

Article

The Sign and Strength of Plant-Soil Feedback for the Invasive Shrub, *Lonicera maackii*, Varies in Different Soils

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Abstract: Plants alter soil characteristics causing changes in their subsequent growth resulting in positive or negative feedback on both their own fitness and that of other plants. In a greenhouse study, we investigated whether the sign and strength of feedback changed across two distinct soil types, and whether effects were due to shifts in biotic or abiotic soil traits. Using soils from two different locations, we examined growth of the exotic invasive shrub, *Lonicera maackii* and the related native shrub, *Diervilla lonicera*, in unconditioned soils and in soils conditioned by previous growth of *L. maackii*, *D. lonicera*, and *Fraxinus pennsylvanica*. In a sandy acidic soil, *L. maackii* showed positive feedback in unsterilized soils, but its growth decreased and positive feedback became negative with sterilization in this soil. In a loamy circumneutral soil, *L. maackii* displayed neutral to negative feedback in unsterilized soils, but sterilization significantly increased growth in all conditioning treatments and caused feedback to become strongly negative. Native *D. lonicera* displayed negative feedback in unsterilized soil of both the sandy and loamy types, but sterilization either eliminated or reversed feedback relationships. Soil conditioning by *L. maackii* and *F. pennsylvanica* had very similar feedbacks on *L. maackii* and *D. lonicera*. While some abiotic soil traits varied across soil types and were affected by conditioning, soil biota sensitive to sterilization were apparently important mediators of both positive and negative feedback effects.

Keywords: *Lonicera maackii*; *Diervilla lonicera*; plant-soil feedback; invasion; soil type; soil microbes; Biolog[®]; phenolics

1. Introduction

Changes in soil properties are an increasingly recognized impact of invasive species that may leave lasting effects in the soil [1–7]. During plant growth, the soil rhizosphere develops characteristics, such as changes in pH, mineral composition, or microbial community composition, that can have effects that feed back on the plant's own fitness, and on that of other species [1,3,8–10]. The sign and strength of plant-soil feedback can affect plant distributions, patterns of dominance, succession and invasion [1,9–12].

Plant growth is highly regulated by soil organisms, both beneficial and pathogenic, and many plant-soil feedback effects are due to alterations in microbial interactions. In their native ranges, invasive plants are often suppressed by soil organisms such as parasitic fungi and nematodes, but in introduced habitats, they may not be exposed to these same natural enemies [3,13]. Plants in introduced habitats may also profit from “enhanced mutualisms”; beneficial microbial allies or mutualists that they have not encountered in their native regions [13,14]. Plants can also affect the presence of nutrient cycling microbes which in turn can create positive feedback mediated by effects on nutrient cycling [3,15–18]. The tendency for plant soil feedbacks to be positive in introduced habitats (*versus* negative in native habitats) may be partly responsible for nonnative plants becoming invasive in introduced areas [4,19,20].

Most plants produce secondary metabolites of various classes, including phenolics, which may have a role in plant-soil feedbacks. Phenolic compounds, as a group, are known to play a role in plant-soil interactions through allelopathic effects on plants and soil microbes, as well as through effects on soil pH and mineral availability [4,18,21–23]. A species in its introduced range may use such biochemical weapons to inhibit neighbors directly or disturb other ecosystem properties giving the invader some advantage. These same weapons may be ineffective against neighbors in its native range, which are adapted to cope with such allelochemicals [22–28]. Phenolic-containing extracts of the invasive shrub, *Lonicera maackii*, for example, can inhibit seed germination, delay plant development, and inhibit plant responses to enhanced nutrient availability [18,24–26].

Studies of plant-soil interactions in the context of plant invasions are increasing but many studies focus on single mechanisms [4]. Holistic examinations of plant-soil interactions would provide much better understanding of how invasive species may alter plant and microbial communities. Invasive plants may have the ability to influence their surroundings differently from native competitors, ultimately leading to changes in community structure which may affect ecosystem processes. Knowledge of nutrient availability, microbial community structure, and soil chemistry in different soil types can provide insight on how exotic invasion occurs. Knowing the influence of soil type (structure and texture) may be helpful in determining why certain areas are more vulnerable to invasion than others. Soil texture (balance of sand, silt and clay) and structure (size and shape of particles and how they aggregate) can determine the rate of water flow and nutrients through the system and can contribute to soil community dynamics which may affect the invasion process [15,27].

Lonicera maackii (Rupr.) Maxim (Amur honeysuckle: Caprifoliaceae), hereafter referred to as *Lonicera*, is a nonnative invasive deciduous shrub found throughout much of the midwestern and Eastern United States. This shrub negatively affects individual plants and plant communities through such mechanisms as competition for light and soil resources and allelopathy [17,18,26,28–30]. The

potential role of plant-soil feedbacks in its invasive success has never been examined. We conducted a greenhouse study to determine the extent to which growth of *Lonicera*, the related native shrub, *Diervilla lonicera*, and the widespread native tree, *Fraxinus pennsylvanica*, had positive or negative feedbacks on their own fitness and how their growth affected fitness of the other species. In addition, we investigated whether the sign and strength of the feedback changed across two distinct soil types, and whether effects were due to shifts in biotic or abiotic soil traits by using soil sterilization. We hypothesized that *Lonicera* would experience more positive feedback in its own conditioned soils than native species would in their own conditioned soils, but that a sandy-acidic soil type would dampen positive feedback effects compared to loamy-circumneutral soils that were expected to have higher nutrient levels and organic matter. We also expected that each species would generally experience more negative feedback in its own soil than in either unconditioned soil or soil conditioned by other species. *Lonicera* would likely cause bigger changes than other species in soil chemistry and microbial profiles due to its known allelochemical effects. This effect was expected to be less pronounced in sandy soils than in loamy soils, possibly due to less organic material to bind allelochemicals resulting in less negative feedback for plants grown in *Lonicera*-conditioned soils.

2. Methods

2.1. Soil Sources

The study took place at Wright State University in the laboratory and greenhouse. Experiments were conducted in two types of soil, both collected in June 2010, from microsites uninhabited by any of the experimental species. We collected bulk soil samples from multiple locations in the Wright State Woods in Dayton, Ohio and in Shawnee State Park in West Portsmouth, Ohio and pooled them by location for use during the conditioning stage. Soil textures were determined using the Pipette method [31]. Soils collected in the Wright State Woods, located at the southern edge of Ohio's glaciated region, were of a circumneutral loam with limestone bedrock, and are hereafter referred to the *loamy* soil. This part of Ohio, and this forest, has been invaded by *Lonicera* for several decades. Soils collected from Shawnee State Park, located in the Western Allegheny Plateau, were of an acidic sandy loam, and are hereafter referred to as the *sandy* soil. Soils there are derived from weathered sandstone, siltstone, and shale sedimentary rock [32,33]. This part of Ohio is as yet relatively uninhabited by *Lonicera*.

2.2. Plant Species

In addition to *Lonicera*, we used *Diervilla lonicera* P. Mill. (Caprifoliaceae), hereafter referred to as *Diervilla*, the native northern bush honeysuckle, as a test species because it is closely related to *Lonicera* and occupies similar habitats [34]. Related species may have similar evolutionary traits that contribute to their behavior and distribution patterns whereas unrelated species may have different characteristics. Its distribution patterns are similar to those of the invasive *Lonicera*, but with a greater shade tolerance, and it is less abundant where it is found [30]. We also used *Fraxinus pennsylvanica* Marsh. (Oleaceae), hereafter referred to as *Fraxinus*, green ash, a deciduous tree native throughout the United States and Canada east of the Rocky Mountains. This species was chosen to represent a

mid-successional species whose local distribution overlaps the geographic ranges and habitat of both *Lonicera* and *Diervilla*. It tolerates soil pH from 5.0 to 8.1 [30].

2.3. Soil Conditioning

The soil conditioning phase ran from August, 2010 through March, 2011. First year *Fraxinus* seedlings were collected from a naturally growing population at Kiser Lake State Park in Conover, Ohio, in July 2010. *Lonicera* and *Diervilla* seedlings used for the conditioning phase were grown from seed and were 12 weeks old. All plants used for soil conditioning were first grown in sterilized ProMix BX potting mix without mycorrhizae (Premier Horticulture Inc., Quakertown, PA, USA) in 1 L pots. Plants were grown in a temperature-controlled greenhouse under ambient light supplemented with fluorescent lights between 0700 and 2100. On July 27 2010, we transplanted several individuals of each species in plastic tubs of each field soil type. We also maintained an unconditioned soil control that contained no plants. Field soils were first mixed to ensure homogeneity and sand (QUIKRETE™ washed, screened and dried play sand) was mixed into soils in each tub (1:5, sand to soil) to inhibit compaction. Each tub contained 25 L of soil/sand mixture into which 10–15 of each species were planted, matched to achieve similar biomass across species. Plants were grown in the greenhouse for 6 months and all tubs (including unconditioned/unplanted control) were rotated weekly to ensure even light exposure and watered as needed with deionized water. No fertilizer was added to the tubs.

2.4. Feedback Experiment

At the end of the conditioning phase, plants used for soil conditioning were removed from soils, the soils mixed within the tubs and then half of each soil was removed and sterilized by fractional sterilization (Tyndallization). Sterilizing soil is a way to study microbial effects as it destroys bacteria and fungi without greatly changing chemical or physical properties of the soil [35]. The moist soil was heated in an autoclave to 100 °C for 1 h on 3 successive days. Three repetitions were needed to trigger heat-resistant spores to germinate and subsequently be destroyed in the next stages. This lower temperature sterilization technique preserves soil structure and quality better than autoclaving at 121 °C [36]. We then transferred sterile soil to ethanol-sterilized containers and the cooled soil was used immediately in the feedback experiment. Successful sterilization was confirmed by culturing soil extracts from unsterilized and sterilized soils from both locations. Soil dilutions were prepared in sterile saline and the lowest dilutions (1:10,000) plated on Tryptic Soy Agar plates (2 reps). Cultures were incubated at room temperature for 72 h and examined for microbial growth [35]. Microbial growth was absent on plates inoculated with extracts from sterilized soils, and abundant on plates inoculated with extracts from non-sterile soils of both soil types. Several unidentified bacterial and fungal species were noted on plates containing extracts from unsterilized soils.

In January 2011, *Lonicera* seeds were surface sterilized by soaking in a 10% chlorine bleach solution for 10 min and rinsed with autoclaved water and then germinated in petri dishes on Whatman No. 2 filter paper in an incubator at 24 °C, using 100 mg/L concentration of gibberellic acid to hasten sluggish germination rates [37]. *Diervilla* seeds were purchased from Gardens North (wild collected in Canada), Annapolis Royal, NS, Canada, and germinated in petri dishes in an incubator at 24 °C on autoclaved sand moistened with autoclaved deionized water, per supplier's recommendation. In

February, 2011, we planted all viable germinated seeds of both species in 300mL propagation cell packs, contained in self-watering trays, purchased from BFG, using sterilized ProMix BX potting mix without mycorrhizae (Premier Horticulture Inc., Quakertown, PA, USA). Plants were maintained in a temperature-controlled greenhouse under ambient light supplemented with fluorescent lights between 0700 and 2100. The response of *Fraxinus* was not examined in the feedback experiment.

On 27 March 2011, we removed seedlings of each species from cell packs and disposed of any loose potting mix. We planted seedlings in 0.5L pots in each possible combination of soil type, soil conditioning treatment and sterilization treatment. There were 8 replicates of each treatment combination, totaling 256 pots across both response species (2 soil types \times 2 sterilization treatments \times 4 conditioning treatments \times 8 replicates). Plants were haphazardly assigned a location on tables in the greenhouse and rotated biweekly to minimize microclimatic effects. Height and basal stem diameter (BSD) were measured at the start of the experiment and biweekly thereafter. All plants were harvested after 12 weeks of growth (June, 2011), separated from soils by rinsing under running water until roots were clean, and dried at 60 °C for 48 h before weighing roots and shoots individually. Total biomass was calculated as the sum of root and shoot biomass. Root and shoot biomass, root/shoot ratios, height, and BSD of each species were compared among soil types, conditioning treatments, sterilization treatments and their interactions with three-way ANOVA. Means within conditioning, sterilization and soil types were compared using Tukey's honestly significant difference tests. The effects of the same factors on changes in height and BSD were analyzed with repeated measures MANOVA. Correlations between all end-of-season measures (total dry biomass, root and shoot biomass, root/shoot ratios, height, and BSD) were made using Pearson correlations (results not shown). Since root, shoot, and total biomass were highly correlated, only effects on total biomass are presented. Likewise, basal stem diameter (BSD) was significantly correlated with height so we only discuss effects on height here. These statistical analyses were performed using SAS (Version 9.2).

2.5. Soil Chemical Properties

In order to determine how each species affected nutrient content and other soil attributes, we collected several soil samples from within each conditioning tub at the end of the conditioning stage for each soil type. Soil samples were pooled within a tub, yielding a 225 g sample per conditioning treatment in each soil type. Analyses for pH, organic matter, total N, NH₄, NO₃, available P, exchangeable K, Mg, and Ca, Cation Exchange Capacity (CEC), and percent base saturation, were performed on pooled soil samples by Spectrum Analytic, Washington Court House, Ohio.

In order to examine how putative allelochemicals varied among soil types and conditioning treatments, we quantified total soluble phenolic concentrations of soil, modified from Scharfy [38]. We made soil extracts by adding 5 mL of 50% ethanol to 1 g of fresh soil and placed them on a shaker at 200 rpm for 1 h. Samples were then centrifuged at 10,000 rpm for 5 min and the supernatant retained. We diluted a 3-mL aliquot of this extract with 2 mL of autoclaved deionized water and added 100 μ L Folin-Ciocalteu-reagent followed by 300 μ L of 2 M Na₂CO₃ after 8 min. Phenolics producing absorbance at 760 nm were detected in a microplate reader after 1 h. A standard curve for phenolics was prepared with gallic acid. Results of the soil analyses for N, P, K, and pH and total phenolics were

not analyzed statistically because of the absence of biological replication, but patterns among soil types and conditioning treatments are discussed.

2.6. Community Level Physiological Profiles (CLPP) Using Biolog[®] EcoPlate[™]

The Biolog[®] EcoPlate[™] is used by microbial ecologists to analyze microbial community footprints over time and is a good tool for analyzing changes in response to soil conditioning. The EcoPlates[™] were designed for the ecological study of whole microbial communities rather than indentifying individual species or strains. The EcoPlates[™] contain 31 carbon substrates (with 3 technical replicates each) and allow measurement of substrate utilization by microbial communities. Microorganisms utilize the substrates causing changes in the color formation of the tetrazolium dye that can be followed in microplate reader [39].

After conditioning, we collected soil samples (10 g dry weight, approximated from moist soil) from each tub of soil, first by taking core samples randomly from the tub, mixing them thoroughly and weighing the required amount. Samples were kept on ice and shaken for 60 min in 20 mL of a 10 mM Bis-Tris (C₄H₁₁NO₃) solution (pH 7) and allowed to settle for 30 min. We decanted the extracts immediately. We first made serial dilutions and then added 100 µL of the 1:1000 diluted solution to each microplate well and incubated it at 22 °C. Substrate utilization was monitored by measuring light absorbance at 590 nm. Measurements were made immediately following inoculation and at 12h intervals for 6 days during March, 2011. EcoPlates[™] were held in an incubator maintained at 23 °C between readings. We accounted for background absorbance by subtracting the absorbance of the least utilized substrate, which varied by conditioning treatment, to prevent negative values [40]. The corrected absorbance values were then used to calculate the average well color development (AWCD), which was 0.42. The time point chosen for analysis (60 h) was based on the reading that exhibited the same mean as the AWCD, which best represents the optimal incubation time based on substrate utilization [39]. Community level physiological profiles at 60 h were analyzed by Principal Component Analysis (PCA) in R (V. 2.14.1). Data were log-transformed to improve normality. Community average metabolic response (AMR) depicts the average respiration of carbon substrates. AMR of conditioned soils was calculated by averaging the mean difference between the absorbance value of the substrate wells and the control well (value of the least used substrate). Community functional richness (CFR) reflects the number of substrates that the culturable microbial community can metabolize from the EcoPlate[™], and is calculated by summing the total number of positive responses after incubation. A positive response was established based on observed purple coloration of the wells. The threshold was set at an absorbance of 0.1. Both AMR and CFR were graphed as a function of incubation time.

3. Results

3.1. Effects of Soil Type and Conditioning on Soil Properties

Conditioned soils of both types were analyzed for pH, nutrient and phenolic levels, and other properties, but were not compared statistically because only one pooled sample per conditioning treatment per soil type was analyzed, but several general patterns were observed in the data. The loamy

soil generally had a higher pH, more organic matter, and greater cation-exchange capacity than sandy soil (Table 1). The loamy soil treatments had greater nutrient availability than the sandy soil treatments after conditioning, though the sandy soil had higher levels of Mg than loamy soil. Interestingly, P levels, albeit low to start, did not vary in sandy soil treatments yet decreased by at least 20% with conditioning in the loamy soil. Calcium/magnesium ratios were twice as high in loamy soil compared to sandy soil. Soil phenolic concentrations tended to be higher overall in loamy soil than in sandy soil (Table 1).

Conditioning also appeared to impact pH levels. In both soils, conditioning by all three species tended to result in a higher pH than that of unconditioned soils, with a minimum increase of 0.4 in loamy soil and a minimum increase of 0.8 in sandy soil (Table 1). Conditioning also appeared to influence nutrient levels. For instance, K and P levels in the loamy soil decreased with conditioning when compared to unconditioned soils. Calcium increased with conditioning in the loamy soil, akin to the increase in soil pH. In sandy soils, *Fraxinus*-conditioned soil had the most K and *Diervilla*-conditioned and *Lonicera*-conditioned soils had lower K than unconditioned soil. NH₄ and NO₃ levels varied widely with conditioning treatments. Ca:Mg tended to increase with conditioning in both soils, but more so in loamy soil. The trend seen in phenolics was different among conditioning treatments. The highest phenolic level was in loamy, *Diervilla*-conditioned soil and the lowest was in sandy, *Fraxinus*-conditioned soil. Phenolic levels seemed to be highest in *Diervilla*-conditioned soil in both soil types. In loamy soil, *Fraxinus*-conditioned soil had the second highest phenolic level (Table 1).

Table 1. Effects of soil conditioning by three different plant species in two soil types on soil properties. Several plants of each species were first grown in containers of each soil type for six months to condition soil, while an unconditioned soil for each soil type was maintained in the same manner. Conditioning treatments: DL = *Diervilla lonicera*; FP = *Fraxinus pennsylvanica*; LM = *Lonicera maackii*; UN = unconditioned.

Soil variables	Loamy soil				Sandy soil			
	DL	FP	LM	UN	DL	FP	LM	UN
pH	7.9	7.8	8.0	7.4	7.0	6.9	7.1	6.1
Organic matter (%)	1.3	1.8	1.5	1.9	1.3	1.6	1.2	1.4
Total N (%)	0.2	0.55	0.14	0.21	0.37	0.29	0.24	0.40
NH ₄ (ppm)	20	8	7	1	7	9	4	8
NO ₃ (ppm)	7	9	8	43	5	48	6	65
Available P (ppm)	37	34	30	46	4	3	3	3
Exchangeable K (ppm)	104	103	84	163	44	64	48	62
Exchangeable Mg (ppm)	285	286	258	368	205	257	252	192
Exchangeable Ca (ppm)	3451	3549	3779	3010	1163	1559	1535	1010
CEC	15.3	15.6	16.2	14.3	6.9	9.2	8.5	5.3
K (% BS)	1.5	1.4	1.1	2.4	1.4	1.5	1.2	2.5
Mg (% BS)	13.7	13.4	11.6	18.8	21.9	20.5	21.8	26.4
Ca (% BS)	84.8	85.2	87.2	78.7	63.6	63.5	67.9	71.1
Phenolics (mg g ⁻¹ soil)	0.206	0.180	0.158	0.173	0.181	0.125	0.165	0.126

3.2. Effects of Soil Type and Conditioning on Biomass of *Lonicera*

Soil type had a significant effect on biomass of *Lonicera*, but its effect depended upon conditioning treatment (Table 2, Figure 1a). For example, in unsterilized loamy soils, biomass of *Lonicera* plants was similar or lower in conditioned soils, relative to unconditioned soils, while in unsterilized sandy soils, biomass of *Lonicera* was generally higher in conditioned soils than in unconditioned soils. There was a highly significant interactive effect between soil type and sterilization (Table 2, Figure 1a). Sterilizing soils significantly increased total biomass of *Lonicera* across all conditioning treatments in loamy soils, but had an overall negative effect in sandy soil (Table 2, Figure 1a). Finally, soil conditioning and sterilization had a significant interactive effect on total biomass (Table 2, Figure 1a). Sterilization tended to increase biomass more in unconditioned and in *Diervilla*-conditioned soils than it did in *Lonicera*- and *Fraxinus*-conditioned soils, a pattern seen most clearly in the loamy soil type.

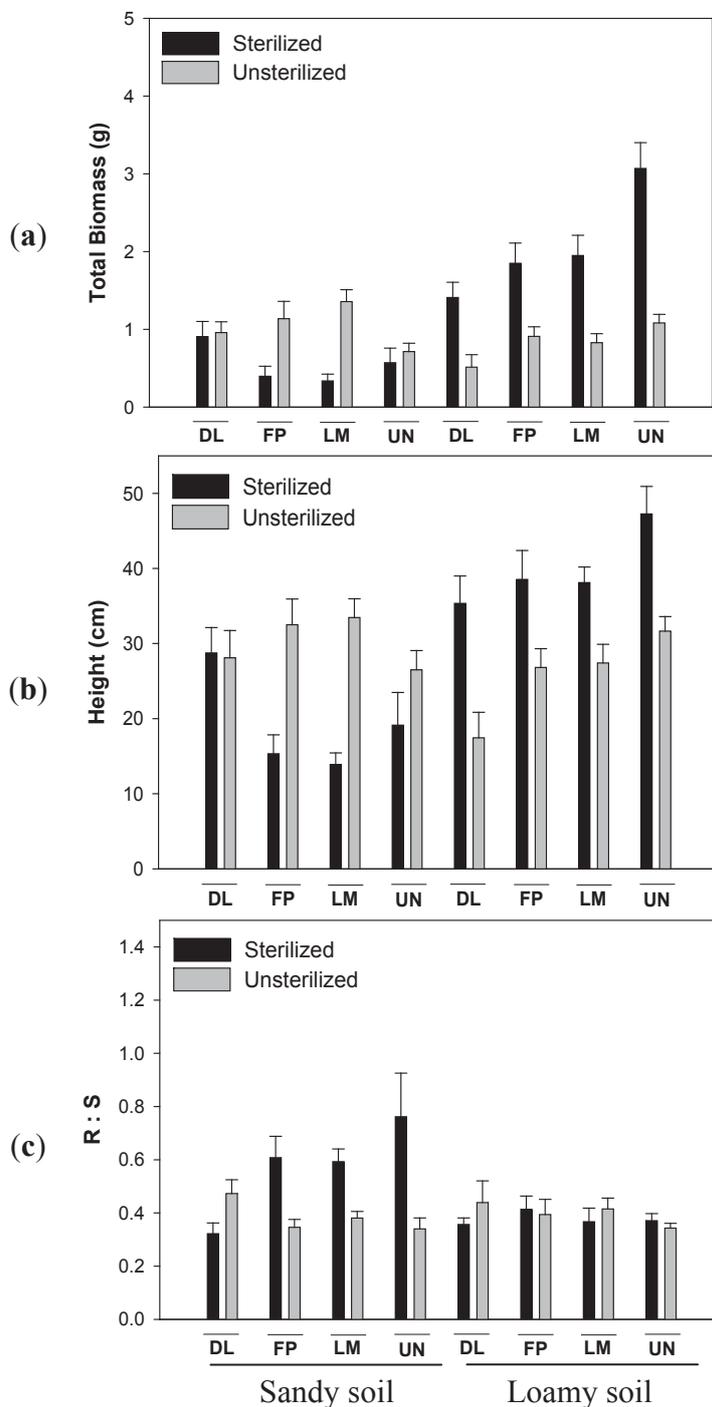
Table 2. Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on total biomass, root/shoot ratio, and height of *Lonicera maackii*.

Factors	df	Total biomass		Height		Root/Shoot	
		F	p	F	p	F	p
Soil Type (T)	1	47.14	<0.0001	23.89	<0.0001	8.65	0.004
Condition (C)	3	1.81	0.1480	0.65	0.5874	0.58	0.6278
<i>T</i> × <i>C</i>	3	8.52	<0.0001	6.19	0.0006	1.45	0.2316
Sterilization (S)	1	6.68	0.0101	0.08	0.7730	7.01	0.0093
<i>T</i> × <i>S</i>	1	87.05	<0.0001	57.86	<0.0001	11.00	0.0012
<i>C</i> × <i>S</i>	3	3.60	0.0150	5.69	<0.0001	5.92	0.0009
<i>T</i> × <i>C</i> × <i>S</i>	3	1.57	0.2000	0.82	0.4873	2.76	0.0457
Error	112						

Lonicera plants were generally taller at the end of the experiment in loamy soil than in sandy soil, across the other treatments (Table 2, Figure 1b). Conditioning had no independent effect, but there was a significant interactive effect between soil type and conditioning (Table 2). The tallest plants grew in unconditioned loamy soil, while the shortest plants grew in sandy soil conditioned by *Fraxinus* and *Lonicera* (Figure 1b). Sterilization had significant interactive effects with soil type on height (Table 2). Sterilization increased height in all loamy soil treatments, but generally decreased height in sandy soil (Figure 1b). There was also an interactive effect between conditioning and sterilization on plant height (Table 2). Sterilization had the most positive effect on height in *Diervilla*-conditioned soils overall, which was also the only conditioning treatment in sandy soil in which sterilization did not decrease height (Figure 1b).

During the experiment, the effects of soil type and sterilization on height varied through time, and sterilization interacted with both soil type and with conditioning (Table 3, data not shown). For example, plants in unconditioned and sterilized loamy soil were as tall as those in other treatments at the start, but had grown to be much taller than the others by day 28. Conversely, height of plants in *Lonicera*-conditioned and in unsterilized loamy soils grew to be shorter than the other treatments through time (Table 3, data not shown).

Figure 1: Mean (+1 SE) (a) total dry biomass; (b) end of season height; and (c) root/shoot ratio of *Lonicera maackii* in response to soil sterilization and soil conditioning by three different species in two soil types. Conditioning treatments: DL = *Diervilla lonicera*; FP = *Fraxinus pennsylvanica*; LM = *Lonicera maackii*; UN = unconditioned.



Root/shoot ratios of *Lonicera* were higher in sandy soil than in loamy soil, across the other treatments (Table 2, Figure 1c), but there were significant interactive effects of soil type and sterilization, conditioning and sterilization, and a significant three way interaction (Table 2). Sterilization increased root/shoot ratios in all conditioning treatments in the sandy soil, with the

exception of a decrease in *Diervilla*-conditioned soils, but in the loamy soil, sterilization did not affect root/shoot ratios across conditioning treatments (Figure 1c).

Table 3. Results of Repeated measures MANOVA with Wilks' lambda test (W) for the effect of time and its interactions with soil type, soil conditioning, and soil sterilization on height of both species.

Factors	df	<i>Lonicera maackii</i>			<i>Diervilla lonicera</i>		
		W	F	<i>p</i>	W	F	<i>p</i>
Time	6	0.047	354.94	<0.0001	0.013	1252.34	<0.0001
Time × Soil Type (T)	6	0.692	7.92	<0.0001	0.701	2.13	0.0054
Time × Condition (C)	6	0.778	1.56	0.0701	0.701	2.13	0.0054
Time × T × C	18	0.816	1.25	0.2201	0.621	2.91	<0.0001
Time × Sterilization (S)	6	0.717	7.01	<0.0001	0.451	20.47	<0.0001
Time × T × S	6	0.666	8.92	<0.0001	0.810	3.94	0.0014
Time × C × S	18	0.729	1.99	0.0101	0.548	3.76	<0.0001
Time × T × C × S	18	0.807	1.32	0.1739	0.831	1.07	0.3819
Error	106						

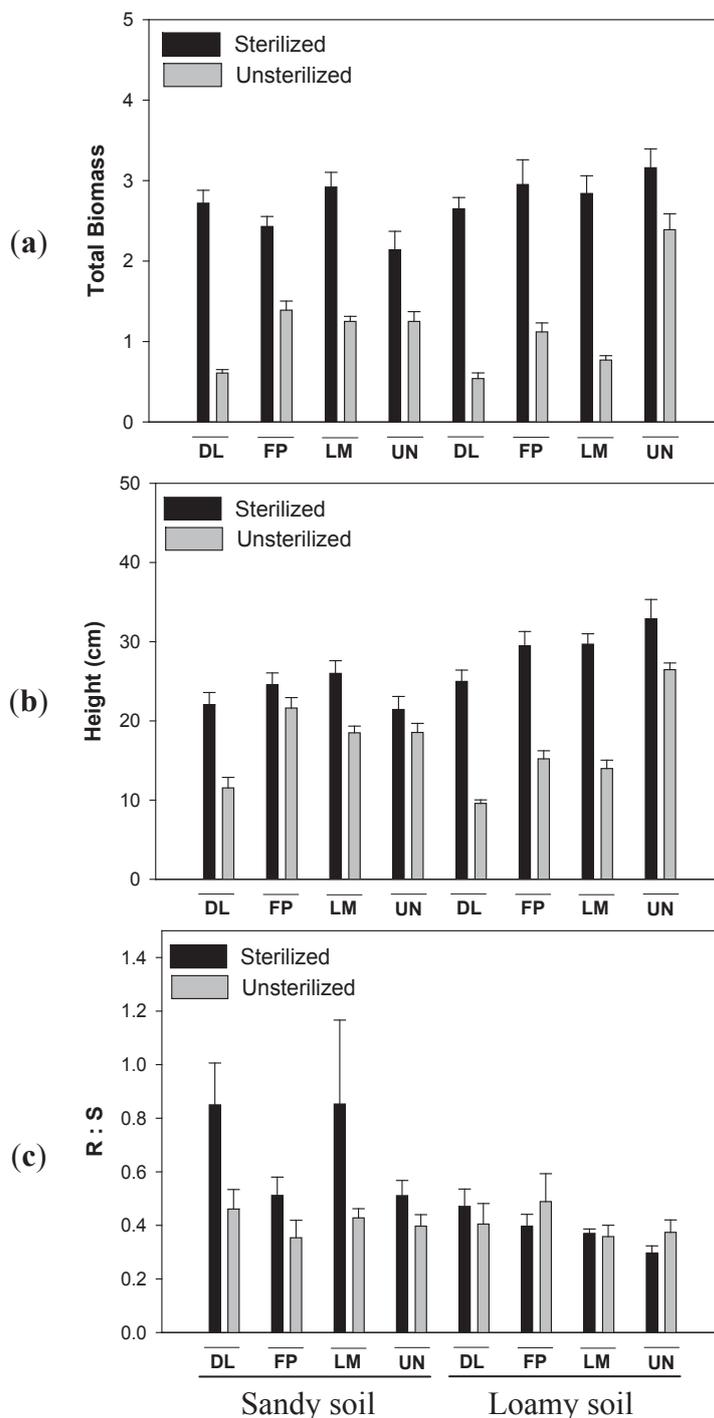
3.3. Effects of Soil Type and Conditioning on Biomass of *Diervilla lonicera*

Soil conditioning had a significant independent effect on total biomass of *Diervilla*, but its effects varied by soil type (Table 4, Figure 2c). Across both soil types, total biomass of *Diervilla* was significantly lower in its own conditioned soil than in all other conditioning treatments. In loamy soil, however, they performed best in the unconditioned treatment, while in sandy soil; they performed best in *Lonicera*-conditioned soils (Figure 2a). Sterilization had a highly significant effect and resulted in increased biomass overall (Table 4, Figure 2a), but its effects varied among conditioning treatments. Sterilization had the most positive impact on biomass of *Diervilla* in its own conditioned soils, and the least positive impact in unconditioned soils (Figure 2a).

Table 4. Results of three-way ANOVA of soil type, soil conditioning, and soil sterilization on total biomass, root/shoot ratio, and height of *Diervilla lonicera*.

Factors	df	Total biomass		Height		Root/Shoot	
		F	<i>p</i>	F	<i>p</i>	F	<i>p</i>
Soil Type (T)	1	2.76	0.0998	2.99	0.0867	10.55	0.0016
Condition (C)	3	15.73	<0.0001	32.69	<0.0001	2.05	0.1112
T × C	3	15.58	<0.0001	13.74	<0.0001	1.52	0.2127
Sterilization (S)	1	426.27	<0.0001	222.11	<0.0001	6.59	0.0117
T × S	1	3.02	0.0854	26.75	<0.0001	9.01	0.0033
C × S	3	20.87	<0.0001	16.52	<0.0001	1.18	0.3197
T × C × S	3	2.53	0.0614	2.59	0.0564	0.04	0.9881
Error	106						

Figure 2: Mean (+ 1SE) (a) Total dry biomass; (b) end of season height; and (c) root/shoot ratio of *Diervilla lonicera* in response to soil sterilization and soil conditioning by three different species in two soil types. Conditioning treatments: DL = *Diervilla lonicera*; FP = *Fraxinus pennsylvanica*; LM = *Lonicera maackii*; UN = unconditioned.



Conditioning alone had a significant effect on height, but its effects varied among soil types (Table 4). *Diervilla* plants generally grew tallest in unconditioned soils and shortest in *Diervilla*-conditioned soils, and grew taller in unconditioned loamy soil than in unconditioned sandy soil (Figure 2b). Sterilizing soils significantly increased plant height overall, but plant heights were increased by sterilization more strongly in loamy soil than in sandy soil (Table 4, Figure 2b). There was a significant three way

interaction between sterilization, soil type, and conditioning (Table 4). For example, plants were taller in unsterilized and conditioned soils of the sandy type than unsterilized and conditioned soils of the loamy type. However, sterilization increased height more strongly in loamy soil than in sandy soil, and had the most positive effect on height in *Diervilla*-conditioned soil.

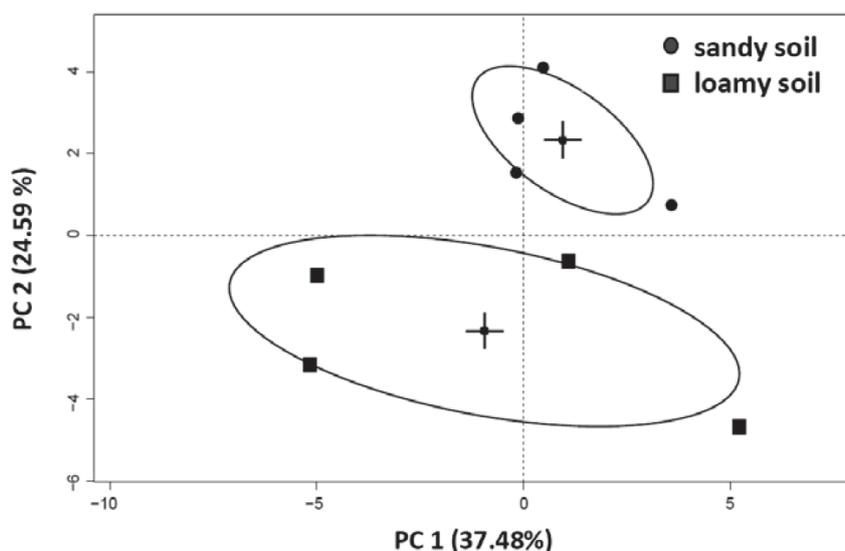
Changes in the height of *Diervilla* through time significantly varied with every other factor, the exception being the four way interaction (Table 3, data not shown). For instance, plants in sterilized and unconditioned soils of the loamy type were indistinguishable from other treatments at the start, but surpassed height of plants in all other treatments on day 70 and were tallest at harvest. Plants in unsterilized, loamy, *Diervilla*-conditioned soils grew the least in height throughout the experiment.

Root/shoot ratio of *Diervilla* was significantly higher in sandy soil than in loamy soil across the other treatments (Table 3, Figure 2c). Sterilization significantly impacted root/shoot ratios, but its effects varied with soil type. Root/shoot ratios were generally positively impacted by sterilization in sandy soils, but not in loamy soils (Table 3, Figure 2c).

3.4. Effects of Soil Type and Conditioning on the Soil Microbial Community

Principal component analysis (PCA) of Ecoplate™ data revealed no major differences in microbial functional community composition among conditioning treatments, but it revealed significant variation in community composition based on soil type. The first two principal components explained 63% of the variation in Ecoplate™ data (PC1: 37.48%, PC2: 24.59%) (Figure 3).

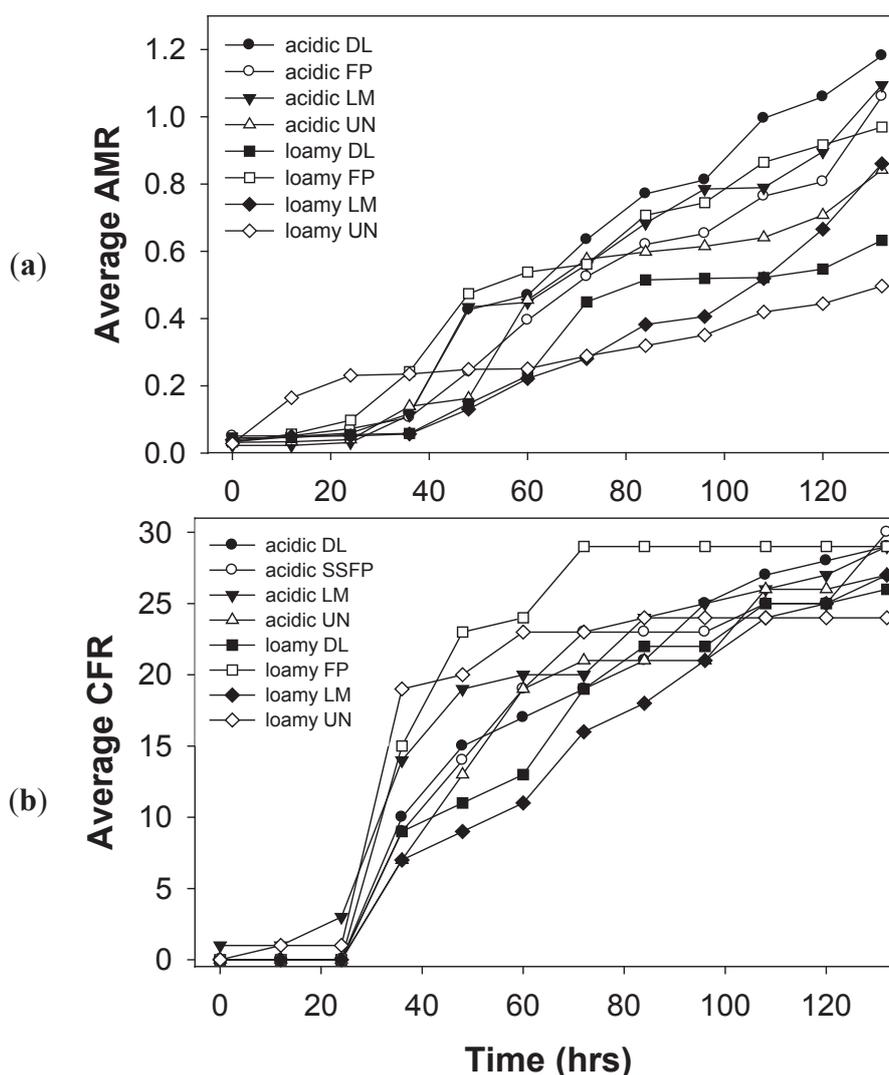
Figure 3. Principal components (PC 1 and PC 2) of the microbial communities cultivated in two different soil types either conditioned by the growth of three species or unconditioned, with 95% CI. Percent of explained variance is in parentheses. The optimal AWCD chosen for analysis was 0.42.



Visual inspection of cultures from the acidic and loamy soil revealed clear differences in the culturable bacterial and fungal community, but the identities of these microbes were not determined. Average metabolic response (AMR) was higher overall in sandy soils and highest in *Diervilla*-conditioned soils. Unconditioned loamy soil was the first to show a metabolic response (substrate utilization indicated by

development of tetrazolium dye); however it had the lowest substrate utilization at the end of incubation compared to all other treatments (Figure 4a). Interestingly, unconditioned sandy soil and *Lonicera*-conditioned loamy soil had nearly the same final metabolic response, though their patterns over time differed considerably (Figure 4a). Community functional richness (CFR) was generally higher in sandy soils. *Fraxinus*-conditioned soils cultivated the highest community functional richness in both soil types. *Diervilla*-conditioned sandy soils, *Lonicera*-conditioned sandy soils and *Fraxinus*-conditioned loamy soils had similar functional richness levels at the end of incubation, but differed in their development over time. *Fraxinus*-conditioned loamy soils maintained the highest functional richness from 48 h through 120 h, only to be surpassed at the last observation by *Fraxinus*-conditioned sandy soils. Unconditioned loamy soils cultivated the lowest community functional richness (Figure 4b).

Figure 4: Average Metabolic Response (AMR) (a) and Community Functional Richness (CFR); (b) of the soil microbial community in two soil types either conditioned with growth by three different species or unconditioned. Soil type: acidic or loamy. Conditioning treatments: DL = *Diervilla lonicera*; FP = *Fraxinus pennsylvanica*; LM = *Lonicera maackii*; UN=unconditioned.



4. Conclusions

4.1. Effects of Soil Type and Sterilization on Plant-Soil Feedbacks

Our results indicate that both soil type and soil biota had important influences on the sign and strength plant-soil feedbacks. Plants often display more negative feedback in their native soils and more positive feedback in nonnative soils [1,9,13,14]. In accordance with predictions, *Lonicera* indeed showed positive feedback in unsterilized sandy soil from its invasive range, growing almost twice as much in its own soil *versus* unconditioned soil. However, feedback was neutral to slightly negative for *Lonicera* in unsterilized loamy soils from its invasive range. Thus, variation in soil physical, chemical and/or biological properties found even within the invasive range altered the sign and strength of plant-soil feedbacks in this study. Our findings indicate that growth of *Diervilla lonicera*, a native species related to *Lonicera*, was generally limited by negative feedbacks in soil from its native range. This plant displayed especially strong negative feedbacks in response to conditioning with itself and any other species in unsterilized loamy soils, and significant negative feedback on itself in sandy soils. These findings are consistent with other research on native species in native soils [12] and indicate that local soil biota may be a key factor limiting the growth and abundance of a native plant species [1,13]. Importantly, effects of soil conditioning detected in this study were caused almost exclusively by root- and rhizosphere-associated effects, as plant litter and throughfall from leaves were generally not present in the study. Leaves and/or leaf extracts, along with throughfall, have been shown to contribute to allelopathic or soil nutrient effects reported for *Lonicera* and other species [17,25,41], and may contribute to variation in the sign and strength of plant-soil feedbacks in the field along with root-associated effects.

Soil biota affected by sterilization was an apparently large contributor to plant-soil feedbacks [9]. In loamy soil, *Lonicera* showed neutral to negative plant-soil feedbacks, but it responded very positively to soil sterilization. The loamy soil was from a location where *Lonicera* has been invasive for several decades, and the microbial community that it cultures from this soil currently has a net neutral to inhibitory effect on its growth. In the sandy soil, however, sterilization reversed the positive plant-soil feedback that *Lonicera* displayed in unsterilized soils, indicating that it had benefitted from soil microbes that it cultured in this soil type that were destroyed by sterilization. In addition to varying from the loamy soil in texture and other quality parameters, this soil type was collected from a location as yet relatively uninvaded by *Lonicera* and soils there are relatively naive to its presence. Sterilization generally eliminated evidence of negative feedback for *Diervilla* in both soil types, indicating that sterilization released *Diervilla* from inhibitory soil microbes that it generally cultures. There were no obvious shifts in microbial community functional composition caused by conditioning that were detected in the PCA; however, there was variation in functional composition based on soil type. Community level physiological profiles showed that sandy soils contained microbial communities with higher average metabolic response and community functional richness than loamy soils, but EcoplateTM results should be interpreted with caution because they do not fully represent species diversity or richness. Others have found that invasive plants initially benefit from the soil biota in nonnative regions, but over time the soil microbial community becomes inhibitory [14]. Indeed, Kardol *et al.* [42] found that mid-successional plant species (like *Lonicera*) typically display neutral feedback. Reinhart and Callaway [14] found

increased benefit from mutualisms when invasives have escaped natural enemies. *Lonicera* may have experienced positive feedback in unsterilized sandy soils by culturing a microbial community with a net positive effect (perhaps due to the presence of mutualists); the opposite being true in the loamy soil from a region with a history of *Lonicera* invasion. Thus, both soil characteristics and invasion history may contribute to variation in soil feedbacks seen across environments.

While sterilization improved growth of *Lonicera* in the loamy soil overall, it caused feedback to become even more negative. This pattern was made clearer because *Lonicera* plants grown in unconditioned loamy soils responded so positively to sterilization. This suggests that root-associated phytochemicals may somehow limit the growth of *Lonicera* in conditioned, but sterilized soils; an effect that the presence of a live microbial community may dampen. On the other hand, nutrient limitation caused by plant conditioning may be particularly noticeable in sterilized soils. Although abiotic soil attributes, such as nitrate, calcium, magnesium concentrations and pH were seemingly affected by conditioning, the much larger effect of sterilization *versus* just conditioning alone indicate that biotic factors had the most important impacts on growth of plants in this study. However, variation in biotic attributes of the soil can also affect abiotic traits, like nutrient status [43], thus it can be difficult to disentangle the two effects. We did not compare changes in soil abiotic and biotic attributes between sterilized and unsterilized soils during the testing phase of the experiment. It is important to mention that sterilization can release nutrients into the soil and it is often controlled for by fertilization [43]. However, because we observed different responses to sterilization in *Lonicera* and *Diervilla*, effects of this treatment are unlikely to be due to simple nutrient release caused by sterilization, which would likely affect most species positively. By using lower sterilization temperature, we attempted to minimize nutrient and phenolic conversion effects, and we confirmed that sterilization effectively eliminated microbes by culturing soil extracts.

4.2. Effects of Soil Type and Sterilization on Root/Shoot Ratios

Low root/shoot ratio is a trait associated with many invasive plants [15], which can also respond to plant-soil feedbacks [44]. Despite these assertions, *Lonicera* and *Diervilla* had generally similar root/shoot ratios, which were largely unaffected by conditioning in unsterilized soils. Sterilization, however, increased root/shoot ratios of both *Lonicera* and *Diervilla* in sandy soils, with the exception of *Lonicera* in *Diervilla*-conditioned soils, but sterilization had little effect in loamy soils. *Lonicera* is known to display plasticity in resource allocation [45,46], and exotic invasives can be more plastic than native species when not limited by resources [47]. Here, *Lonicera* and *Diervilla* both allocated more resources to root biomass in sterilized sandy soils where sterilization presumably destroyed beneficial microbes. Increased allocation to root biomass in sterilized sandy soils may have been particularly important for both species because of the poorer soil nutrient profile in the sandy soil type.

4.3. Conspecific versus Heterospecific Feedbacks and Allelopathy

We expected more growth of species in soil conditioned by other species than in their own conditioned soil. Our findings support this for *Diervilla*, which grew significantly smaller in its own conditioned soil than in other conditioning treatments across both soil types. *Lonicera* promoted its own growth in one soil type but not in the other, but feedback effects on itself were largely similar to

effects caused by heterospecifics. While *Lonicera* is not prevalent in the sandy area at present time, this positive feedback implies that it will promote its own success in this area if given a chance. Allelopathic compounds are known to cause changes in microbial communities and vice versa [9,19,22], both allelochemicals and microbes have effects on nutrient cycling [3,15] and these interactions can affect ecosystem feedbacks and species composition [1,3,9,13,14,44]. We expected *Lonicera* to cause large changes in soil chemistry and microbial profiles due to its documented allelopathic effects [17,18,26], and to have significant heterospecific impacts. It had a large heterospecific impact on *Diervilla* in unsterilized loamy soils, but no effect on *Diervilla* in unsterilized sandy soils (where *Diervilla* exhibited negative feedback on itself). Several soil quality traits, such as nutrient levels, were apparently affected by conditioning which were likely due to nutrient uptake and losses to biomass production of the plants used to conditioned soils. Changes in such quality traits could contribute to feedback effects. However, soil phenolic levels were actually lower in *Lonicera*-conditioned soils than in unconditioned sandy soils, and they were higher overall in loamy soils. Thus, total phenolic levels in soils do not seem to correlate with *Lonicera*'s heterospecific impacts, but this measure is crude and *Lonicera* contains other putatively bioactive compounds in other compound classes, such as iridoids [48]. Different soil microorganisms degrade or magnify allelochemicals differently, so microbial metabolism of allelochemicals and other soil inputs from plants may explain differences seen between soil types or conditioning treatments [19]. Though it has not been well studied, some phenolic compounds are known to be oxidized by high heat [49] so the net effect of allelopathy may not be as observable with sterilization for some species. Interestingly, unsterilized *Diervilla*-conditioned soil generally had the highest total phenolics in both soil types and generally produced the most negative conspecific or heterospecific feedback effects. Potential autotoxic or allelopathic effects have never been studied for this plant, but it appears to hold the potential to be allelopathic, and phenolics may play a role in that effect. It is related to *Lonicera*, however, so it likely displays similarities in other bioactive compound classes too.

Interestingly, conditioning by *Lonicera* and *Fraxinus* had nearly identical effects on both *Lonicera* and *Diervilla* in both soil types in both sterilization treatments. This indicates that these two unrelated species modify soils in ways with very similar consequences for subsequent plant growth. It is known that some aspects of their secondary chemistry are similar [18,50]. In fact, *Fraxinus* is invasive in Hungary where research showed evidence of reduced germination rates and growth of white mustard (*Sinapis alba* L.) caused by green ash extracts [51]. However, similarities in the microbial community cultured by these two species could also be responsible for their similar heterospecific impacts. Using multiple plant species to first condition soil allows us to make predictions about patterns of invasion based on current ecosystem composition. It appears that *Lonicera* has no different effects than a widespread and unrelated tree, but its responses to soil type, conditioning and sterilization clearly vary from even a native relative.

5. Future Research

In this study, we attempted to link changes in soil chemistry and microbial communities to feedback effects of different plant species in two contrasting soil types. Our results indicate that both soil type and soil microorganisms play a large role in plant-soil feedback, and that *Lonicera maackii*, an

important invasive shrub, indeed can experience positive feedback in some soils, but negative feedback in others. Because there were so many significant interactions in this study with soil type, it is important that studies consider accounting for variation due to soil attributes. Conducting studies for adequate growth periods and taking measurements throughout the study is also critical, as we noted significant differences in effects on growth through time. For instance, if we had stopped midway through our experiment, when the height of *Lonicera* in sterilized, unconditioned loamy soils was similar to that in other loamy soil treatments, we would have failed to detect the significant effect of sterilization that was evident at the end of the experiment. A more thorough assessment of soil chemistry as well as variation in soil microbial communities and their responses to plant growth using molecular techniques would also enhance our understanding of mechanisms driving microbially-mediated plant-soil feedbacks.

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Conflict of Interest

The authors declare no conflict of interest

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