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Effects of soil biota from different ranges on *Robinia* invasion: acquiring mutualists and escaping pathogens

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Abstract. The net effects of soil biota on exotic invaders can be variable, in part, because net effects are produced by many interacting mutualists and antagonists. Here we compared mutualistic and antagonistic biota in soils collected in the native, expanded, and invasive range of the black locust tree, *Robinia pseudoacacia*. *Robinia* formed nodules in all soils with a broad phylogenetic range of N-fixing bacteria, and leaf N did not differ among the different sources of soil. This suggests that the global expansion of *Robinia* was not limited by the lack of appropriate mutualistic N-fixers. Arbuscular mycorrhizal fungi (AMF) from the native range stimulated stronger positive feedbacks than AMF from the expanded or invasive ranges, a biogeographic difference not described previously for invasive plants. *Pythium* taxa collected from soil in the native range were not more pathogenic than those from other ranges; however, feedbacks produced by the total soil biota were more negative from soils from the native range than from the other ranges, overriding the effects of AMF. This suggests that escape from other pathogens in the soil or the net negative effects of the whole soil community may contribute to superior performance in invaded regions. Our results suggest that important regional evolutionary relationships may occur among plants and soil biota, and that net effects of soil biota may affect invasion, but in ways that are not easily explained by studying isolated components of the soil biota.

Key words: arbuscular mycorrhizal fungi, AMF; black locust tree; exotic plant invasion; feedbacks; mutualism; nitrogen fixation; pathogens; phylogeny; Pythium *sp.;* Robinia pseudoacacia; *soil biota.*

Introduction

Soil biota may impede or accelerate exotic plant invasion (van der Putten et al. 2005, Wolfe and Klironomos 2005). However, most studies find the net effect of soil biota in the native ranges of an invader to be more negative than soil biota in invaded ranges of the same species (Reinhart and Callaway 2006, Kulmatiski et al. 2008). Depending on the relative intensities of the total pathogenic and mutualistic effects of microbes in soil, the inhibitory or beneficial effects of soil biota on plants can increase or decrease over time as different soil organisms accumulate (Bever 2002, van der Putten et al. 2007). These "feedbacks" can promote invasion (Callaway et al. 2004*b*), and transformed soil communities may exacerbate the impacts of invaders on resident plant communities (Vogelsang and Bever 2009) and affect reestablishment by native species.

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Invasive species can escape soil-borne natural enemies when they are introduced into new regions of the world (Klironomos 2002, Agrawal et al. 2005, Reinhart et al. 2010; but see Parker and Gilbert 2007). Biogeographic differences in pathogenic effects of soil may result from the effect of the invader on pathogen densities (Mangla et al. 2007), pathogen community composition (i.e., species identity and species richness; Mitchell and Power 2003), the virulence of different pathogenic species or genotypes (Reinhart et al. 2010), and phylogenetic relatedness of the invader relative to resident species susceptible to resident pathogens (Gilbert and Webb 2007). For example, an invader may be unaffected by resident pathogens because they are not adapted to the invader, relative to resident plant species (Agrawal et al. 2005), which may affect their ability to locate, colonize, and ultimately cause disease symptoms.

The effects of soil mutualists on invasions are less well understood than those of pathogens, but they also can have important ecological effects. For example, invaders may be limited by the absence of appropriate mutualists in their new ranges (Parker 2001) or may benefit from soil mutualists that they encounter in invaded ranges (Marler et al. 1999, Reinhart and Callaway 2004, Parker

et al. 2007). Invaders can also suppress soil mutualists of other plant species in invaded ranges more aggressively than mutualists in their original range (Callaway et al. 2008). Like some pathogenic interactions, mutualistic interactions can be highly specific among some taxa, but most mutualisms appear to be quite general (Bronstein 2003). Many mutualisms do not seem to have tight, long coevolutionary relationships, and invasive plants can form mutualisms as effective or more effective in the new ranges than in the old range (Richardson et al. 2000, Parker et al. 2007). However, a fundamental unanswered question is whether the benefits of new mutualist partnerships in invaded regions are generally stronger, weaker, or similar to mutualistic interactions in native regions.

We used *Robinia pseudoacacia* L. (black locust) as a focal species to test the effects of the total soil biota from different ranges as well as specific pathogenic and mutualistic components. *Robinia pseudoacacia* is a large leguminous tree native to North America, but globally invasive in temperate regions. It is colonized by arbuscular mycorrhizal (AM) fungi, ectomycorrhizal fungi, and soil-borne pathogens (e.g., *Armillaria*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Verticillium*) (Farr et al. 1989), and harbors N_2-fixing bacteria that alter soil nitrogen cycling in different parts of its now global range (Boring and Swank 1984, Dzwonko and Loster 1997, Rice et al. 2004). The original native range of *Robinia* is the Appalachian and Ozark mountains, where it is primarily an early-successional species (Boring and Swank 1984). It is a component of mature forest in some places but it is not a community dominant in its native range. During the last 200 years, *Robinia* has expanded its range throughout North America, where it now occurs in all contiguous states and throughout southern Canada. It is officially listed as invasive in Connecticut and Massachusetts, and is described as invasive in other states (Uva et al. 1997, California Invasive Plant Council 2006). *Robinia* has also been widely planted around the world and is now considered invasive in a number of European countries (Global Invasive Species Database, *available online*).[6] It is considered to be one of the top 100 worldwide woody plant invaders (Cronk and Fuller 1995) and its negative impacts on native species have been reported in Japan (Maekawa 1991), Hungary (Matus et al. 2003), and Italy (Celesti-Grapow et al. 2009). Therefore one way to describe the biogeographic distribution of *Robinia* is that it occurs in a "native" range, an "expanded" range throughout much of North America, and an "invasive" range in many parts of the world.

We hypothesized that *Robinia* escapes soil pathogens in its expanded and invasive ranges. Concomitantly, we hypothesized that *Robinia* disproportionally benefits from mutualists in its invasive range or at least is not limited by a lack of associations with suitable mutualists in its invasive range. We examined these hypotheses by collecting soils from all three ranges of *Robinia* and testing the effects of whole-soil biota, different soil fractions, AM fungi, and soil-borne *Pythium*, and conducted a detailed investigation of N_2-fixing taxa found in nodules that developed in the different soils.

Methods

We collected soil from forests at seven sites within the native range of *Robinia*, six sites in the expanded range, and 11 sites within the invaded range of Europe (Appendix A). At each site we collected soil in forests containing *Robinia*, but in locations that were at least 20 m from *Robinia* trees. Sampling away from *Robinia* trees was intended to minimize the effect of ongoing plant–soil microbial feedbacks in the field and to sample the broad potential for feedbacks caused by soil microbes in these forests, not specifically the soil community only associated with *Robinia*. At each site, soil was collected from 6–10 haphazardly chosen locations scattered over a 1–2 ha area and was combined into one 10–15 L sample per site. Each collection was 10 cm deep and included the O horizon and the portion of the A horizon required to reach 10 cm. We did not collect litter or portions of the B horizon. These soils were collected over a period of 11 months. Collected soils were then slowly air-dried at room temperature until mass was stable to encourage soil biota to enter a dormant stage and survive storage, and then soils were stored in sealed Ziploc bags. We do not know the disturbance histories of these stands, but all forests except one, the site in the Great Smoky Mountains National Park (GSMNP), were secondary forest. *Robinia* densities were not measured, but *Robinia* clearly appeared to occur at much higher densities in the invasive ranges, and at the lowest density in the GSMNP.

Soil feedbacks

On 5 July 2006 we planted 10 *Robinia* seeds collected near Hagerstown, Maryland (UTM easting and northing coordinates: E −77.7471, N 39.6355; no soil was collected here) in each of 2–3 2.4-L pots per site sampled. At the same time we placed the same amount of soil per site into the same number of pots and left these pots unplanted. All pots were kept 30 cm from each other but intermixed within the growing space; the pots were rotated often. Once seeds germinated, we thinned seedlings to three per pot and grew these for 127 days in a greenhouse in Missoula, Montana. We then measured seedling height, diameter, and leaf number and counted all root nodules visible at 10× magnification on each individual. The means of these measurements for all seedlings grown in soil from a particular site were analyzed in single ANOVA (SPSS 15.0) with range (native, expanded, invaded) as a fixed factor. Then, *Robinia* seeds and soil (referred to as "trained soil") from each treatment at each site were pooled and

[6] ⟨http://www.issg.org/database/welcome/⟩

sent to the University of Guelph, where a second plant–soil feedback experiment was conducted. In this second experiment, the experimental unit consisted of a 1-L pot filled with sterile silica sand. To each unit we also added one of the following: (1) 50 g of soil (either trained with *Robinia* or trained without *Robinia*), (2) an AM fungal spore fraction from 50 g of soil (either trained with *Robinia* or trained without *Robinia*), (3) a <20-μm microbial fraction from 50 g of soil (either trained with *Robinia* or trained without *Robinia*), or (4) a 20–200 μm microbial fraction from 50 g of soil (either trained with *Robinia* or trained without *Robinia*). We made preliminary observations to determine what biota were present in each of the soil fractions added. The trained soil contained all biota from the previous experiment; the AM fungal spore fraction contained arbuscular mycorrhizal spores >45 μm in diameter (which includes most spores of the Glomeromycota) as well as some attached fungal hyphae; the <20-μm fraction contained fungal spores and hyphal fragments in the ascomycota, basidiomycota, and zygomycota, as well as bacteria, and thus included saprobic, parasitic, pathogenic, and perhaps some mutualistic microbes; the 20–200 μm fraction included some AM fungal spores, nematodes, and microarthropods, such as collembolans and mites. These fractions were prepared as in Klironomos (2002), except for the 20–200 μm fraction, which was collected between a 200-μm and a 20-μm sieve. Also, in the AM fungal spore fraction, we isolated 100 randomly chosen AM fungal spores, rinsed them in distilled water, and added only these to the pots. These additions were mixed thoroughly with the silica sand prior to planting a one-week-old *Robinia* seedling into each pot. Plants were grown for 112 days in a greenhouse. We then harvested the plants, dried them at 60°C for 48 hours, and measured total biomass for each plant. We also counted the number (and biomass) of nodules that developed on the roots of each plant, and measured the N concentration of plant leaves. Feedback for each of the different additions was calculated as the percentage difference in plant biomass between the treatments with and without *Robinia* training.

Soil pathogen virulence

Pythium has been shown to be a factor in other tree invasions (Reinhart et al. 2010) and this pathogen was collected in field soil (as just described) from the different ranges on *Robinia*. The *Pythium* isolates were used in a controlled pathogenicity trial. We predicted that isolates from the historic native range would have the strongest negative effects. Globally, *Pythium* (kingdom Stramenopila, phylum Oomycota) are important plant pathogens infecting plant seeds or seedlings prior to emergence from the soil (Hendrix and Campbell 1973), and they are known to affect *Robinia* (Farr et al. 1989). *Pythium* species often have a wide host range, can severely reduce plant fitness, and can survive as saprophytes in the soil (Jarosz and Davelos 1995). A series of standard techniques for the culturing of *Pythium* were used to acquire pure cultures of *Pythium* (Abad et al. 1994). The isolation, pathogenicity trials, and analyses followed the methodology described previously (Reinhart et al. 2010).

In 2007, *Pythium* isolates were obtained from soil collected at six of the seven sites from the native range ($n = 8$ isolates total); five of the six sites from the expanded range ($n = 7$ isolates total), and eight of the 11 sites from the nonnative range in Europe ($n = 9$ isolates total). In a few cases, two isolates were collected from a single site and were used in the pathogenicity trial. The pathogenicity experiment used *Robinia* seed from Kentucky (Sheffield's Seed Company, Locke, New York, USA). We tested the effect of individual isolates on eight recently germinated seedlings contained in an experimental vessel ($n = 3$ vessels per isolate). After 30 days, the survival, shoot biomass, and root necrosis of seedlings were quantified. We tested the effect of *Pythium* origin (native historic, native expanded, vs. exotic) on root necrosis and stem biomass using Proc GLIMMIX and Proc MIXED, respectively, in SAS version 9.13 (SAS Institute, Cary North Carolina, USA), with origin as a fixed effect and isolate (origin) as a random effect. Survival was 100% across treatments and was not analyzed.

Nitrogen-fixing mutualists

Because far more is known about the genetics, phylogenetics, and physiology of nodule-forming N_2-fixing mutualistic bacteria than other microbial mutualists, we studied them in more detail. We produced a phylogeny of the N_2-fixing bacterial mutualists found in the nodules of *Robinia* formed in soils from all three ranges to determine the taxonomic relationships and breadth of these mutualists across the three ranges. We collected 10–15 nodules from *Robinia* saplings planted in soil from each site; nodules were pooled and analyzed at the Microbiologia del Suelo y Sistemas Simbioticos, Estación Experimental del Zaidín, CSIC, Granada, Spain for phylogenetic relationships among the N-fixing bacterial components of the nodules. Nodules were surface-sterilized with 2.5% $HgCl_2$ for 3–5 min, rinsed thoroughly with sterile distilled water, placed on a petri dish, and crushed in a drop of sterile water with a sterile glass rod. The resulting suspension was streaked onto petri dishes containing either yeast extract mannitol (YEM; Vincent 1970) medium, peptone–mineral salts–yeast extract (PSY; Regensburger and Hennecke 1983) medium, or triptone soybean agar (TSA) medium. To test the surface-sterilization process, aliquots of the sterile distilled water used in the final rinse were plated onto each YEM, PSY, and TSA media. Plates were incubated at 30°C for 7–10 d. Then, morphologically different colonies were checked for purity by repeated streaking of single-colony isolates on the same medium.

Genomic DNA isolated from bacterial cells using RealPure Genomic DNA Extraction kit (Durviz,

Valencia, Spain) was used as template for each repetitive extragenic palindromic (REP) polymerase chain reaction (PCR) and 16S rRNA gene amplifications. REP-PCR was performed using primers REPIR-I and REP2-I according to de Bruijn (1992). After the reactions, the PCR products were separated on 1.5% agarose gels in TBE (tris-borate-EDTA) buffer (Trizma base, 10 g/L; boric acid, 5.5 g/L; and EDTA, 0.93 g/L, pH 8.5) at 6 V/cm, stained in a solution containing 0.5 μg/mL ethidium bromide, and photographed under UV light. Molecular Marker III (Roche Applied Science, Barcelona, Spain) was used as a size standard. Aliquots of loading solution (40% sucrose and 0.25% bromophenol blue) were added to each sample. PCR amplifications of 16S rRNA gene fragments were done using template DNA and the two opposing primers, 41f and 1488r, as described previously (Herrera-Cervera et al. 1999). Aliquots of PCR products were supplemented with loading buffer, electrophoresed on 0.7% agarose gels in TBE buffer, and stained and photographed as previously described. PCR products were further purified with a QIAquick PCR purification kit (Quiagen, Valaencia, California, USA) and sequenced directly using primers 41f and 1488r. The sequence reactions were performed on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) using a BigDye terminator version 3.0 cycle sequencing kit (Applied Biosystems) as supplied by the manufacturer. The sequences obtained were compared with those from GenBank using the UW-BLAST program through EMBL-EBI (*available online*).[7] Sequences were aligned using the multiple-sequence alignment program ClustalW2 from EMBL-EBI (*available online*).[8] Phylogenetic analyses were performed with the PHYLIP computer program package, version 3.67 (Felsenstein 1993). The distances were calculated according to Kimura's (1980) two-parameter model. Phylogenetic trees were inferred using the neighbor-joining algorithm (Saitou and Nei 1987). Bootstrap analysis was based on 1000 resamplings. Trees were rooted using *Bacillus subtilis* as an outgroup, and were visualized with TreeView (software *available online*).[9]

Results

Robinia grown in soil collected ≥20 m away from conspecifics in the native, expanded, and invaded ranges did not differ in the number of nodules produced per sapling, leaf number, or stem diameter. However, saplings grown in soil from the invaded range (21.0 ± 2.4 cm, mean ± SE) were significantly taller than saplings from the expanded (11.1 ± 2.7 cm) or native (15.7 ± 2.4 cm) ranges (for range, $F = 4.66$, df = 2, 21, $P = 0.021$), consistent with their reported invasive success in invaded European forests relative to forests in North America.

Soil feedbacks

Robinia grown in soils from the three ranges, but after they had been occupied by another *Robinia*, showed no difference in the number of nodules produced per plant (native = 4.17 ± 0.78 nodules/plant, mean ± SE; expanded = 3.27 ± 1.03; invaded = 4.76 ± 0.64; for range, $F = 8.04$, df = 2, 21, $P = 0.433$), the mean mass per nodule (native = 21.6 ± 1.3 mg, mean ± SE; expanded = 22.0 ± 1.2 mg; invaded = 21.4 ± 0.6 mg; for range, $F = 0.08$, df = 2, 21, $P = 0.926$), or total leaf N concentration (native = 3.32% ± 0.16 %, mean ± SE; expanded = 3.10% ± 0.10 %; invaded = 3.25% ± 0.09 %; for range, $F = 0.759$, df = 2, 21, $P = 0.475$). Thus the ability to form nodules and the benefits of N_2-fixing mutualists did not differ across ranges. See Appendix B for mean nodule number and mass for treatments and regions.

Soil feedback effects on total *Robinia* mass using the total biota were more than twice as negative for *Robinia* seedling mass in soils from the native range (−19.6% ± 3.2% reduction in biomass) than for soils collected in either the expanded or invaded ranges (−7.8% ± 1.7% vs. −6.0% ± 1.1%, respectively; Fig. 1 and Appendix B). This pattern was the same for feedbacks using the 20–200 μm fraction of the soil biota. The effects of the <20-μm fraction were equally negative among all three ranges and, surprisingly, were about as negative as the total fraction (−10.2% to −15.7%), even without the larger components of the soil biota in this treatment. Tested alone, the AM fungal fraction from the native range showed a strong positive feedback effect (+18.1% ± 4.2% biomass increase); whereas the AM fraction from the expanded and invaded ranges showed no feedback effects (−1.0% ± 2.9% vs. −3.4% ± 1.4%, respectively). Because the filtering treatments cannot eliminate all bacteria, some nodules formed in all treatments, even the "AMF" and "20–200 micron" treatments, although there were fewer nodules in these treatments (Appendix B).

Soil pathogen virulence

Despite the regional differences in total soil feedbacks, seedling survival was unaffected (100% survival) by *Pythium* isolates. Overall, disease symptoms were uncommon and there was no effect of origin of *Pythium* isolates on root necrosis of *Robinia* seedlings (GLIMMIX, $F_{1,25.3} = 0.13$, $P = 0.72$). However, there was a significant effect of isolate origin on shoot biomass of *Robinia* seedlings (ANOVA, $F_{2,21} = 4.33$, $P = 0.027$), but not in the direction predicted if *Pythium* played a role in invasion success. Instead, shoot biomass of seedlings interacting with *Pythium* isolates from the nonnative range were smaller (0.052 ± 0.002 g, mean ± SE) than those interacting with isolates from the historic native range (0.060 ± 0.002 g) and recently expanded range in the United States (0.058 ± 0.002 g).

Nitrogen-fixing mutualists

After incubation in TSA, YEM, and PSY medium, 68 strains forming morphologically different colonies were

[7] ⟨http://www.ebi.ac.uk/Tools/sss/⟩
[8] ⟨http://www.ebi.ac.uk/Tools/msa/clustalw2/⟩
[9] ⟨http://taxonomy.zoology.gla.ac.uk/rod/treeview.html⟩

selected for the REP-PCR analysis. Because bacterial strains that show the same REP-PCR pattern belong to the same species (de Bruijn 1992), this technique is a good tool for grouping bacteria in order to select representative strains from each group for further 16S rRNA gene sequencing. Accordingly, strains that showed the same REP-PCR profile were grouped and a representative strain from each group was selected for further 16S rRNA gene sequencing. Variation in REP-PCR patterns revealed 26 different isolate groups. The nearly complete sequence of 16S rRNA genes from each representative isolate was obtained and compared with those held in GenBank. Our results showed that a wide range of N-fixing taxa occupy *Robinia* nodules, and there was no biogeographic pattern in the total phylogeny. Twenty-three isolates clustered in 10 REP-PCR groups that were members of the family Rhizobiaceae of the α-Proteobacteria (Fig. 2). Strain RP2 belongs to the genus *Bradyrhizobium*, and showed a 97.8% and a 97.7% similarity with *B. canariense* BC-C2 and *B. betae* type strains, respectively. The remaining 22 isolates were classified into the genus *Mesorhizobium*. Strains RP14 (together with isolates RP4, RP61, and RP63 of the same REP-PCR group), RP20 (together with isolates RP6 and RP17 of the same REP-PCR group), RP47, RP48, and RP50 (together with isolate RP49 of the same REP-PCR group) showed 98.8%, 99.6%, 98.6%, 99.6%, and 99.3% similarity with strain Rob23 isolated from root nodules of *R. pseudoacacia* growing in Germany (Ulrich and Zaspel 2000). Rob23 showed strong similarity with *Mesorhizobium* sp. 88B, a strain isolated from *Lotus corniculatus*. Based on the 16S rRNA sequences, strain R88 had five nucleotide mismatches with that of *M. loti* type strain NZP2213 (Sullivan et al. 1996). The closest relative species to strains RP1 (together with isolate RP52 of the same REP-PCR group), RP8 (together with isolate RP3 of the same REP-PCR group), RP18 (together with isolates RP55 and RP59 of the same REP-PCR group), and RP26 (together with isolates RP11, RP57, and RP65 of the same REP-PCR group) was *M. amorphae*, with 99.6%, 98.8%, 98.4%, and 99.7% similarity, respectively.

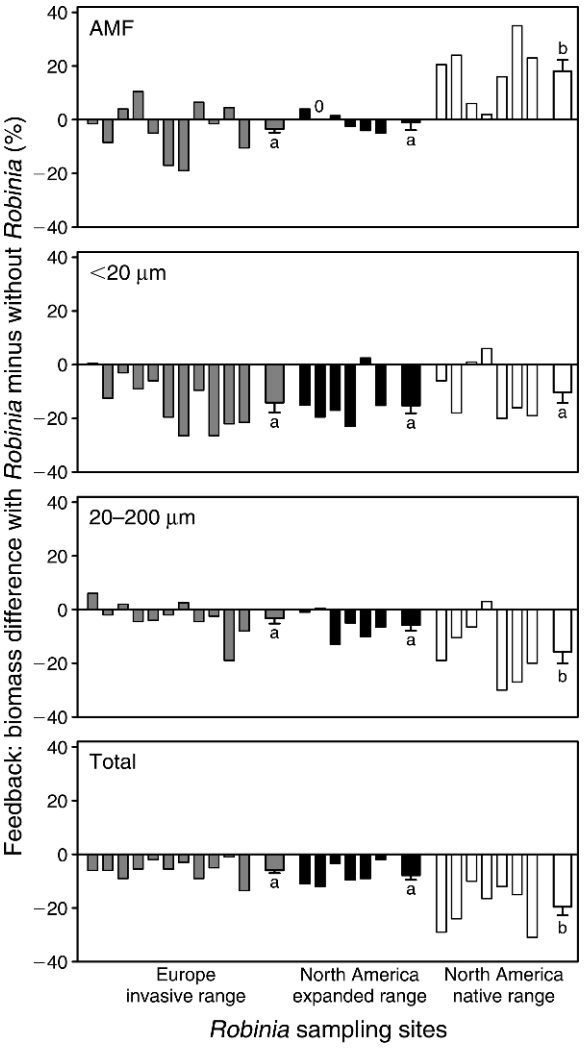

FIG. 1. Soil feedback effects for the AMF (arbuscular mycorrhizal fungi) spore fraction, the <20-μm size fraction of the soil biota, the 20–200 μm size fraction of the soil biota, and total soil. Bars represent the percentage difference in plant biomass between soils trained with the black locust *Robinia pseudoacacia* and those trained without *Robinia*. Narrow bars show the feedback strength for each site in the order presented in Appendices A and B, and the thicker bars show the mean and 1 SE for each region. Shared lowercase letters within a graph indicate no statistical difference as determined by ANOVA with region as a fixed factor and post-ANOVA Tukey tests, $P < 0.05$

DISCUSSION

Robinia acquired N-fixing mutualists from all ranges, with no differences among the ranges in nodule production or leaf N. In contrast, the most beneficial AM fungi were from the native range. Despite these biogeographical patterns in mutualist effects, the net effects of soil and soil feedbacks on *Robinia* mass were much more negative for soil from the native range than for soils from the expanded or invaded ranges, indicating that the most important net ecological processes related to soil biota was escape from enemies in the native range. To our knowledge, no other study has tested potential biogeographic variation in the effects of different soil components as we have, but there are a number of general parallels for net soil biota effects in other systems (reviewed in Reinhart and Callaway 2006). For example, van der Putten et al. (2007) found that an invasive savanna grass showed neutral to positive soil feedbacks, but native grasses showed neutral to negative feedbacks. In a meta-analysis of studies on plant–soil feedbacks, Kulmatiski et al. (2008) also found that exotic invasive plants demonstrated much less negative plant–soil feedbacks than either native plants or noninvasive exotic species.

Soil feedback studies typically characterize feedback interactions for a single site and compare growth of plants when grown in soil previously "trained" by

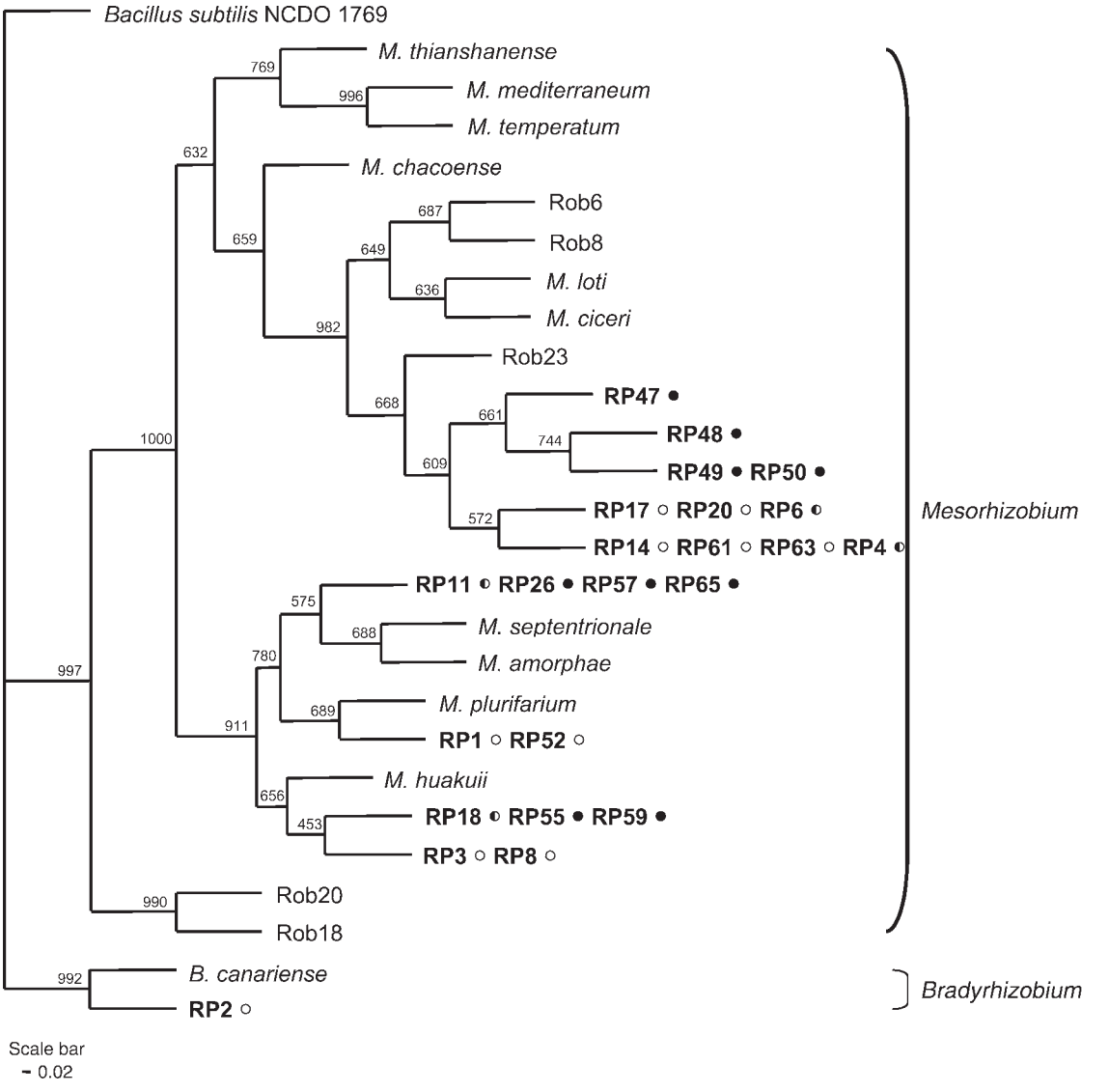

FIG. 2. Phylogenetic tree generated by the neighbor-joining method using 16S rRNA sequences. Bootstrap values (1000 replicates) are indicated above the branches. Horizontal branch length in the PHYLIP program (Felsenstein 1993) reflects the number of nucleotide (nt) substitutions per site; the scale bar (0.02) indicates 2 nt substitutions per 100 nt. Taxa without circles to the right have been chosen from the literature to represent known sequences to accurately order the phylogeny. Taxa in boldface (RP) with circles to the right were identified from nodules in this study from *Robinia pseudoacacia*. Black circles indicate that the isolates were found in the native range, black and white circles represent taxa found in the expanded range, and white circles indicate taxa found in the invaded range of *R. pseudoacacia*. The nucleotide sequence of isolates RP1, RP2, RP8, RP14, RP18, RP20, RP26, RP47, RP48, and RP50 have been deposited in GenBank under the accession numbers EU999231, EU999232, EU999233, EU999234, EU999235, EU999236, EU999237, EU999239, EU999240, EU999241. Isolates Rob6 (accession number AJ271898), Rob8 (AJ27189), Rob18 (AJ271901), Rob20 (AJ271902), and Rob23 (AJ271900) were published by Ulrich and Zaspel (2000). *Mesorhizobium* and *Bradyrhizobium* strains (with superscript T indicating the type strain) included: *M. tianshanense* USDA 3592 (AF041447), *M. mediterraneum* UPM Ca36^T (L38825), *M. temperatum* SDW 018^T (AF508208), *M. chacoense* PR5 (AJ278249), *M. lotI* LMG 6125^T (X67229), *M. ciceri* UPM Ca7^T (U07934), *M. septentrionale* SDW 014^T (AF508207), *M. amorphae* ACCC 19 665^T (AF041442), *M. plurifarium* LMG 11 892^T (Y14158), *M. huaukii* IFO 15 243^T (D13431), and *B. canariense* BC-C2 (AY577427). *Bacillus subtilis* NCDO 1769 (X60646) was used as an outgroup.

conspecifics vs. other species (heterospecifics) present at the site. We performed a soil feedback calculation comparing growth in soil previously trained by conspecifics vs. pots without plants but originally collected away from any *Robinia* trees. This was not ideal, but was necessary because our study included soil from 24 sites from around the world, and we were unable to identify an alternate species that co-occurred with *Robinia* across all sites. We compared the total biomass of *Robinia* seedlings grown in untrained soil from the different sites and found no differences among regions after feedbacks were initiated (see Appendix B), suggesting that the

percentage changes reported in Fig. 1 do not mask inherent strong regional differences in soil biota effects not reflected in plant–soil feedbacks. Whether the results represent true feedback patterns vs. general soil biota effects driven primarily by other resident species and not *Robinia*, the reported biogeographical differences still have implications for understanding *Robinia* establishment and invasion.

Our results for the potential effects of N_2-fixing or AM fungi (AMF) mutualists are mixed, but neither provides evidence for the enhanced mutualists hypothesis (Reinhart and Callaway 2006) or corresponds well with the general patterns observed for the total soil biota. For example, *Robinia* grew larger with AMF from its native range than with AMF from either the expanded or invaded ranges, suggesting a possible evolved AMF–*Robinia* relationship in the native range, but not one that can explain invasion or the net effects of the soil biota or feedback results for the total soil (Fig. 1). However, clear evidence for such evolutionary relationships would require experimentation with *Robinia* seed sources from different populations in the invaded and native ranges (e.g., Seifert et al. 2009). Although the AM fungi results suggest some biogeographical variation in AMF interactions depending on their origins, *Robinia* was readily colonized by many nodulating mutualist taxa in all three ranges. Furthermore, we found no evidence for functional variation among the isolates from the different ranges; neither nodule number nor leaf nitrogen content varied between ranges. The phylogeny for these mutualists showed no biogeographic pattern, with three primary genera of nodule-forming bacteria being associated with root nodules inoculated with soils from different ranges. However, functional variation may occur in ways not well represented by leaf nitrogen. Rhizobia may synthesize chemicals that are costly to plants in other ways, impairing plant growth even when leaves accumulate a normal concentration of total N. Second, in our experimental design, plants developed nodules based on inoculation with soil. Thus, our net effects of rhizobia on leaf N could be confounded by the effects of AMF or pathogens.

The ecological black box of soil biota appears to be difficult to study with reductionist approaches that focus on specific components of the soil in isolation from each other without synergistic effects. We found that AM fungi from the native range caused more positive feedbacks than did AM fungi from the expanded or invasive range, but the total soil effects swamped these AM fungi effects (e.g., Klironomos 2002). This suggests that the effects we observed for soil biota may be produced by complex interactions among multiple taxa in the soil and are not necessarily attributable to single taxa, especially when the components are likely to vary spatially among trees at a site and among sites (e.g., Reinhart and Clay 2009).

To our knowledge, our results are the first to show that AM fungi, notoriously promiscuous in their mutualistic relationships, from the native range of a plant species have disproportionally more beneficial effects on their plant partner than do AM fungi from outside the native range. In other comparisons of plant–soil feedbacks among native species and exotic species at a site involving AM fungi, Klironomos (2002) found that total soil biota feedbacks for the exotics were positive, but were negative for relatively rare natives. However, the feedback effects of AM fungi ranged from neutral to positive for both exotic and native species. *Centaurea maculosa*, an invasive perennial forb in North America, is more inhibited by soil biota in its native European range than by soil biota in its invaded range (Callaway et al. 2004*b*). However, this invader is highly infected by AM fungi in North American soils and appears to benefit competitively from the relationship with these AM fungi (Marler et al. 1999, Callaway et al. 2004*a*, Carey et al. 2004). Recent studies have shown that introduced North American populations of *Hypericum perforatum* respond less to inoculation with AM fungi than do European populations, suggesting evolution toward decreased AM fungi dependence (Seifert et al. 2009). Such an evolutionary shift could explain our findings for *Robinia* and AM fungi. Furthermore, despite the fact that AM fungal taxa often infect a wide range of plant taxa, the direction and magnitude of the plant responses depend on the combination of plant and fungal species (Klironomos 2003) and the environment in which they interact. In this study, the range of positive and negative plant responses to AM fungi was greater for plants and fungi from the same region than when plants and fungi from different regions were mixed, suggesting, as do our results, that regional evolutionary relationships among plants and AM fungi may contribute to plant species coexistence. However, by only using one *Robinia* genotype from the native range for all tests (although not from a place where soil was collected), the broad regional differences may be affected by local adaptation between the specific genotype of *Robinia* we used and native soil biota. The only option for testing this would be a very large experiment in which seeds from large number of *Robinia* populations in each of the three ranges were grown in all soils from the three regions.

Of course, other biogeographic differences in biota or habitat conditions are also likely to affect *Robinia* invasion. Variation in competitive and allelopathic effects may affect the invasion success of other plants (Callaway and Aschehoug 2000), and *Robinia* extracts have been reported to be allelopathic (Nasir et al. 2005). To our knowledge, however, no biogeographic comparisons of competitive or allelopathic interactions have been explored for *Robinia*. *Robinia* appears to be outcompeted in its native range by late-successional species (Boring and Swank 1984); this may be related to the greater species richness in late-successional forest

communities in North America than in Europe. Also, there are many generalist and specialist consumers that attack *Robinia* in the native range (Hoffard and Anderson 1982, Hargrove 1986). These include the locust borer (*Megacyllene robiniae*), locust leaf miners (*Chalepus dorsalis*, *Parectopa robiniella and Phyllonorycter robiniella*), the locust twig borer (*Ecdytolopha insticiana*), and heart rot (*Fomes rimosus*) that often follows borer damage (Anderson et al. 1981). *Parectopa robiniella and P. robiniella, t*wo monophagous leaf-mining moths, have become widespread in Europe. These consumers and others are likely to have important effects on *Robinia* invasion.

Our results indicate that the soil biota with which *Robinia pseudoacacia* interacts are different in the native, expanded, and invaded ranges of the species, and that these differences may contribute to the success of the species in the expanded North American range and in the invasive range in Europe. The effects of soil biota were complex, and no isolated component that we examined provided much insight into the net effects of soil biota on invasion. However, the net effect of soils from the native range was much more negative than that of soils from the other ranges. These biogeographical differences in soil effects suggest the occurrence of important regional evolutionary relationships among plants and soil biota, and that the biota of soil communities often function to affect invasion as a whole in ways that are not easily explained through reductionists approaches of individual soil components.

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APPENDIX A

Sampling locations for soils (*Ecological Archives* E092-086-A1).

APPENDIX B

Biomass and nodule results for each soil/treatment combination in feedback experiments (*Ecological Archives* E092-086-A2).