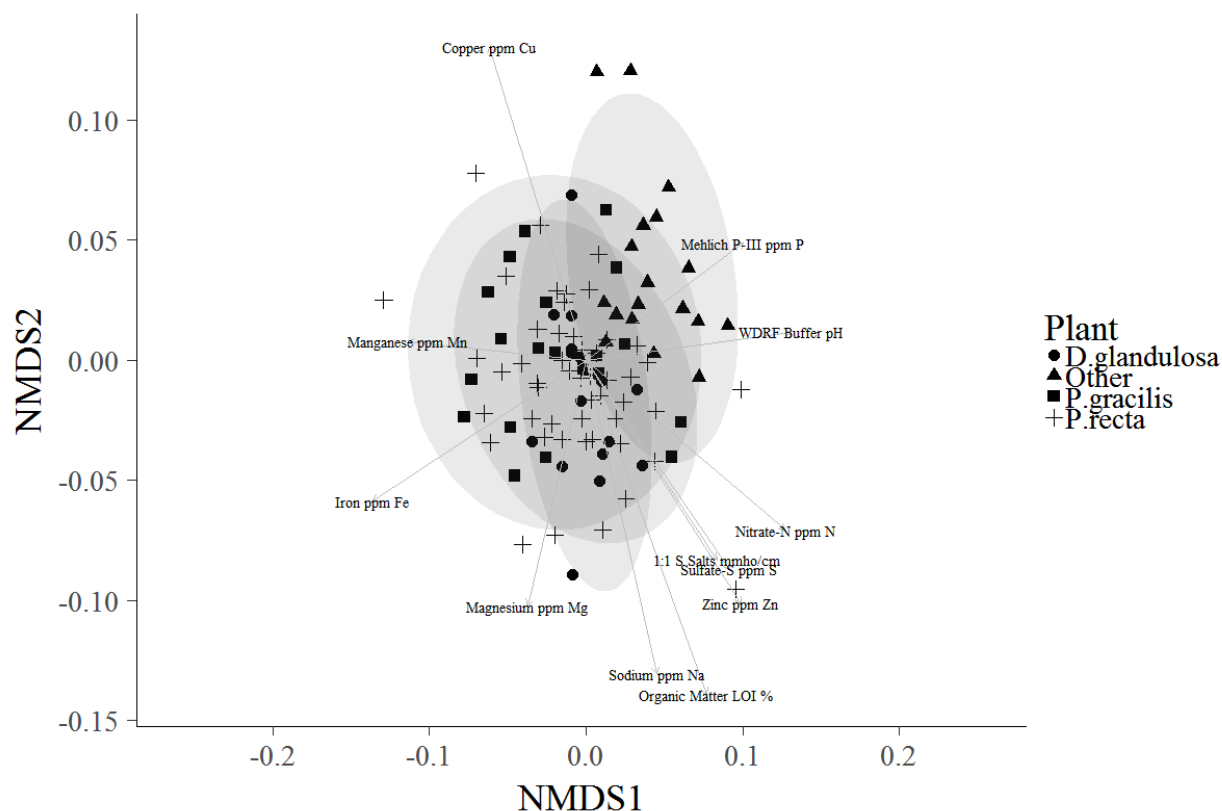


Figure 8. Ordination plot of the ITS OTU matrix by host plant with significant ($p < 0.001$) soil chemistry variables. Ordination stress was low (0.248, Non-metric $R^2 = 0.939$). Ellipses denote the 95% confidence interval around the centroid (weighted mean of the group). $N = 104$.

Table 3. Summary of Adonis results comparing the fungal species by host plant, is comparable to MANOVA results. Adonis partitions sums of squares using dissimilarities.

	<i>df</i>	<i>SS</i>	<i>F</i>	<i>p</i>
Plant	3	3.61	5.77	0.001
Residuals	100	20.87		

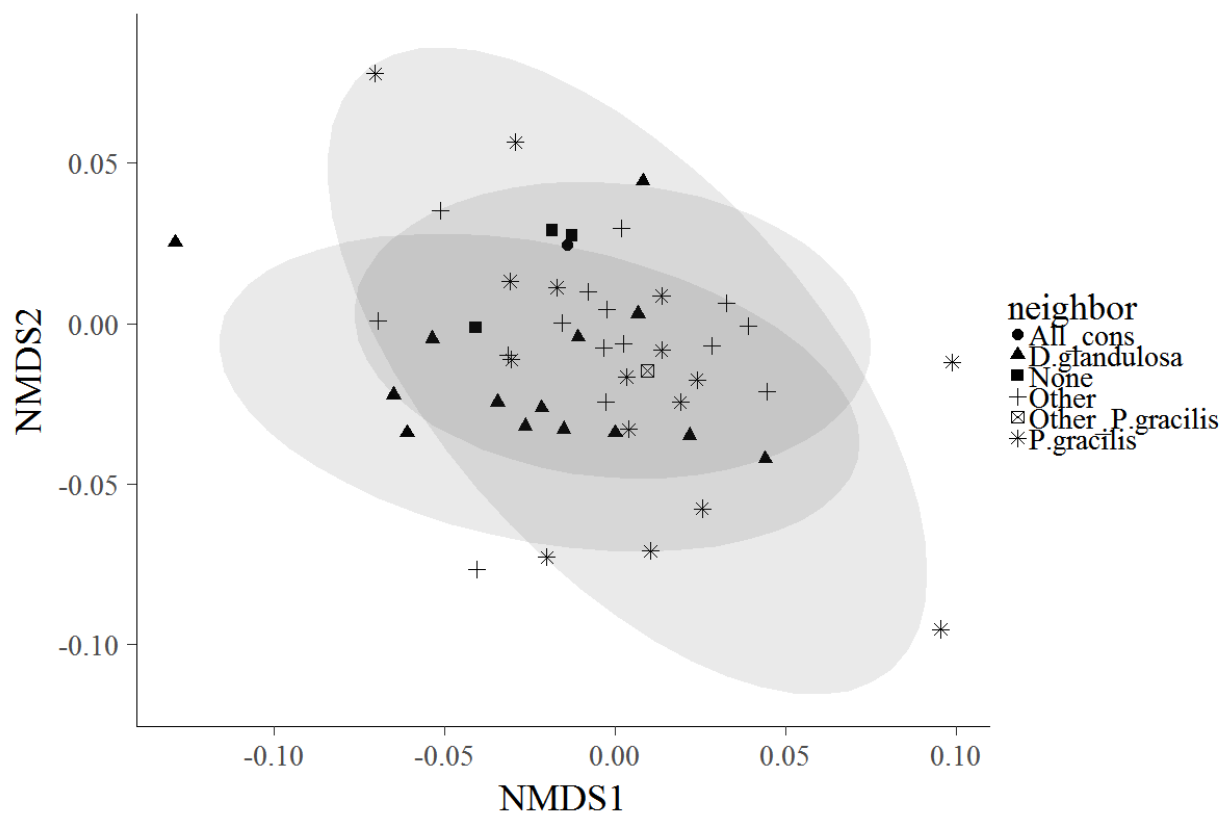


Figure 9. Ordination plot of the ITS OTU matrix for *P. recta* only by neighbor. Ordination stress was low (0.236, Non-metric $R^2 = 0.94$). Ellipses denote the 95% confidence interval around the centroid (weighted mean of the group). N = 50.

Table 4. Summary of Adonis results comparing the fungal species within *P. recta* by neighbor, is comparable to MANOVA results. Adonis partitions sums of squares using dissimilarities.

	<i>df</i>	<i>SS</i>	<i>F</i>	<i>p</i>
Neighbor	5	1.13	1.05	0.33
Residuals	44	9.42		

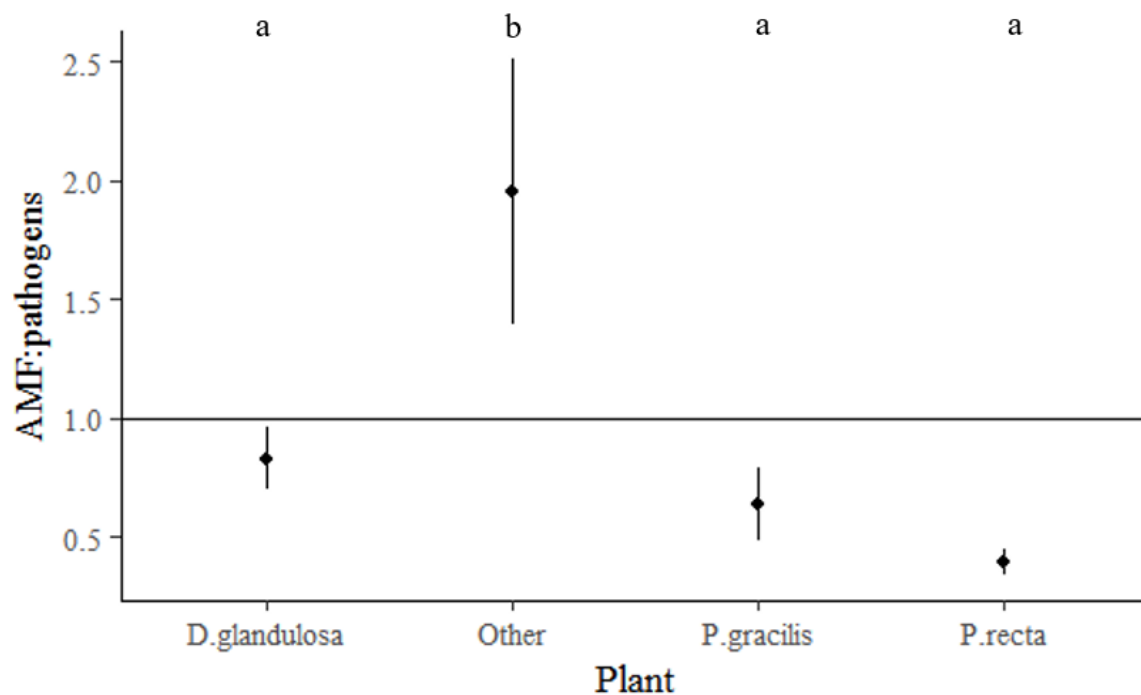


Figure 10. The ratio of AMF to pathogens relativized by total OTU abundance ($F_{3,100} = 9.42$, $p < 0.001$). Center point is the mean and lines are one standard error from the mean. Different letters represent significantly different groups determined by Tukey's HSD ($p < 0.05$).

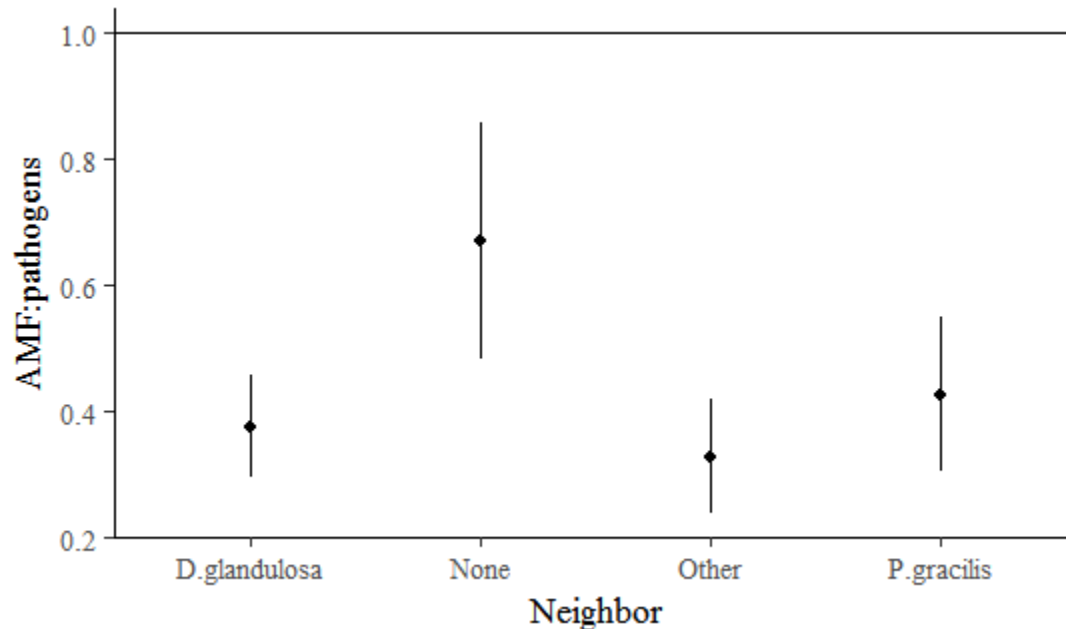


Figure 11. The ratio of AMF to pathogens relativized by total OTU abundance within *P. recta* roots by neighbor ($F_{5,44} = 0.52$, $p = 0.75$). Center point is the mean and lines are one standard error from the mean. Tukey's HSD ($p < 0.05$) found no differences by neighbor identity.

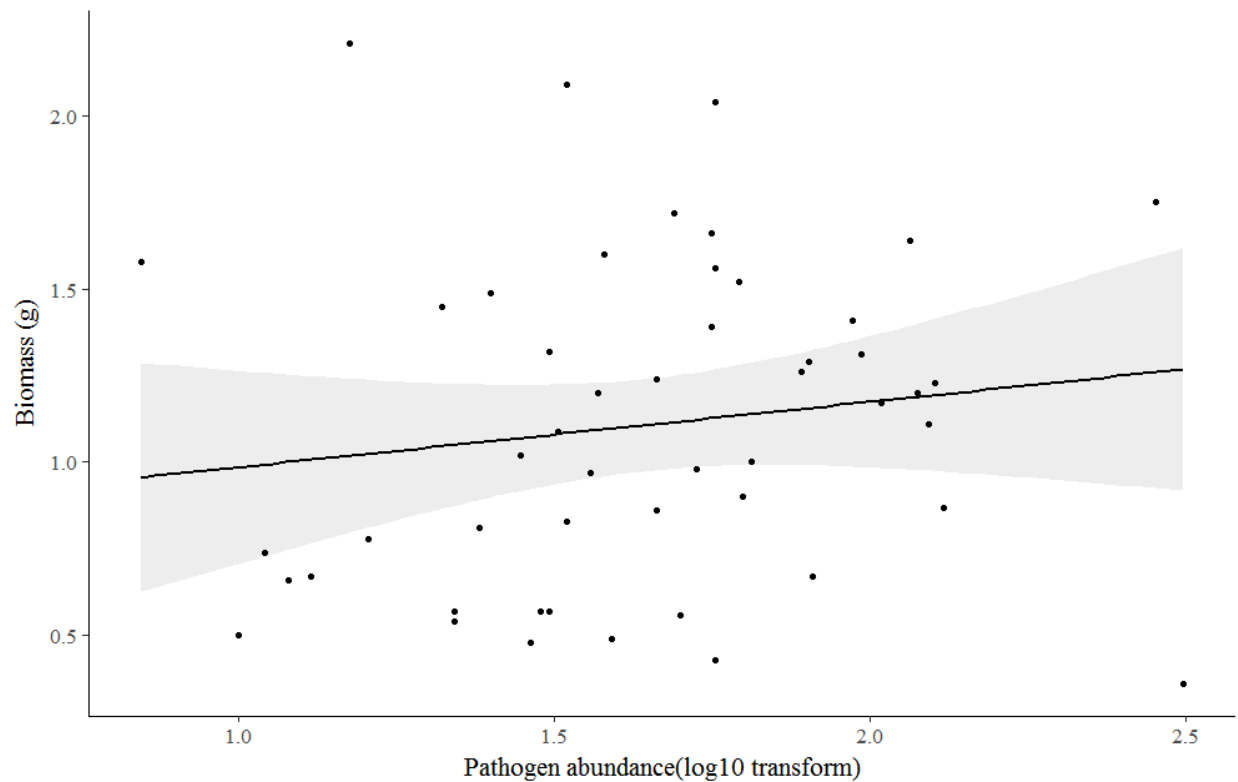


Figure 12. Pathogen abundance (log 10 transformed to meet the assumption of normality) did not predict *P. recta* biomass ($R^2 = 0.02$, $p = 0.32$). Grey shading denotes the 95% confidence interval.

3.2 Greenhouse Study

3.2.1 Whole and small microbe effects

When all microbe fraction treatments were compared, *P. recta* grown in the whole microbial community treatment were significantly less in biomass (g) than plants growing in both the small microbial community and the sterilized no microbe treatments (Figure 13, Table 5). We found parallel results for the roots, shoots, and root to shoot ratio (descriptive statistics in Table 1S). On average total biomass was reduced by $20 \pm 2.8\%$ by the whole relative to the no microbe treatment. Differences did not exist among source plants (Congener, Forb, Grass, *P.*

recta; Table 5). Because we did not pool our soil inocula, we were about to directly compare the biomass of plants grown in each microbial treatment by replicate. The whole soil effect is the ratio of total biomass of a single plant grown in the whole microbial treatment to a single plant growing in the corresponding no microbe treatment. Similarly, the small microbe effect is the ratio of total biomass of a single plant grown in the small microbe treatment to the corresponding no microbe treatment. There were no significant differences in the whole soil effect (whole:none) or the small soil effect (small:none) for total biomass by plant source and (Table 5, Figure 14). As described in the methods section 2.8.3, we grouped all the small effect (small:none) data to test if the mean for the entire group was different from one. We found that the small microbe fraction did not affect the growth of *P. recta* ($t_{39} = -0.47$, $p = 0.63$, $\mu_0 = 1$). The whole soil microbe treatment reduced the biomass of *P. recta* more than the small microbe, relative to the no microbe treatment (Figure 9, 14). Grouping non-congeners, we found that they reduced *P. recta* biomass more than congeners and *P. recta* as a group ($F_{1,38} = 4.65$, $p = 0.037$, partial $\eta^2 = 0.109$).

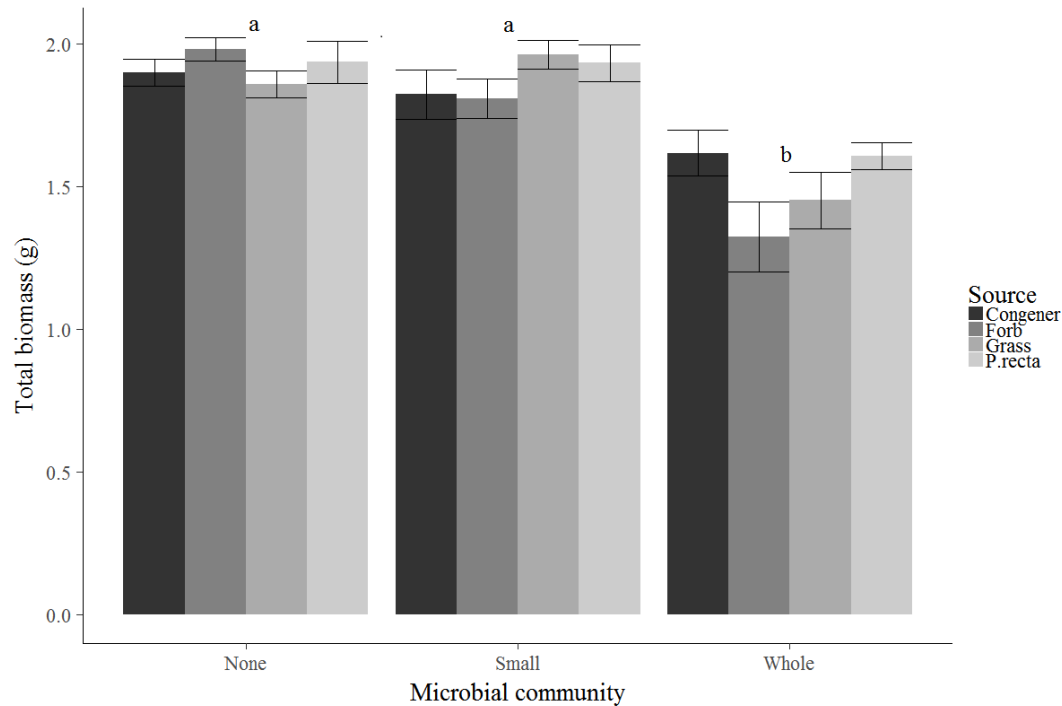


Figure 13. Total biomass mean \pm one standard error by microbe source and microbial community fraction. There were significant differences among the microbial community fractions but not among the source plants, nor their interaction (Table 5). Letters represent significantly different groups determined by Tukey's HSD post hoc analysis ($p < 0.05$).

Table 5. Analysis of variance tables for the greenhouse experiment (full factorial), and plant source within the whole community only.

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>p</i>
Greenhouse experiment full factorial (2-way)					
Source plant	3	0.04	0.01	1.66	0.18
Microbe community	2	0.68	0.34	39.5	<0.001
Source:community	6	0.10	0.02	2.12	0.06
Error	108	0.01	0.01		
Greenhouse experiment Whole microbe tmt only (total biomass)					
Source plant	3	0.20	0.07	2.30	0.09
Error	36	1.05	0.03		

df = degrees of freedom, *SS* = sum of squares, *MS* = mean square.

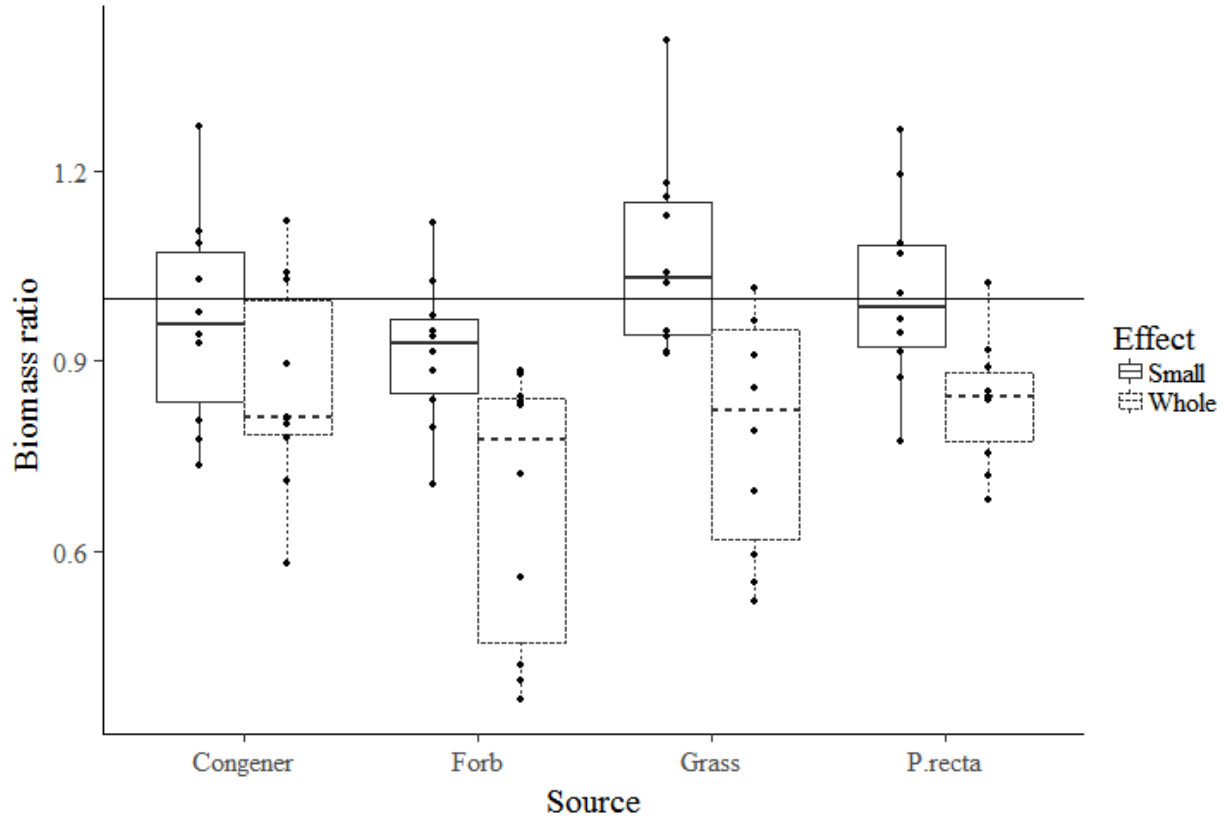


Figure 14. Biomass ratios for the whole (whole:none) and small (small:none) effects. The center horizontal line is the median and the vertical lines extending from each box are the maximum (top) and minimum (bottom) values, $n = 10$, $N = 40$ per effect. For the whole effect there was no difference by source plant ($F_{3,36} = 2.30$, $p = 0.09$), there were no pairwise difference determined by Tukey's HSD post hoc analysis. For the small effect there was no difference by source plant ($F_{3,36} = 1.88$, $p = 0.15$), there were no pairwise difference determined by Tukey's HSD post hoc analysis.

3.2.2 Root colonization by arbuscular mycorrhizal and other fungi

Root colonization by AMF from all source plants was low $15 \pm 2.1\%$ (mean \pm standard error; Figure 15, Table 6). As described in the methods section (2.6.4), we calculated the proportion of colonization by AMF as the ratio of intersections where any AMF structures were

present to total intersections scored. We then multiplied by 100 to calculate the percent colonized. We found percent of root colonization by AMF differed by source plant ($F_{3,36} = 2.825$, $p = 0.052$, partial $\eta^2 = 0.191$) and total biomass was negatively correlated with AMF colonization ($R^2=0.33$, $F_{1,38} = 20.06$, $p<0.001$, $N=40$; Figure 16). We found AMF colonization only differed between by plants with microbes sourced from *P. recta* and the other native forbs (Tukey's HSD $p = 0.04$; Figure 15). Resting sporangia of *Olpidium* sp. were found in *P. recta*'s roots with microbes sourced from 6 of 10 congener, 7 of 10 *P. recta*, 1 of 10 forb, and 1 of 10 grass root samples (Figure 17).

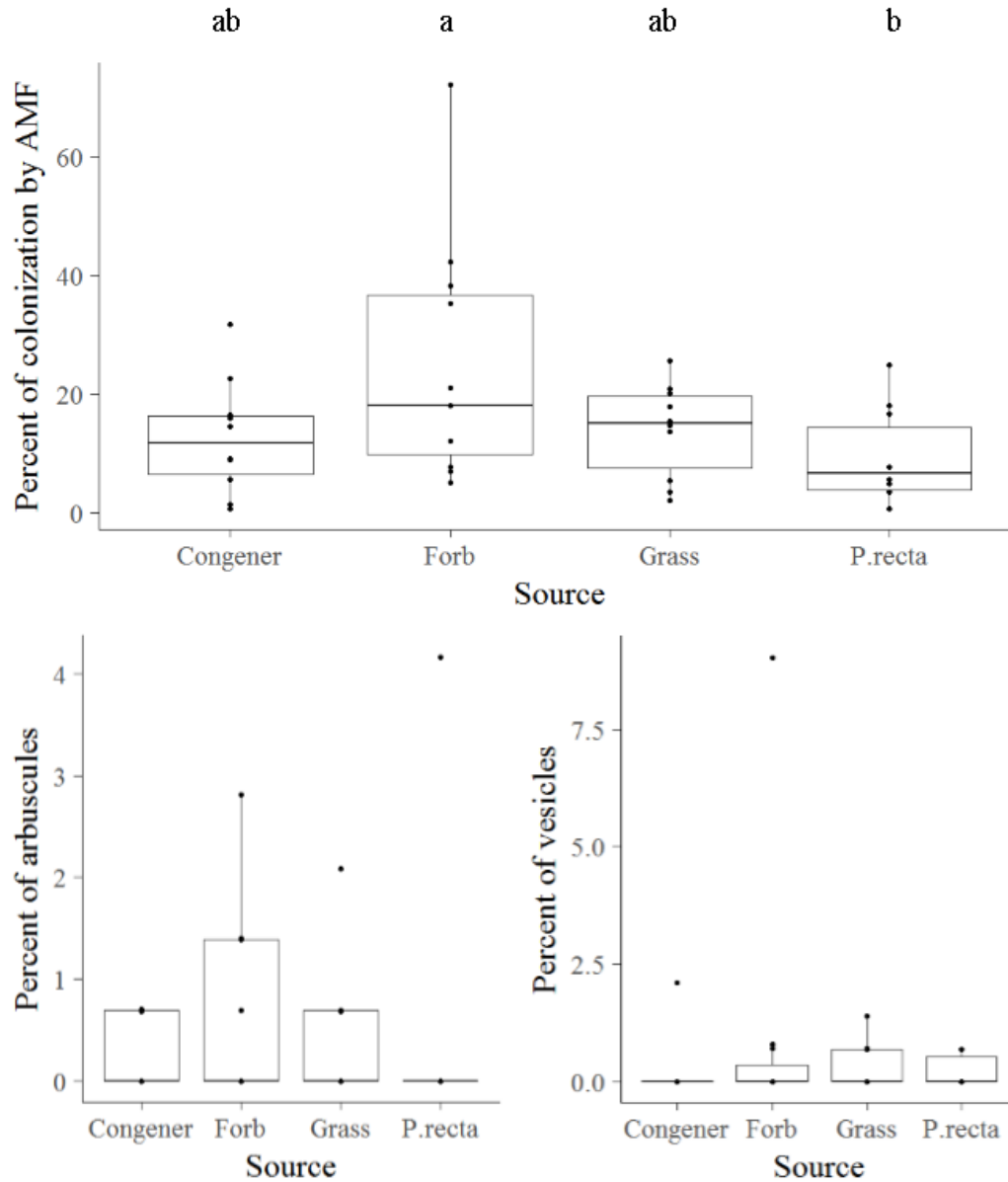


Figure 15. The top plot shows the percent of colonization by AMF by source plant ($F_{3,36} = 2.825$, $p = 0.052$, partial $\eta^2 = 0.191$); the center is the median. The bottom left shows the percent of arbuscules found in the whole treatment roots by source plant. The bottom right shows the percent of vesicles found in the whole treatment roots by source plant. In total, we scored roots from 40 plants, 10 from each microbial source plant.

Table 6. Mean and standard error of fungal structure percentages from the whole treatment roots by source plant. Statistical comparisons were not performed among source plants due to low and unbalanced n.

Structure	Congener	Forb	Grass	<i>P. recta</i>
Arbuscules	0.28 ± 0.11	0.70 ± 0.28	0.42 ± 0.21	0.42 ± 0.42
Vesicles	0.21 ± 0.21	0.96 ± 0.81	0.35 ± 0.16	0.21 ± 0.11
<i>Olpidium</i>	7.0 ± 3.6	0.76 ± 0.76	0.55 ± 0.55	3.9 ± 1.3

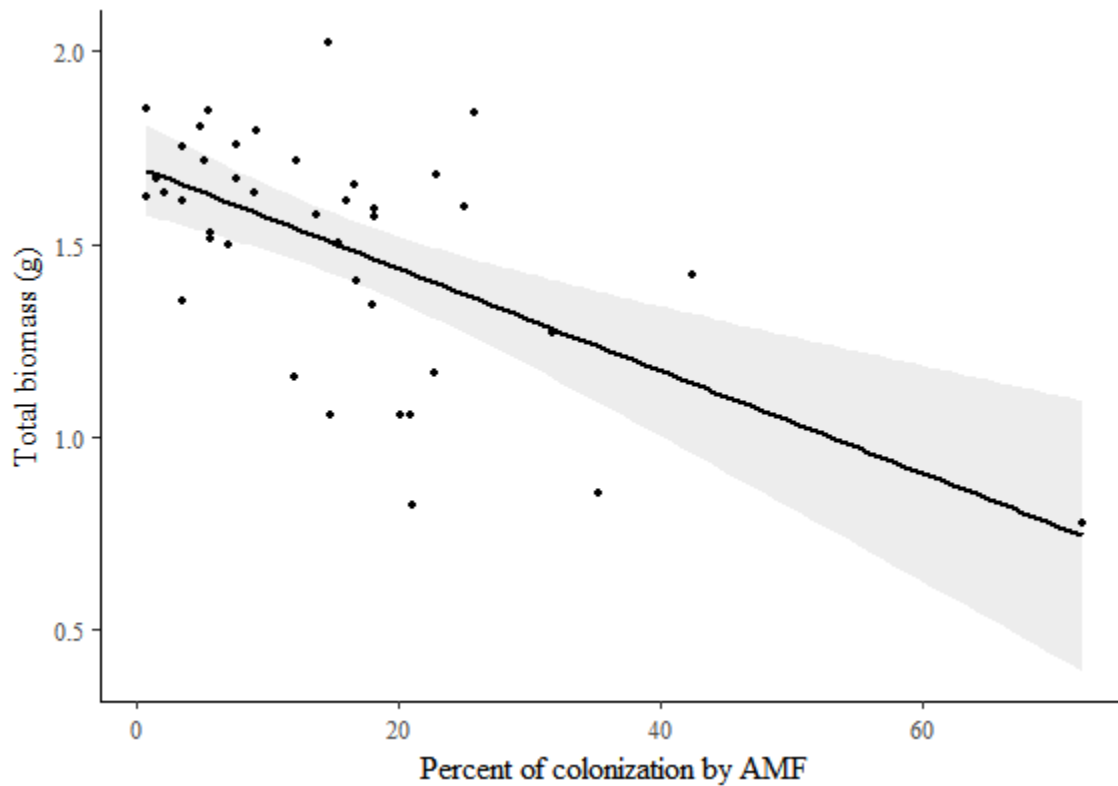


Figure 16. Total biomass (g) was negatively correlated with percent of AMF colonization ($R^2=0.33$, $F_{1,38} = 20.06$, $p<0.001$, $N=40$). Grey shading denotes 95% confidence interval.

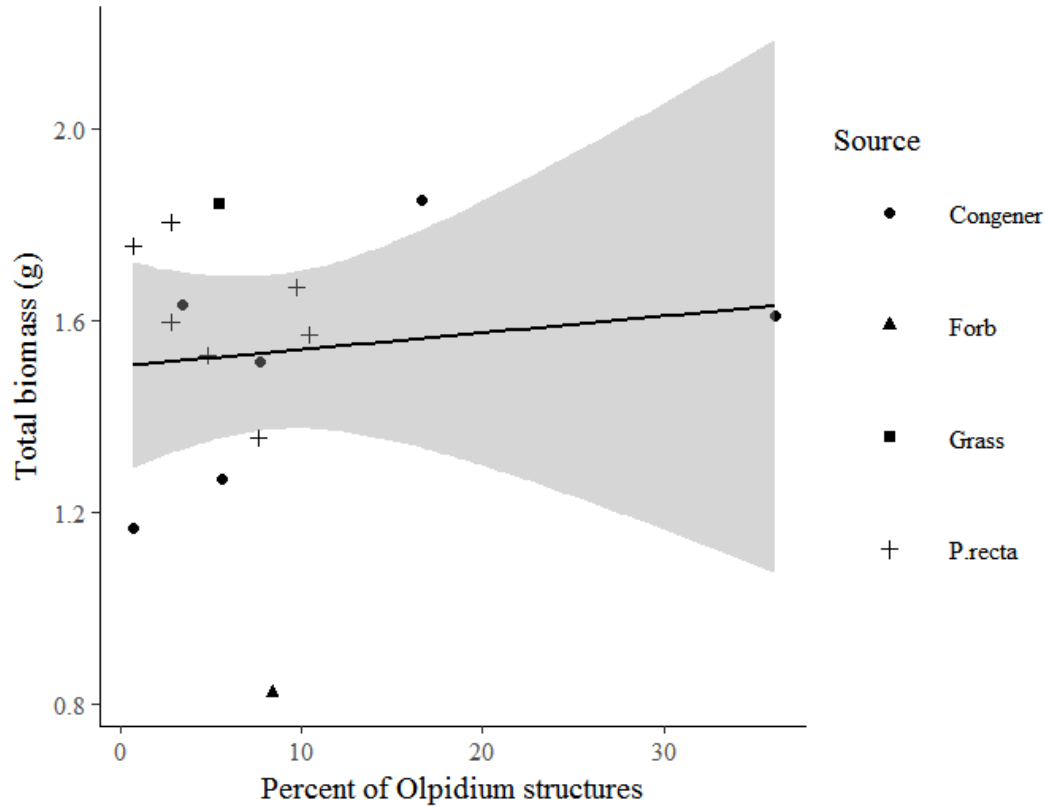


Figure 17. Percent of *Olpidium* resting sporangia did not predict total *P. recta* biomass ($F_{1,13} = 0.16$, $R^2 = 0.01$, $p = 0.70$). *Olpidium* sp. resting sporangia were found in 6 out of 10 congener, 7 out of 10 *P. recta*, 1 out of 10 forb, and 1 out of 10 grass root samples from the whole microbe treatment, $N=40$.

3.3 Field Studies

3.3.1 Plant-to-plant level (neighbor survey)

Relative distance to the congeners ranged from -0.82 to 42 cm, but we found no evidence that relative distances differed among focal plant ($p = 0.571$, Table 4). Though, when the congeners are separated by species we observed a trend that *P. recta* was distributed farther from *P. gracilis* than *D. glandulosa* while the opposite was true for the other three native non-congener forbs (Figure 18). However, this trend was marginally significant ($t_{88} = -1.63$, $p =$

0.11). This trend did not hold when we analyzed *P. recta* and *P. gracilis* co-occurrence at the community and landscape levels.

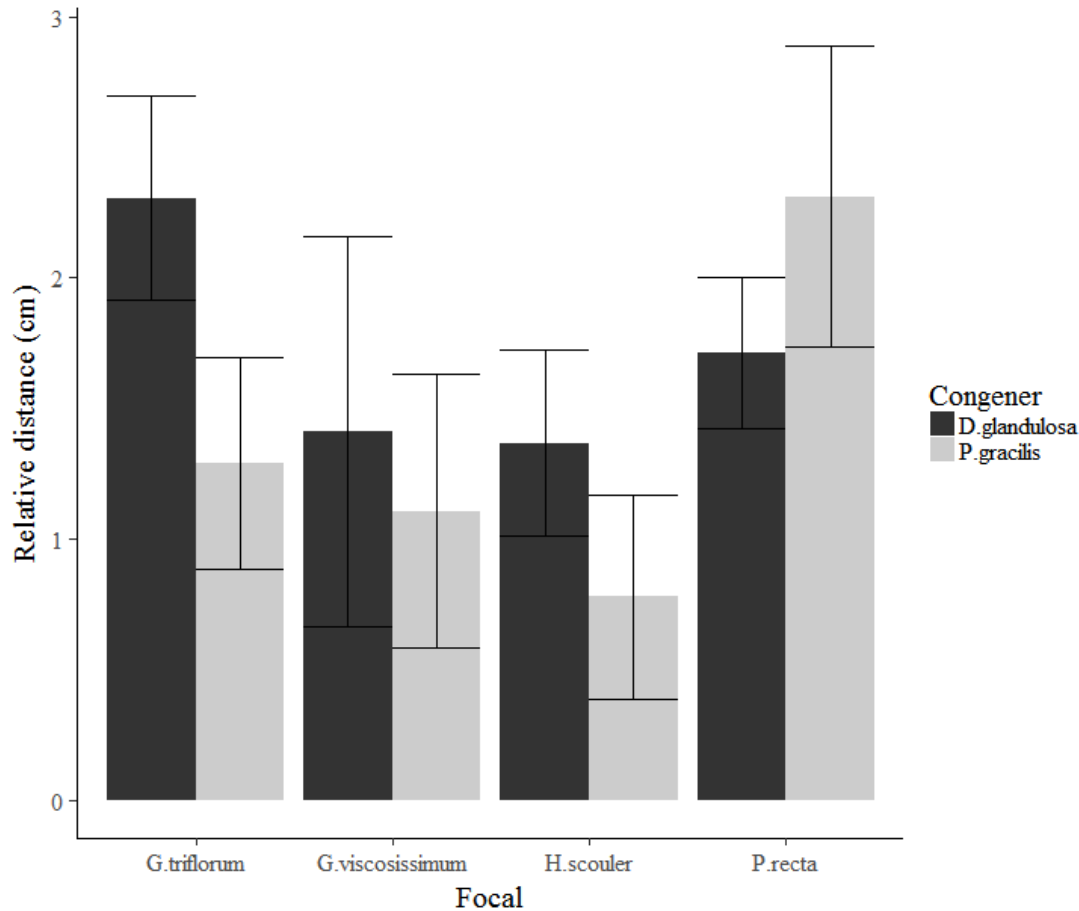


Figure 18. Plant-to-plant level: mean relative distance [(distance to nearest congener – distance to nearest non-congener forb) ÷ distance to nearest non-congener forb] to the congeners from *P. recta* or three other focal forbs. Error bars represent one standard error from the mean. There were no significant differences for the relative distances between focal plants ($N = 281$; *G. viscosissimum* $n = 48$, *G. triflorum* $n = 76$, *H. scouler* $n = 30$, *P. recta* $n = 127$, $F_{3,276} = 0.67$, $p = 0.571$).

3.3.2 Community level (abundance survey)

Total abundance of the focal plants ranged from 0% to 35%. *P. recta* had a mean abundance of 3.45% and a range of 0 – 25% (Table 7). We define co-occurrences as two plants

occurring in the same survey plot (1 m² or along a one of four 15.24 m transects). At the community level, *P. recta* and *P. gracilis* were predicted to co-occur 8 times, the actual instance of co-occurrence was 12. This suggests that the co-occurrence of *P. recta* and *P. gracilis* was positively associated, compared to random chance ($p(gt) = 0.03$), meaning they co-occurred more than expected based on their total number of occurrences. Yet, *P. recta* and *D. glandulosa* were predicted to co-occur 18 times and the observed instance of co-occurrence was 17. Therefore, we found the co-occurrence of *P. recta* and *D. glandulosa* to be no different from random chance ($p(gt) = 0.76$).

Table 7. Mean \pm SE and range for the abundance (percent cover in 1m²) of *P. recta* and the three focal plants at sites A, B, C, and D (N=59).

	cover (%)	
	Mean \pm SE	Range
<i>P. recta</i>	3.45 \pm 0.68	0 - 25
<i>P. gracilis</i>	1.77 \pm 0.57	0 - 25
<i>D. glandulosa</i>	4.74 \pm 0.81	0 - 25
<i>G. triflorum</i>	6.15 \pm 0.94	0 - 30
<i>G. viscosissimum</i>	5.20 \pm 1.04	0 - 35
<i>H. scouler</i>	1.83 \pm 0.59	0 - 20

3.3.3 Landscape level (complete MPG vegetation survey)

Although at the community scale we found a positive pattern of co-occurrence between *P. recta* and *P. gracilis*, at the landscape level we found no evidence of a pattern ($p(lt) = 0.78$; Table 8). Consistent with the findings at the community level, we found *P. recta* and *D. glandulosa* to co-occur 25 times and the expected number of co-occurrence was 21; therefore, the two co-occurred randomly ($p(lt) = 0.99$).

Table 8. Occurrence and co-occurrence of the native congeners with *P. recta* from landscape data (N=428). We define co-occurrences as two plants occurring in the same survey plot (1 m² or along a one of four 15.24 m transects). The probability that the two species would co-occur at a frequency less than the observed number of co-occurrence sites if the two species were distributed randomly of one another is $p(lt)$.

Species	Occurrence	Cooccurrence with <i>P. recta</i>	Expected cooccurrence with <i>P. recta</i>	$p(lt)$
<i>P. recta</i>	70			
Congeners				
<i>D. glandulosa</i>	20	5	3.3	0.91
<i>P. gracilis</i>	10	2	1.6	0.78

4 Discussion

Our field and greenhouse studies allowed us to evaluate the effect conspecific microbes have on the invasive *Potentilla recta*. Our study aimed to answer three questions: (1) how do the fungal communities within the roots of *P. recta* compare to the fungal communities within the roots of neighboring congeners and forbs, (2) what are the effects of the whole microbial community (microflora, microfauna, and some mesofauna <2 mm), and the small microbial community (microflora, <20 μ m) on *P. recta*, (3) is there evidence that conspecific soil microbes mediate the distribution of *P. recta* in the field? To address question one, we compared the fungal communities within the roots of *P. recta*, its native congeners (*P. gracilis* and *D. glandulosa*), and neighboring grassland forbs. To address our second question, we conducted a greenhouse experiment testing the effects of microbe fraction (none, small, whole) and source plant (congener, forb, grass, and *P. recta*) on *P. recta* biomass. To address our third question, we observed and analyzed the distribution of *P. recta* in relation to its congeners in the field. In general, we found little support for conspecific inhibition of *P. recta*.

4.1 *How do the fungal communities within the roots of P. recta compare to the fungal communities within the roots of neighboring congeners and forbs?*

Molecular analysis of the fungal communities within the roots of *P. recta*, its congeners, and other neighboring forbs suggests that the congener and *P. recta* fungal communities are similar to each other, but different from those of other neighboring forbs (Figure 8, 10 and Table 3). This provides support for a mechanism behind the findings of Callaway et al. (2013) that conspecific microbes inhibit *P. recta*. Further supporting this mechanism, we found *P. recta* and

its congeners to have a higher relative proportion of pathogens to mutualists than the other neighboring forbs (Figure 10).

In other invasive plant research on MPG, Gibbons et al. (2017) found *Bromus tectorum* (cheatgrass) to increase pathogen load in invaded field locations. Different from our findings and also on MPG, Lekberg et al. (2013) found invasive forbs, *Centaurea stoebe* and *Euphorbia esula*, to have higher AMF associations (percent AMF root colonization and OTU richness) than the native plant community. *P. recta* is a forb, and we would expect its fungal community composition to be more similar to previous findings of other invasive forbs than grasses, but that is not what we found. In paired native and invaded field plots also in Western Montana, *P. recta* invasion did not increase aboveground net primary productivity or extractable soil nitrate even though the ammonia oxidation bacterial gene abundance was significantly higher in invaded compared to native field plots. *B. tectorum*, *C. stoebe*, and *E. esula* did increase aboveground net primary productivity and extractable nitrate and had significantly higher ammonia oxidation bacterial gene abundance in invaded compared to native field plots. This suggests that *P. recta* may interact differently with soil microbes than the other problematic invasive plants in Western Montana.

Within *P. recta*, the fungal communities and the ratio of mutualists to pathogens were not different by neighbor (Figure 11 and Table 4). This suggests that although *P. recta*'s root fungi are more similar to those of the congeners than other neighboring forbs, *P. recta*'s root fungi are not influenced by the identity of neighboring plants. This limits our ability to state that *P. recta* is affected by the fungal communities of its neighbors whether the neighbor is a congener or a noncongener forb. *P. recta* may be influenced by specific microbes from its neighbors, but the

whole fungal community was generally similar. Yet, *P. recta* biomass did not correspond to pathogen abundance (Figure 12). Thus, we observed no mechanism for conspecific fungal inhibition of *P. recta*.

We propose two reasons why *P. recta* biomass did not correspond to pathogen abundance. First, the fungi identified as pathogens, may produce disease in some plants, but may function as endophytes within *P. recta* where *P. recta* is asymptomatic of disease (Porrás-Alfaro and Bayman 2011). This has been found for the pathogen, *Verticillium dahlia*, that has a wide range of asymptomatic host plants (Malcolm et al. 2013). Similarly, through molecular methods, the U.K. native dune grass *Ammophila arenaria* was found to host many potential pathogens, but displayed no evidence of disease in its non-native ranges of New Zealand and Australia (Johansen et al. 2017). Second, not all pathogens are identified on FUNGuild, the database used to assign functional traits to our fungal OTUs. Therefore, *P. recta* may be inhibited by conspecific pathogens that have not been identified on that database and in this analysis. We note that the effects of other plant pathogens such as viruses, bacteria, and those that infect leaves and stems were not captured in this study. The effects of those potentially conspecific pathogens could be detrimental to *P. recta*. Thus, to isolate the effects of conspecific soil pathogens we conducted a greenhouse experiment and we used microbes smaller than 20 µm to represent a small-spored pathogen treatment and microbes smaller than 2 mm as a whole soil microbe treatment.

4.2 What are the effects of the whole microbial community (microflora, microfauna, and some mesofauna <2 mm), and the small microbial community (microflora, <20 µm) on *P. recta*?

In general, we found negative effects from the whole microbial community and neutral effects from the small microbial community (reduced total biomass from the whole relative to the no microbe treatment and similar biomass between the small and no microbe treatment). This suggests that *P. recta* are more responsive to the whole microbial community than the small. We found no effect of microbe source plant (congener, forb, grass, *P. recta*) from the whole or small microbe treatments on the biomass of *P. recta*. This suggests that *P. recta* are not inhibited by conspecific soil microbes whether large (< 2 mm) or small (< 20 µm). Furthermore, we did not observe signs of disease (lesions or decay) on any of *P. recta*'s roots.

In the whole microbe treatment, through microscopy, we found *Olpidium* sp. (Chytridiales order) resting sporangia in the roots of plants with microbes from *P. recta* and the congeners (Figure 17). *Olpidium bornovanus* and *Olpidium brassicae* are vectors of viruses such as lettuce big vein virus, tobacco necrosis virus, and cucumber necrosis virus (Webster and Weber 2009). We found the occurrence of these resting sporangia did not correspond to trends in total plant biomass. This suggests that although *P. recta* and its congeners may culture *Olpidium*, the presences of *Olpidium* resting sporangia does not affect *P. recta*'s biomass production. As proposed by (Malcolm et al. 2013), we suggest that within *P. recta*, *Olpidium* functions as an endophyte. *Olpidium* were found through molecular methods in the mycoheterotrophic orchid *Epipogium aphyllum*, the authors did not note if the plants appeared diseased (Roy et al. 2009). Interestingly, we did not detect *Olpidium* in our molecular analysis of field collected *P. recta* roots from 2014. The general eukaryotic primer ITS4 that we used has been used previously to

amplify *Olpidium*, therefore it was either not present in our 2014 field samples, or not identified on the FUNGuild database (Sasaya and Hiroki 2006). We note that *Olpidium* may influence *P. recta*'s competitive ability, seed production, or tolerance to other stressors (Mordecai 2011), but those responses were not directly measured in this study.

Within the whole microbial community treatment, AMF colonization was low. AMF are generally thought to form mutualistic associations with plants (Smith and Read 2008). Interestingly, we found biomass to be negatively correlated with colonization by AMF. Additionally, we found *P. recta* grown with microbes sourced from itself were less colonized by AMF than when grown with microbes sourced from other native forbs. Klironomos (2003) also found reduced biomass of *P. recta* when colonized by AMF compared to noncolonized controls. This suggests that *P. recta* may not benefit much from AMF associations. However, Klironomos (2003) also noted that the amount of colonization did not correspond to plants' responsiveness to AMF.

The interaction between AMF, low light levels, and elevated nutrient concentrations may explain the reduction in *P. recta* biomass found in the whole microbe treatment. To our knowledge, the effects of light exposure time, light intensity, and the interaction with AMF on *P. recta* have not been investigated directly. However, Johnson et al. (2015) found in the C4 grass, *Andropogon gerardii*, reduced biomass in mycorrhizal versus non-mycorrhizal treatments when under low light (66% reduction from 610-1047 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and with elevated nitrogen and phosphorus concentrations. The reduced light intensity used by Johnson et al. (2015) is comparable to the mean light intensity in our greenhouse experiment that was 201 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Similarly, our growing medium's nitrogen and phosphorus (methods section 2.6.2)

concentrations were also comparable to those used by Johnson et al. (2015). It is important to note that forbs and C4 grasses respond differently to AMF associations in the field, in the greenhouse, and under different nutrient concentrations (Hoeksema et al. 2010). A recent review on the effect of AMF under low-light conditions found 13 cases of reduced biomass in mycorrhizal versus non-mycorrhizal controls with decreasing light intensity, eight cases independent of light intensity, and two cases where non-mycorrhizal plant biomass was reduced more with decreasing light intensity (Konvalinková and Jansa 2016). Research has also demonstrated a decrease in AMF abundance in plants with decreased light and fertilization (Liu et al. 2014, Shi et al. 2014). This is in line with the low levels of AMF colonization that we observed. To summarize the findings of our greenhouse experiment we found no evidence for conspecific microbial inhibition of *P. recta*. Thus, we look to our field observational studies to find evidence that congeners inhibit *P. recta*.

4.3 *Is there evidence that conspecific soil microbes mediate the distribution of P. recta in the field?*

4.3.1 Plant-to-plant level (neighbor survey)

At the plant-to-plant level because *P. recta* and other co-occurring grassland forbs were equally distant from *P. recta*'s congeners, we have concluded that *P. recta*'s distribution was unaffected by the presence of its congeners (Figure 18). This suggests that the distribution of *P. recta* and its congeners are affected more by external factors than each other. Yet, in Figure 18, we can see that for all focal plants the relative distance to *D. glandulosa* is farther than the relative distance to *P. gracilis*, except for *P. recta* where the pattern is reversed. These data provided weak evidence (marginal statistical significance) that the relative distance between *P.*

gracilis and *P. recta* is larger than the relative distance between *P. gracilis* and the other target forbs. This indirectly supports the hypothesis that *P. recta* are inhibited by conspecific microbes, but the effect was only present for the more related congener (*P. gracilis*) to *P. recta*. Although this pattern is marginally significant, we discuss it here because the inocula used in our greenhouse experiment were collected from the rhizospheres of both congeners. Therefore, in our greenhouse experiment, any inhibition by *P. gracilis* microbes may have been diluted by *D. glandulosa* microbes. This possibly explains why we did not detect differences in *P. recta* biomass from different source plants (forb, congener, grass, *P. recta*). Our next question is if *P. recta* is truly inhibited by *P. gracilis*, then does this have any effect on *P. recta*'s distribution in the plant community and at the landscape level?

4.3.2 Landscape and community levels

To evaluate the observed compared to the expected instances of co-occurrence between *P. recta* and the native congeners we used vegetation survey data from two scales: community (2016) and landscape (2011). We define co-occurrence as two species occurring in the same surveyed plot either 1 m² for the community or along one of four 15.24 m transects for the landscape. We failed to find evidence of a positive or negative association between *P. recta* and either congener at the landscape level. Thus, if *P. recta* is farther from *P. gracilis* at the plant-to-plant level, this pattern does not scale up to observable landscape level inhibition. This is consistent with meta-analytical findings of (Ma et al. 2016) where invasive species did not do as well when the invasive plant and native plant community was more related at the local scale (i.e. plant-to-plant), but the invasive was unaffected by relatives at the larger regional scale such as the landscape. The lack of evidence for conspecific inhibition at the landscape level could be due

to the generally low abundance of native congener species. On the landscape, the congeners occurred a combined 30 instances out of 428, that is less than 10% of the surveyed plots. *P. recta* are prolific seed producers (Tuitele-Lewis 2004), and seeds landing on ground free of congeners and their microbes is more likely than seeds landing on ground with congeners. We found a positive association between *P. recta* and *P. gracilis* at the community level (co-occurred more often than chance). This does not support the hypothesis that *P. recta* is inhibited by conspecific soil microbes. The positive association between *P. recta* and *P. gracilis* at the community level may be an artifact of the surveyed sites because *P. recta* and its native congeners were known by MPG staff to co-occur at those locations and that is why they were surveyed, even though generally on the landscape the congeners are less common.

4.4 Additional considerations

We note that our methodologies did not capture *P. recta*'s growth rate, fecundity, or stress tolerance so negative effects of conspecific microbes could manifest in these or other plant responses. In our greenhouse experiment, after approximately 6 weeks some leaves began to yellow and we acknowledge that light intensity, temperature, pathogens, and other environmental factors can induce changes in leaf color (Lers 2007). To relieve what we interpreted as nutrient stress, we fertilized the plants with a low phosphorus Hoagland's solution, possibly exacerbating the interactive effects of excess nutrients, light, and AMF. Furthermore, the congener inocula were a composite of both *P. gracilis* and *D. glandulosa* soil microbes, therefore we cannot isolate the possible effects of each congener. However, the lack of evidence for inhibitory effects of conspecific microbes on *P. recta* may reflect a true null as our molecular findings suggest that the fungal communities within *P. recta* roots are unaffected by neighbor identity and at the

landscape level *P. recta* and either congener were found to co-occur as we would predict given their overall occurrences.

4.5 *Differences between our findings and previous P. recta research*

Our greenhouse study differed from the work of Callaway et al. (2013) in that we used soil inoculum collected directly from the field and they used inoculum that had been cultured by a host plant for one generation. Our method potentially introduced additional variation to our experiment, but was inherently more reflective of field conditions. Additionally, Maron and Mahler's (2008) research used experimental monocultures of common grassland forbs, but this is not how many of these plant species are distributed in the field. Although our field studies were observational, and not experimental, we quantified how *P. recta* were naturally distributed from its native congeners relative to other neighboring grassland forbs. Finding that *P. recta*'s distribution was unrelated to the distribution of its congeners whether at the plant-to-plant or landscape levels. Synthesizing previous findings and our own, we conclude that *P. recta* may be inhibited by its congeners when they are densely established or have experimentally cultured soil microbes, but in the field, we did not detect any conspecific inhibition of *P. recta* or a fungal mechanism for conspecific inhibition.

4.6 *Synthesis*

Counter to previous studies, we found little evidence for negative effects of conspecific microbes on *P. recta*. We found higher proportions of pathogens relative to mutualists for *P. recta*, but pathogen abundance did not predict *P. recta* biomass. Nor was *P. recta*'s fungal community composition different by its neighboring plant. This does not support a fungal

pathogen mechanism for the conspecific inhibition found by Callaway et al. (2013). In the greenhouse *P. recta* had reduced biomass from the whole microbial community and not the small microbial community. However, we found no difference in this effect among the microbe source plants in either the whole or small microbe treatments. This suggests that *P. recta* is not inhibited by the conspecific soil microbial community. Furthermore, in the greenhouse, we found *P. recta* had low colonization by AMF, but experienced parasitism from AMF possibly due to the interactive effects of low light levels and nutrient fertilization. In the field, *P. recta* and other common forbs were similarly distant from *P. recta*'s congeners. This does not support the hypothesis that native congeners inhibit *P. recta* because we would expect *P. recta* to be farther from its congeners than the other forbs are from *P. recta*'s congeners. At the landscape level, we found no evidence that the instance of co-occurrence between *P. recta* and its congener are related. Again, this does not provide support for inhibition of *P. recta* by native congeners. At the community level, we found *P. recta* and *P. gracilis* to positively associate within 1 m² areas, directly contradicting what we would expect if conspecific microbes inhibited *P. recta*. Taken as a whole, our combined findings suggest that *P. recta* is not functionally inhibited by conspecific soil microbes.

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5 Appendix A:

Table 1S. Mean \pm standard error, and range for biomass (g) of the roots, shoots, and root to shoot ratio by source plant and microbial fraction whole (<2mm), small (<20 μ m), and none.

Biomass	Microbe source plant	Whole		Small		None	
		Mean \pm SE	Range	Mean \pm SE	Range	Mean \pm SE	Range
Roots (g)	Congener	0.912 \pm 0.043	0.697 - 1.101	1.031 \pm 0.050	0.881 - 1.308	1.078 \pm 0.047	0.904 - 1.364
	Forb	0.754 \pm 0.070	0.467 - 1.058	1.049 \pm 0.048	0.752 - 1.233	1.133 \pm 0.029	0.900 - 1.221
	Grass	0.828 \pm 0.054	0.579 - 1.084	1.131 \pm 0.036	0.973 - 1.378	1.029 \pm 0.035	0.825 - 1.154
	<i>P. recta</i>	0.886 \pm 0.029	0.694 - 1.014	1.100 \pm 0.052	0.806 - 1.365	1.097 \pm 0.043	0.848 - 1.341
Shoots (g)	Congener	0.706 \pm 0.047	0.470 - 0.940	0.793 \pm 0.041	0.620 - 0.990	0.823 \pm 0.021	0.700 - 0.920
	Forb	0.569 \pm 0.054	0.310 - 0.770	0.760 \pm 0.031	0.590 - 0.920	0.850 \pm 0.017	0.770 - 0.930
	Grass	0.624 \pm 0.046	0.410 - 0.800	0.830 \pm 0.022	0.690 - 0.940	0.830 \pm 0.026	0.670 - 0.920
	<i>P. recta</i>	0.720 \pm 0.020	0.630 - 0.840	0.833 \pm 0.024	0.750 - 1.010	0.839 \pm 0.031	0.690 - 0.990
Root:Shoot	Congener	1.322 \pm 0.071	0.968 - 1.779	1.309 \pm 0.042	1.077 - 1.449	1.321 \pm 0.079	1.028 - 1.948
	Forb	1.338 \pm 0.048	1.152 - 1.603	1.390 \pm 0.062	1.108 - 1.612	1.335 \pm 0.033	1.140 - 1.540
	Grass	1.343 \pm 0.048	1.096 - 1.573	1.368 \pm 0.044	1.209 - 1.646	1.251 \pm 0.054	0.948 - 1.519
	<i>P. recta</i>	1.232 \pm 0.028	1.052 - 1.320	1.326 \pm 0.063	0.960 - 1.576	1.309 \pm 0.027	1.147 - 1.440

6 Appendix B: detailed bioinformatics methods from MPG Ranch

Bioinformatics

We performed all bioinformatic analysis using QIIME (Quantitative Insights Into Microbial Ecology, version 1.9.0, Caporaso et al. 2010). Raw forward and reverse PE ITS reads were trimmed at the 220 and 180 base pair position, respectively, before joining paired-ends using the fastq-join method (Aronesty 2013) with a minimum overlap of 20 nucleotides and allowing for 10% mismatch in the region of overlap. All low quality (phred<25) short (<200bp) reads were removed during demultiplexing and remaining reads were clustered into operational taxonomic units (OTUs) based on a 97% sequence similarity threshold using Uclust (Edgar 2010). Sequences from the ITS reference database UNITE (Koljalg et al. 2013) were used as seeds for clusters and OTU taxonomic assignment. Any read that did not match a reference sequence was clustered *de novo* and compared against sequences from Genbank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the BLAST algorithm (Altschul et al. 1990). Reads that matched with Fungi in Genbank were retained for analysis but were not assigned taxonomy. OTUs with a sequence abundance of less than 0.05% of the total number of sequences were removed in order to decrease artifactual OTUs generated through sequencing error (Bokulich et al. 2013). Function was then assigned to OTUs using the FUNGuild reference database (Nguyen et al. 2016).

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