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Root Exudates Regulate Soil Fungal Community Composition and Diversity▽

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Plants are in constant contact with a community of soil biota that contains fungi ranging from pathogenic to symbiotic. A few studies have demonstrated a critical role of chemical communication in establishing highly specialized relationships, but the general role for root exudates in structuring the soil fungal community is poorly described. This study demonstrates that two model plant species (*Arabidopsis thaliana* and *Medicago truncatula*) are able to maintain resident soil fungal populations but unable to maintain nonresident soil fungal populations. This is mediated largely through root exudates: the effects of adding in vitro-generated root exudates to the soil fungal community were qualitatively and quantitatively similar to the results observed for plants grown in those same soils. This effect is observed for total fungal biomass, phylotype diversity, and overall community similarity to the starting community. Nonresident plants and root exudates influenced the fungal community by both positively and negatively impacting the relative abundance of individual phylotypes. A net increase in fungal biomass was observed when nonresident root exudates were added to resident plant treatments, suggesting that increases in specific carbon substrates and/or signaling compounds support an increased soil fungal population load. This study establishes root exudates as a mechanism through which a plant is able to regulate soil fungal community composition.

Interactions between plants and soil microbes are highly dynamic in nature and based on coevolutionary pressures (6, 7, 10, 15, 16, 20, 21, 29). Thus, it is not surprising that rhizosphere microbial communities differ between plant species (2, 13, 26, 34), between genotypes within species (17), and between different developmental stages of a given plant (22, 33). At a community scale, microbial diversity in the soil has been linked to plant diversity, though it is unclear whether this is through increased habitat heterogeneity, the increased plant biomass commonly observed with highly diverse plant communities, or increased diversity of carbon substrates and signaling compounds provided by the plants (36).

Fungi are heterotrophic organisms, depending on exogenous carbon for growth, and different clades of fungi utilize different carbon substrates. Plant root exudates contain simple carbon substrates, including primary metabolites, such as sugars, amino acids, and organic acids, in addition to a diverse array of secondary metabolites that are released into the rhizosphere and surrounding soil (14).

Previous research suggests that secondary metabolites in root exudates are critical in specialized associations between plants and individual species of soil microbes ranging from mutualistic to pathogenic. For example, *Rhizobium* spp. are bacterial symbionts of legumes that are responsible for nitrogen fixation, and communication between the two organisms is mediated in part through root-secreted flavones (28). Saponin ginsenosides from American ginseng stimulate the growth of two specialist soilborne fungal pathogens, although these same compounds are identified as possessing general antifungal properties (24). A sesquiterpene from *Lotus japonicus* induces hyphal branching in an arbuscular mycorrhizal fungus, which is thought to be essential for the establishment of a symbiotic relationship between the two species (1). Many studies on the role of plant secondary metabolites or root exudates analyze only a specific group of fungal species (often mycorrhizal fungi or pathogenic fungi), making interpretation of the results challenging in the context of soil fungal biodiversity. A detailed analysis of the effects of novel plant species and the role of their root exudates in the soil fungal community is a necessary step in the investigation of how plants regulate biological diversity in the soil and the nature of the interactions between plants and intact fungal communities.

The purposes of this study were to (i) examine the effect of a novel plant species on an existing soil fungal community and (ii) determine the relative importance of root exudates in structuring the soil fungal community. The results of these experiments demonstrate that root exudates play a heretofore underappreciated role in shaping the soil fungal community.

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MATERIALS AND METHODS

Experiment 1. Experiment 1 compared the effects of resident and nonresident plants, or their root exudates, on the soil fungal communities from various natural soils. The experiment consisted of a factorial design ($n = 10$) with two plant species (*Arabidopsis thaliana* Col-0 and *Medicago truncatula* 'Jemalong') and three natural soils obtained from Illinois, Texas, and Oregon. This design results in two conditions in which a soil supports its resident plant species or its

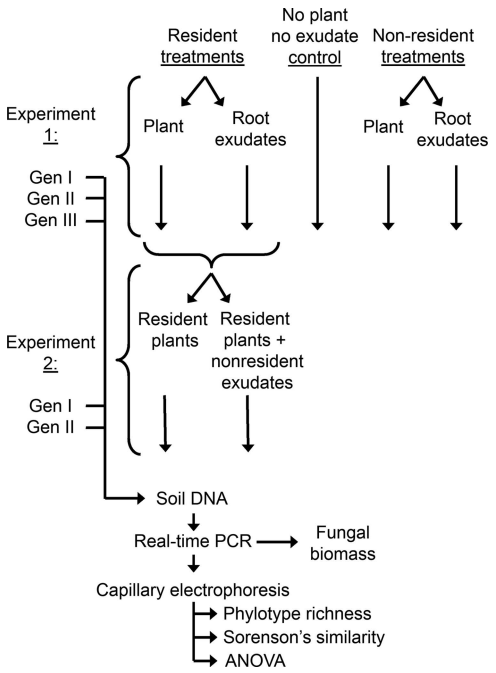

FIG. 1. Schematic representation of the experimental design. Two experiments were performed; experiment 1 consisted of four treatments (resident plant or exudates and nonresident plant or exudates) and one control, which received neither plants nor exudates. Experiment 1 was conducted for three generations (Gen I to III). Resident treatments included *A. thaliana* in Illinois soil and *M. truncatula* in Texas soil. Nonresident treatments included *M. truncatula* in Illinois soil, *A. thaliana* in Texas soil, or either plant species in Oregon soil. Experiment 2 was conducted on a subset of the pots from experiment 1 following the completion of experiment 1. Those pots in experiment 1 that received resident plant or resident exudate treatments were treated for an additional two generations with resident plants or resident plants supplemented with nonresident exudates. Soil samples were taken after each generation, and DNA was isolated from soil. DNA was subjected to real-time PCR with internal transcribed spacer primers to quantify fungal biomass, and amplified products were subjected to length heterogeneity analysis by capillary electrophoresis to estimate differences in phylotype richness, community similarity, and relative abundance via ANOVA.

root exudates (*Arabidopsis thaliana* in Illinois soil and *Medicago truncatula* in Texas soil), four conditions in which a soil supports a nonresident plant species or its root exudates (*A. thaliana* in Texas or Oregon soil and *M. truncatula* in Illinois or Oregon soil), and three control conditions (no plants or exudates in Illinois, Texas, or Oregon soil) (Fig. 1). The use of soils from diverse locations increases confidence in the results, as *Medicago truncatula* has very likely not grown recently in the *Arabidopsis thaliana* soil. Further, the properties of the soils are quite different from each other (see below for descriptions), demonstrating that the results observed are a general phenomenon.

The three field-collected soils (see below) were shipped to Fort Collins, CO, air dried, cleaned of plant debris, and thoroughly homogenized. Six (5-g) subsamples were randomly selected from each soil type to establish the pretreatment (generation 0) fungal community composition. The remaining soil was transferred to 9- by 9- by 12-cm pots, the bottoms of which were lined with Whatman 3MM Chr paper to prevent soil particle loss. The pots were then well watered as necessary (two to three times weekly) for 3 to 4 weeks, during which time the soil's existing seed bank seedlings were continuously removed. After the bulk of seedling emergence had passed, each experimental treatment was applied to 10 replicate pots. Pots were maintained in a greenhouse under ambient conditions and watered as needed. At the conclusion of each generation (approximately 10 weeks after germination or the time at which *M. truncatula* plants began flowering), soil from each pot was sampled and analyzed for fungal community composition and total C and N contents, as described below. Any plants (particularly *A. thaliana*) that had begun flowering prior to the 10-week termination point were clipped of their inflorescence to extend the generation time. The aerial portions of the plant were harvested at the end of each generation, the tissue was dried for 3 days at 70°C, and the dry weight biomass was recorded. Plant biomass values are not reported here, as seasonal changes confound the interpretation of generational or fungal community effects. A 2- to 3-week dormancy period (no watering) was applied between each generation to allow the root systems of previous plants to die. The first generation was seeded in July 2005 and harvested in October 2005, the second seeded in late October 2005 and harvested in January 2006, and the third seeded in February 2006 and harvested in June 2006.

Experiment 2. To examine whether the observed changes in soil fungal communities in experiment 1 were due to the loss of the resident plants/exudates or the addition of the nonresident plants/exudates, a second experiment was conducted (Fig. 1). For this experiment, only those pots that had been treated with resident plants or exudates were used. Resident plants ($n = 10$) were grown in either Illinois or Texas soil and amended with the root exudates of the nonresident plant. Controls ($n = 10$) consisted of resident plants only. All other conditions were the same as in experiment 1. The experiment was carried out for a total of two generations. The first generation was seeded in July 2006 and harvested in October 2006, and the second was seeded in late November 2006 and harvested in February 2007.

Description of soils. The Texas soil was collected in 2005 from under *M. truncatula* plants that were seeded at that location in 2000. Loose litter and plant material were removed from the site, and the top 4 inches of soil were collected and shipped to Fort Collins, CO. The soil was collected in 2005 at 28° 36′ 31.59″ N, 97° 39′ 21.43″ W, elevation 322 feet. Other common species growing nearby included burr medic (*Medicago polymorpha*), Bermuda grass (*Cynodon* sp.), huisache (*Acacia* sp.), mesquite (*Prosopis* sp.), and live oak (*Quercus virginiana*) (William Ocumpaugh, Texas Agricultural Experiment Station, Beeville, TX, personal communication). The Texas soil contained 3.38% total carbon and 0.32% total nitrogen before treatment.

The Illinois soil was collected from under *A. thaliana* plants. The soil was collected in 2005 at 42° 05′ 34″ N, 86° 21′ 19″ W, elevation 630 feet. Common co-occurring species included chickweed (*Stellaria media*), mouse-ear chickweed (*Cerastium vulgatum*), vetch species (*Vicia* spp.), sand bittercress (*Cardamine parviflora*), shepherd's purse (*Capsella bursa-pastoris*), houstonia (*Houstonia* spp.), purple dead nettle (*Lamium purpureum*), whitlow grass (*Draba verna*), small-flowered cranesbill (*Geranium pusillum*), dandelion (*Taraxacum officinale*), asters (*Aster* spp.), curly dock (*Rumex crispus*), field peppergrass (*Lepidium campestre*), bedstraw (*Galium* spp.), hop clover (*Trifolium agrarium*), and field violet (*Viola arvensis*) (Joy Bergelson, Joel Kniskern, and Megan Dunning, University of Chicago, IL, personal communication). The Illinois soil contained 2.21% total carbon and 0.19% total nitrogen before treatment.

The Oregon soil was collected in 2005 from Trask Mountain, at 45° 26′ 29″ N, 123° 20′ 39″ W, elevation 2,000 ft. Co-occurring species included an overstory of coastal Douglas fir (*Pseudotsuga menziesii*) and Western hemlock (*Tsuga heterophylla*) and an understory consisting of salal (*Gaultheria shallon*), Oregon grape (*Mahonia aquifolium*), and sword fern (*Polystichum munitum*) (Rick Kelsey, USDA Forest Service, personal communication). The Oregon soil contained 5.13% total carbon and 0.21% total nitrogen before treatment.

Plant material. *Arabidopsis thaliana* Col-0 and *Medicago truncatula* 'Jemalong' were used as the resident or nonresident plant species for this study. *A. thaliana* seeds were surface sterilized for 5 min in 20% bleach solution, rinsed four times with sterile deionized water, and germinated directly in premoistened experimental soils. *M. truncatula* seeds were scarified in sulfuric acid for 5 min, rinsed until the rinsate pH measured >5.5, surface sterilized for 10 min in 20% bleach solution, rinsed four times with sterile deionized water, and directly seeded to premoistened experimental soils.

Root exudate preparation. Plant root exudates were generated under sterile conditions in vitro. *A. thaliana* and *M. truncatula* seeds were surface sterilized as described above and plated on MS agar plates (0.8% agar, 1% sucrose; Caisson Laboratories), and the seedlings transferred to liquid MS medium (MS, 3% sucrose, pH 5.8) and allowed to grow for 2 weeks. After 2 weeks, the MS medium was removed, and the plants were rinsed with Hoagland's medium (a carbon-free and low-nitrogen medium, pH 5.8) and grown for 3 days in Hoagland's medium. After 3 days, the Hoagland's medium containing the exudates from each species was collected and held at −20°C until application. This medium was replaced with MS medium, in which the plants were grown for 4 days. After this time, the procedure was repeated, replacing MS medium with Hoagland's medium for exudate collection. This method was used to allow for rapid growth under laboratory conditions while preventing the application of excess carbon/nitrogen to the soil when root exudates were used as treatments. Though the exudate

profiles of soil-grown plants are likely to be different than those of in vitro-grown plants, attempts were made to minimize this effect by using a number of plants for in vitro exudate generation that was equivalent to that used under greenhouse conditions. The exudate solution was then divided equally (typically, 2.0- to 3.0-ml amounts) among the replicates. Exudates were applied weekly, and watering was scheduled such that water was not applied to the pots within 24 h of exudate application, to prevent immediate exudate leaching through the soil.

Soil sampling. The top 2.0 cm of soil within a 0.7-cm radius around the crown of the plant was collected by using a cork borer sterilized with 20% bleach and rinsed with deionized water. This sampling procedure was used to allow for multigenerational sampling without great soil disturbance between generations. Soil samples were transferred to scintillation vials and stored at −20°C until processing.

Fungal community analysis. Soil samples (500 mg) were subjected to DNA extraction using an UltraClean soil DNA kit (Mo Bio, Carlsbad, CA) according to the manufacturer's instructions, except for the addition of one extra wash with ethanol to remove excess humic acid. The soil DNA was quantified by using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE) and diluted with distilled water to a concentration of 20 ng μl^{-1}.

The fungal DNA in the soil samples was amplified by using fungus-specific primers (ITS1-F and ITS4) previously described (9, 19, 35). The quantity of total fungal DNA in the sample was determined by measuring Sybr green fluorescence (iCycler iQ; Bio-Rad, Hercules, CA) using an external standard curve generated from serial dilutions of fungal DNA obtained from five different fungal species (*Fusarium equiseti*, *Alternaria solani*, *Verticillium* sp., *Rhizoctonia solani*, and *Scletorina sclerotium*). All PCR mixtures contained 5 μl (100 ng) soil DNA, 10 μl 2× jumpstart reaction mix (Sigma, St. Louis, MO), 2.4 μl 25 mM $MgCl_2$, 0.2 μl 1 μM fluorescein, and 0.4 μl 10 μM forward and reverse primers and were brought to 20 μl with distilled water. The PCR products were amplified for 32 cycles (at 95°C for 30 s, 55°C for 30 s, and 72°C for 60 s).

Length heterogeneity analysis of PCR amplicons was achieved by capillary electrophoresis (ABI Prism 310; Applied Biosystems) by means of a 5′ 6-carboxyfluorescein-labeled ITS1-F primer. Briefly, PCR samples from the previously described amplification reactions were diluted with 80 μl of distilled water and 2 μl was added to 13 μl of a loading buffer (12 μl formamide, 0.5 μl 0.3 M NaOH, and 0.5 μl Genescan 2500 [6-carboxytetramethylrhodamine] size standard). The analysis conditions were as follows: Genescan POP4 polymer, 15-s 15-kV injection, and 60-min 10-kV electrophoresis. Scoring of amplicons into unique 2-bp bins (or fungal phylotypes) was performed by using Genemapper software (version 4). For each sample, the phylotype richness was determined as the number of unique fragments. The phylotype abundance was determined by measuring the capillary electrophoretic peak heights of individual phylotypes. Fragments with a peak height below 50 relative fluorescent units were not included in the analysis.

The PCR step of this method selectively amplifies internal transcribed spacer sequences from Ascomycetes, Basidiomycetes, and Zygomycetes. It does not amplify sequences from Oomycetes (19), bacteria, or plants (D. K. Manter, unpublished data). Its template heterogeneity prevents its use as an absolutely quantitative measure, but it is reliable in determining the relative abundances of phylotypes, as the amplicon quantity reflects the abundance of template in the original sample (19). During analysis by electrophoresis, one electrophoretic peak may represent one or more phylotypes. Fragment length heterogeneity analysis likely underestimates the phylotype diversity (19).

Soil carbon and nitrogen analysis. The remaining soil samples not used for DNA extraction were dried at 70°C, and samples for C:N analysis were generated by pooling two to three replicates for each treatment and generating three replicates for C:N analysis. Dried soil (200 mg) was analyzed for total carbon and nitrogen using a LECO CHN1000 analyzer.

Statistical analysis. All analyses were conducted with SAS 9.1 using the mixed model procedure. Significant changes in the measured fungal community characteristics over time (generations) were analyzed by using a repeated-measure analysis of variance (ANOVA), with soil a fixed effect, generation a repeated measure, and individual pots serving as the subjects. Since pretreatment (generation 0) samples were not available for all pots, the average pretreatment value of the six pretreatment subsamples was used. P values of <0.05 are considered significant for all tests. The community characteristics tested included total fungal abundance (quantitative PCR), phylotype richness (i.e., number of fragments), individual phylotype abundance (peak heights for each unique fragment), and Sorenson's similarity indices. Sorenson's similarity indices were calculated for each individual pot in PC-ORD 5.0.

RESULTS

The experiments were designed to determine how the introduction of novel plant species to a soil would influence the soil fungal community previously established under the influence of other plant species. They also examined the role of root exudates in this process. To test these effects, a greenhouse experiment was designed using two noninvasive "model" plant species in a variety of natural soils to monitor the dynamics of change in the soil fungal population. Natural soils originally supporting *Arabidopsis thaliana* (Illinois soil) and *Medicago truncatula* (Texas soil) communities for several generations were collected, along with a third soil (Oregon forest soil) that had not experienced the growth of either *A. thaliana* or *M. truncatula*. Either *A. thaliana* or *M. truncatula* was grown in (or their exudates were applied to) these soils for several plant generations. In this design, *A. thaliana* in Illinois soil and *M. truncatula* in Texas soil are considered "resident" treatments, while *A. thaliana* in Texas soil, *M. truncatula* in Illinois soil, or either species in Oregon soil are "nonresident" treatments (Fig. 1). Control pots receive neither plants nor exudates.

Real-time PCR analysis of soil DNA demonstrated that fungal biomass levels remained elevated through three generations when a resident plant species was present and that fungal biomass levels fell rapidly when a nonresident plant species or no plant was present (Fig. 2A). These effects were most apparent after two or more generations and were consistent for all three soil types: fungal biomass remained elevated only when a plant species that is a resident of that soil was present. However, even in resident treatments, fungal biomass decreased by the third generation.

Many plant secondary metabolites are known to possess antifungal activity, and fungal species have been demonstrated to respond to different plant primary and secondary metabolites that may function as carbon substrates and/or growth-modifying signals. Root exudates may serve as a selective agent through which a plant is able to regulate the fungal community in the surrounding rhizosphere. To test the role of root exudates in structuring the soil fungal community, in vitro-grown root exudates from *A. thaliana* and *M. truncatula* were applied to each of the three soils described above. Real-time PCR analysis of the soil fungal community revealed results similar to those obtained with whole plants: nonresident plant root exudates failed to support the native soil fungal community to the extent observed for resident plant root exudates (Fig. 2A).

These results suggest two nonexclusive scenarios: (i) there is a net positive effect of resident plants or exudates on the biomass of soil fungi, or (ii) there is a net negative effect of nonresident plants or exudates on the biomass of soil fungi. To distinguish between the two, a second experiment was initiated (Fig. 1). This experiment was performed by using soils from experiment 1 that had been treated with resident plants or exudates and following them for two additional generations. In this experiment, resident plants were grown in all pots and half of the pots were supplemented with nonresident exudates. Using this design, one might expect to observe one of three alternative results: (i) the fungal biomass would be expected to accelerate its rate of decline if fungal-growth-inhibiting substances are present in the nonnative root exudates, (ii) the fungal biomass would maintain a comparable rate of decrease

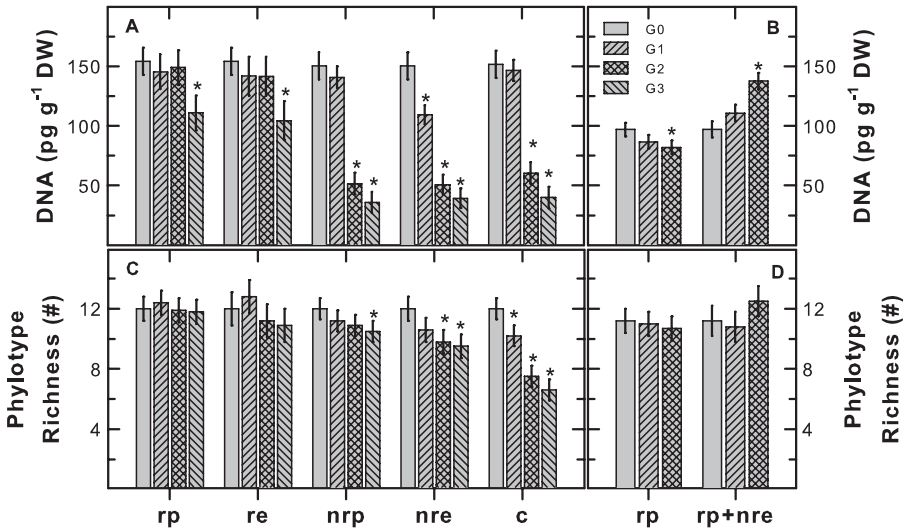

FIG. 2. Change in soil fungal community characteristics in response to *A. thaliana* and *M. truncatula* plants or their exudates. Total fungal DNA estimated from quantitative PCR for experiment 1 (A) and experiment 2 (B). Average phylotype richness for experiment 1 (C) and experiment 2 (D). Treatment codes: c, no plant/exudate control; rp and re, resident plants and resident exudates (*A. thaliana* in Illinois soil and *M. truncatula* in Texas soil); nrp and nre, nonresident plants and nonresident exudates (*A. thaliana* in Illinois or Oregon soil and *M. truncatula* in Texas or Oregon soil). DW, dry weight; G0, pretreatment generation; G1, G2, G3, posttreatment generations 1 to 3. Phylotype richness is based on peak counts following length heterogeneity analysis. Error bars show standard errors of the means. An asterisk indicates a significant difference ($P < 0.05$) from the pretreatment value.

if the sole factor driving the results shown in Fig. 2A was the presence or absence of resident plants and their exudates, or (iii) the fungal biomass would be expected to increase if carbon substrate (metabolite) diversity is shaping the community. Resident plant treatments continued the trend as observed for the first three generations, with slightly decreasing fungal biomass over time (Fig. 2B). However, when resident plants were grown and supplemented with nonresident root exudates, the soil fungal biomass increased significantly (Fig. 2B).

Biomass is only one measure of the soil fungal community and not necessarily representative of community complexity. To gain further insight into the community structure, amplified PCR products from biomass measures were analyzed via capillary electrophoresis to gauge community diversity, as measured by phylotype richness. On average, the pretreatment soils had phylotype richnesses of 17, 14, and 11 (phylotype richnesses of Oregon, Illinois, and Texas soils, respectively). Nonresident plants and root exudates failed to support the diversity of the fungal community as much as resident plants and exudates (Fig. 2C). The examination of phylotype diversity in experiment 2, in which pots were treated with resident plants or resident plants supplemented with nonresident exudates, reveals that fungal phylotype richness tended to increase, though not significantly so (Fig. 2D), when nonresident exudates were applied.

Phylotype richness reports the number of taxa in the soil sample but provides no information on the quantitative trends for individual taxa. To better understand how the community changed quantitatively in response to the treatments, the Sorensen's similarity index was calculated, comparing each sample to generation zero. This index takes into account not only phylotype presence/absence but changes in relative abundance, as measured by capillary electrophoretic peak height, and can respond to both increases and decreases of individual phylotypes. Resident plants and exudates maintained a more-similar fungal community than did nonresident plants and exudates or controls (Fig. 3A). The second experiment revealed that the community similarity rapidly decreased if nonresident exudates were applied in addition to resident plants (Fig. 3B), indicating that the addition of nonresident exudates has a dramatic effect on fungal community structure.

Community measures mask the response of individual fungal phylotypes to the treatments. To dissect these treatment effects for individual phylotypes, each phylotype was analyzed with ANOVA, and the number of significant increases or decreases is plotted in Fig. 4. Treatment with resident plants or exudates resulted in, on average, two phylotypes which decreased in abundance and two phylotypes which increased in abundance between generations zero and three (Fig. 4A). In contrast, treatment with nonresident plants reduces the abundance of ~10 phylotypes while increasing the abundance of 7. Likewise, nonresident exudates reduced the abundance of eight phylotypes while increasing the abundance of six (Fig. 4A). Supplementing resident plants with nonresident exudates resulted in three phylotypes that decreased in abundance and seven phylotypes that increased in abundance (Fig. 4B).

Soil fungi are heterotrophic and, thus, dependent on soil- or plant-derived carbon and/or nitrogen for growth. To ensure that the observed effects were not simply due to altered soil carbon or nitrogen, total soil carbon and nitrogen was quantified on soil remaining from soil samples collected for DNA extraction. The results of this analysis indicate that there was no effect of any treatment on total soil carbon, total soil nitrogen, or the soil carbon-to-nitrogen ratio (data not shown), indicating that the plant species' specific effects on the soil fungal population are due to the specific composition of roots

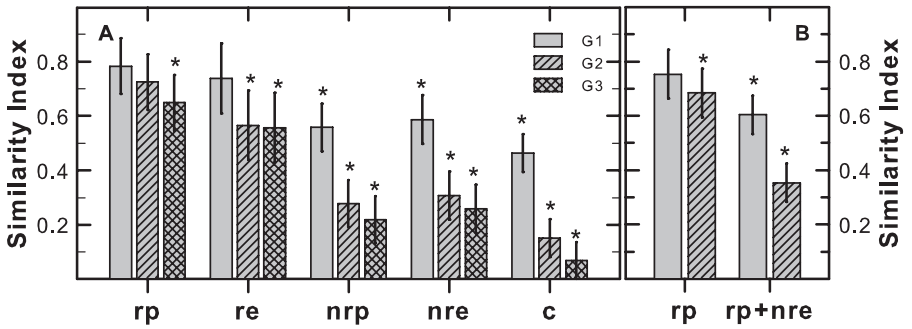

FIG. 3. Similarities (Sorenson's similarity index) of soil fungal communities exposed to *A. thaliana* and *M. truncatula* plants or their exudates to the pretreatment community. (A) Results for experiment 1. (B) Results for experiment 2. Treatment codes below the figure are defined in the Fig. 2 legend. Error bars show standard errors of the means. An asterisk indicates a significant difference ($P < 0.05$) from the pretreatment value.

and their exudates. High-performance liquid chromatography-mass spectrometry profiling revealed dramatic qualitative differences between components of *A. thaliana* and *M. truncatula* root exudates (data not shown).

DISCUSSION

Plants are highly dependent on the soil microbial community, and the relationships between plants and microbes are often highly specific and mediated through chemical communication, as demonstrated for legume/rhizobial symbioses (4, 25, 28, 31, 37). Further, microbes are highly dependent on plants for carbon substrates for growth. This interdependence suggests that selective forces would favor tight regulation of the relationships between them, and numerous examples of fungal species responding to chemical signals from a particular plant species have been documented (1, 24, 27). At the community level, the response of the fungal community to a plant is more poorly documented, with many studies targeting specific taxa or functional categories of fungi, such as mycorrhizae. Likewise, root exudates are often implicated in regulating fungal community structure and abundance (13), but convincing scientific data detailing the role of root exudates are sparse (14).

In an effort to clarify the role of plants and root exudates in structuring the soil fungal community, multiple soils were treated with either resident plants or their root exudates, nonresident plants or their root exudates, or no-plant/no-exudate controls. The results of this experiment demonstrate that resident plants, or their root exudates in isolation, are capable of maintaining the soil fungal community to a much greater extent than nonresident plants over three generations and that the effect of plant species was apparent by the second plant generation. These data establish that root exudates of diverse plant species (an arbuscular mycorrhizal and nodulating legume and a nonmycorrhizal Brassicaceae) can influence the composition of soil fungal communities in diverse soil types in the absence of intact root biomass. However, even when resident plants or exudates were applied, soil fungal biomass declined slightly by the third generation. This could be due to depletion of nutrients in the soil other than nitrogen and carbon over multiple generations (13) or the reduction of plant species diversity influencing the fungal community. The observed changes in the soil fungal community may be a transient response toward a new stable community driven by the novel plant species. A similar effect has been observed under field conditions with invasive plant species (2, 29).

The first experiment displaced resident plant species for nonresident plant species, and therefore, confounding variables (loss of one species and gain of a novel species) limit the interpretation of the results. Experiment 2 was designed to distinguish between positive growth regulation by resident plants/exudates and negative growth regulation by nonresident plants/exudates. The data from this experiment indicate that plant root exudates regulate the soil fungal community by two mech-

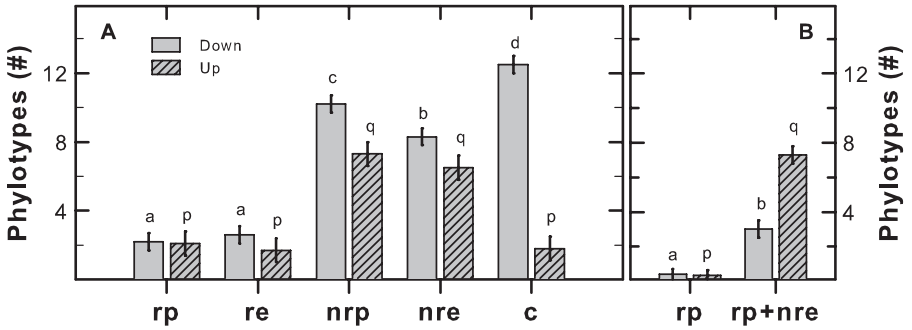

FIG. 4. Number of phylotypes significantly decreasing or increasing between pre- and posttreatment (generation 3) for experiment 1 (A) and experiment 2 (B). Treatment codes below the figure are defined in the Fig. 2 legend. Error bars show standard errors of the means. Means with different letters are significantly different ($P < 0.05$).

anisms that act on specific fungal phylotypes. One mechanism serves to reduce relative abundance through either an antifungal effect of the exudates or a chemical signal that negatively impacts growth, while a second mechanism positively regulates relative abundance, either through growth-inducing chemical signals or the provision of a supply of an appropriate carbon substrate.

Root exudates are estimated to total between 2 and 10 percent of the total fixed carbon for a plant (14). The relatively minor absolute abundance of root exudates compared to total root biomass belies the importance of root exudates in structuring the soil fungal community. Recent evidence suggests that root exudation is an active, ATP-dependent process in *A. thaliana* (18) and soybeans (30). The active nature of root exudation, coupled with our data, suggests that root exudates do not represent a passive loss of carbon to the rhizosphere, as is commonly suggested, but that they play an active role in shaping the soil fungal community. *A. thaliana* is known to accumulate the ubiquitous phenylpropanoids, as well as more phylogenetically restricted glucosinolates, in its roots (3, 32), and many of these compounds and others are found in root exudates (23). *M. truncatula* is known to secrete flavonoids as root exudates (25, 28), and it accumulates numerous triterpene saponins, flavonoids, and isoflavonoids in its root tissue and as exudates from root-derived cell cultures (5, 8, 11, 12). This wealth of secondary metabolites offers ample opportunity for specificity in the response of fungi to plant root exudates, and our liquid chromatography-mass spectrometry profiling results (data not shown) document the qualitative differences between *A. thaliana* and *M. truncatula* exudates. Future studies should focus on identifying both the fungal taxa that are selectively responding and the exudate and/or root components that are responsible for this selection.

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