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REGULAR ARTICLE

Heterogeneity in arbuscular mycorrhizal fungal communities may contribute to inconsistent plant-soil feedback in a Neotropical forest

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Abstract
Background and aims Plant-soil feedback may vary across host species and environmental gradients. The relative importance of these biotic versus abiotic drivers of feedback will determine the stability of plant and microbial communities across environments. If plant hosts are the main driver of soil microbial communities, plant-soil feedback may be stable across changing environments. However, if microbial communities vary with environmental gradients, feedback may also vary, limiting its capacity to predict plant distributions.
Methods We characterized arbuscular mycorrhizal (AM) fungi across tree plantations and a primary Neotropical rainforest. We then performed a plant-soil feedback pot experiment of AM fungi from these plantations on three plant species and related feedback and AM fungal communities in the field.
Results In the field, temporal and spatial variation in AM fungal composition was similar in magnitude to variation across plant host species. Composition of AM fungi in the pot experiment significantly differed from the field plots. Furthermore, differential feedback was explained by shifts in AM fungal composition only for one plant host species (*Hyeronima alchorneoides*) in the pot experiment.
Conclusions Natural AM fungal communities were temporally and spatially heterogeneous and AM fungal communities in the greenhouse did not reflect natural soils. These factors led to heterogeneous and unpredictable feedback responses, which suggests that applying greenhouse derived plant-soil feedback trends to predict plant coexistence in natural systems may be misleading.

Keywords Context-dependency · Environmental variability · Greenhouse · Spatial · Temporal

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Introduction

Plants can cultivate soil abiotic and biotic conditions that in turn affect subsequent plant performance, termed plant-soil feedback (Bever et al. 1997). Despite the prominent role of microorganisms in plant-soil feedback, the drivers of microbial distributions are rarely connected to their feedback effects. However, understanding plant-soil feedback in the broader context of other factors is key to understanding plant and microbial community

trajectories in natural ecosystems. Plant-soil feedback depends on both the host and soil microbial community (Bennett et al. 2017; Teste et al. 2017); therefore when microbial distributions are controlled by abiotic factors (e.g., Chagnon et al. 2013; Coince et al. 2014) more so than by host species (e.g., Klironomos 2002), we might expect feedback to become environmentally context-dependent. This is especially relevant when considering environmental change where plant community trajectories may depend on the resulting shifts in plant-microbe interactions (van der Putten et al. 2013; Classen et al. 2015; van der Putten et al. 2016).

Plant-soil feedback can be the result of relationships with both positive (e.g., mutualists) and negative (e.g., pathogens) microorganisms. Indeed, in a meta-analysis of plant-soil feedback, mostly conducted in temperate ecosystems, the majority of plant-soil feedback was negative (Kulmatiski et al. 2008). Despite the dearth of plant-soil feedback studies from tropical ecosystems, feedback dynamics are likely also important at low latitudes where benign environments create situations where negative biotic interactions are expected to regulate communities more than abiotic filtering (Janzen 1970). Recent work in Panamanian tropical forests supports a role for feedback in these hyper-diverse plant communities: plant-soil feedback was highly positively correlated with the abundance of adult trees (Mangan et al. 2010b). This trend was driven by varying degrees of negative interactions with soil microbiota and in particular arbuscular mycorrhizal (AM) fungi, which can differentially affect the growth of conspecific and heterospecific host plants (Mangan et al. 2010a). Because AM fungal symbiosis with plants can range from parasitism to mutualism (Johnson et al. 1997) and the majority of tropical forest plant species associate with AM-fungi (Averill et al. 2014), AM-fungi can be a causal driver of both positive and negative plant-soil feedback. Therefore, these results are consistent with AM fungi structuring tropical rainforest plant communities. However, the generality of these findings remains unknown. Understanding how feedback relates to the biotic and abiotic drivers of AM fungal distributions can potentially help us extend these findings across the landscape and through time. For instance, in temperate communities, soil resources, particularly phosphorus, are an important driver of AM fungal composition (Johnson 2010) and thus knowing the relationship between phosphorus, AM fungi, and host-AM feedback could help predict low and high resource locations where positive or negative feedback may be more likely (Revillini et al. 2016). In the tropics, however, there is substantial variation in how AM fungi respond to resources (e.g., Treseder and Vitousek 2001; Waring et al. 2016; Schappe et al. 2017) and other abiotic factors (e.g., Guadarrama and Alvarez-Sanchez 1999) and thus drivers of tropical AM fungal communities remain an area of active study.

Predicting plant-AM fungal interactions in the context of environmental and host variation remains challenging. Plant-soil feedback outcomes varied between greenhouse and field experiments based on a meta-analysis of 38 studies (Lekberg et al. 2018), but only four of the studies were done in the field, making it difficult to parse causal mechanisms. In large part this may be due to the frequent disconnect between the greenhouse conditions under which plant-soil feedback experiments are performed and the field conditions where feedback occurs. Greenhouse experiments, by necessity, rarely reflect environmental variation in climate or resources. Furthermore, AM fungal communities in the greenhouse may be a nested subset of true field diversity composed of disturbance-tolerant taxa (Mariadassou et al. 2015) or generalist taxa that thrive in a broad variety of habitats (Barberan et al. 2014). However, the likelihood of greenhouse environmental filtering of AM fungal taxa is unknown because AM fungal composition in greenhouse-based plant-soil feedback studies has never been compared to that of natural environments. When greenhouse experiments either accurately reflect field conditions or when plant-soil feedback is based solely on host species associations that are temporally consistent, plant-soil feedback may predict plant relative abundance in the field (Klironomos 2002; Mangan et al. 2010b). However, feedback to plant seedlings in the greenhouse may not predict plant abundance in field settings when for example, feedback changes over time (e.g., Kardol et al. 2006; Hawkes et al. 2013) or depends on fluctuating resources (Revillini et al. 2016; Van Nuland et al. 2017) or climate (Ren et al. 2015). Another possibility is that host-specific effects on AM fungi are diluted non-additively by diverse plant communities (Kivlin and Hawkes 2011), making it difficult to compare greenhouse experiments to real-world ecosystems with high plant diversity. A greater understanding of what factors control AM fungal distributions in the field and how these fungi relate to plant-soil feedback in the greenhouse will provide a framework for when feedback can influence natural ecosystems.

Here we addressed the biotic and abiotic drivers of AM fungal distributions, their feedback to plants in a Neotropical rainforest, and how the spatial and temporal heterogeneity of AM fungal communities relate to the observed feedback. Given the plant host-specific associations and high spatial and temporal variation of soil-borne saprotrophic fungi (Kivlin and Hawkes 2016a) and bacteria (Kivlin and Hawkes 2016b) at this experimental site, we expected that vegetation type and spatial and temporal heterogeneity would influence AM fungal composition in the field. We hypothesized strong and consistent plant-soil feedback if AM fungal composition in the field plots was largely determined by vegetation type, but weaker or inconsistent feedback if AM fungal composition was heterogeneous across space or time. Finally, we predicted different AM fungal composition and richness in the field versus pot samples given their different environmental conditions and the likelihood that not all AM taxa found in the field would be collected as spores or survive in pots.

Materials and methods

Field experiment

All studies were conducted at La Selva Biological Station, Costa Rica (10°25′53.14”N, 84°0′10.51”W). Mean annual temperature at this site is 25.8 °C while mean annual precipitation is 4142 mm (Sanford Jr. et al. 1994; Clark et al. 2010). Soils and AM fungal communities were collected from monodominant stands of three tree species *Hyeronima alchorneoides* Allemao (Phyllantaceae), *Pentaclethra macroloba* (Willd.) Ktze (Fabaceae), and *Virola koschnyi* Warb. (Myristicaceae), and a mature, primary forest within 150 m. We will refer to the experimental tree plots and primary forests collectively as “vegetation types.” The location of the monodominant stands was slash-burned and planted with *Panicum maxicum* L. and *Melinis minutifolia* Pal. in 1955 and grazed until 1988 when the experiment was established (Fisher 1995). The three tree species were placed in a randomized block design with four blocks and primary forest samples were similarly acquired from four random blocks across the same distance. All blocks and primary forest plots are within 800 m of each other (see Kivlin and Hawkes 2016b for details). Plots within blocks are 0.25 ha in size and were planted with individual trees spaced 3 m apart (Fisher 1995). Soils are derived from andesitic lava flows (Alvarado 1990) and are classified as Mixed Haplic Haploperox (Kleber et al. 2007). Soils are generally acidic (~4 pH) and contain low levels of inorganic phosphorus, base cations, and nitrogen, but relatively high levels of soil organic matter (Russell et al. 2007); however, some of these properties also vary among the experimental tree species (Russell et al. 2010).

To examine AM fungi in the field, we collected soils and roots from five (2.5 cm wide × 10 cm deep) cores in September 2012, February 2013, and September 2013. We used a stratified random sampling design to avoid coring the same exact location over time. The five cores were combined in each plot and within 24 h of collection, roots were separated from soils and frozen at −20 °C for later analysis of field AM fungal communities. Soils were processed for biogeochemical pools as described below.

Feedback pot experiment

For the feedback experiment, we leveraged the vegetation types from the field as a 24-yr conditioning phase to serve as sources of both the AM fungi and soils. Varying different AM inocula addressed how host-specific AM fungal communities affect plant-soil feedback, whereas varying soil sources allowed us to examine the abiotic drivers of plant-soil feedback. The full design included three tree species grown with five AM fungal communities (three tree species, primary forests, and sterile) on three soil origins (from the three tree species) in a fully factorial design. With four replicates per treatment, there were a total of 180 pots. Seeds were collected from the canopy when ripe, stored dry for up to 2 months, and surface sterilized with 1% sodium hypochlorite prior to use. At the same time as the seeds matured, we collected soils from 0 to 15 cm depth separately from each vegetation type in the field. For each soil source, soils were combined across all four blocks of each monodominant tree species and across the four plots in the nearby primary forest plots.

To create the AM fungal treatment inoculum, AM fungal spores were isolated from 1 kg of fresh soil from each plot via sucrose centrifugation (Allen et al. 1979). The supernatant was sieved through a 45-μm sieve to isolate AM fungal spores and discard other saprotrophic fungi and bacteria. Spores isolated from the same vegetation type were combined across blocks for the final AM fungal inoculum treatments. The remaining soils

were used to create the soil treatment: soils were combined by vegetation type, sieved to 4 mm, and sterilized by autoclaving three times at 121 °C for 1 h over three consecutive days. To improve pot drainage, sand (accumulated in a nearby alluvial flat) was similarly sterilized and soil and sand were mixed 1:1. Pots were constructed as follows: (1) ~2.5 L of the sterile soil-sand mix was added to a 2.65 L deep pot (10.2 cm wide × 34.3 cm tall; Stuewe and Sons, Inc., Tangent, OR, USA), (2) spores of AM fungi were added at a uniform density of 1000 spores/pot in 2 ml of sterile water and sterile controls were given the same volume of sterile water, placed approximately 2 cm below the soil surface, (3) one surface-sterilized seed was added to the top of the pot and lightly covered. The pots were also modified with a 20 cm tall clear vinyl ring around the top to prevent cross-pot transfer of AM fungal spores via rain splash.

We focused on matching the AM fungi and soil treatments to seed maturation dates in order to create treatments that seeds would experience. Thus, the timing of each experiment depended on seed maturation: *Pentaclethra* trials began in August 2012 and *Hyeronima* and *Virola* trials began in June 2013. Soil and AM fungal inoculum for each plant species were collected approximately 1 month before the feedback experiments started. All plants were allowed to grow for 6 months in a shadehouse at La Selva Biological station and watered as needed.

At the end of 6 months, we measured plant size as above- and belowground plant biomass. Fungal colonization of roots was quantified by microscopy for aseptate and septate hyphae, arbuscules, and vesicles; roots were cleared in 10% KOH, stained with acid fuchsin, and 100 fields of view were counted at 160× magnification (McGonigle et al. 1990). Extraradical hyphal lengths of both aseptate and septate fungi were determined by extracting hyphae from soil in a 5% sodium hexametaphosphate solution and visualizing under 160× magnification with a gridded reticle (Brundrett et al. 1994). Soils were also analyzed for biogeochemical pools as described below. Roots (~0.75 g) were frozen at −20 °C within 24 h for later examination of AM fungal community composition.

Biogeochemical methods

For both field and pot soils, we extracted ammonium, nitrate, and labile dissolved organic carbon (DOC) in 0.5 M K_2SO_4 using a 1:5 ratio of soil to extractant (Jones and Willett 2006; Makarov et al. 2013). Soluble inorganic phosphorus was extracted by shaking ~10 g of soil with anion resin strips (Membranes International, Ringwood NJ) in 100 ml of water and subsequently extracting the strips in 0.5 M HCl (Lajtha et al. 1999). Ammonium, nitrate, and phosphate concentrations were quantified colorimetrically (D'Angelo et al. 2001; Doane and Horwath 2003). In addition, we measured microbial biomass carbon (MBC) using chloroform fumigation and direct extraction in 0.5 M K_2SO_4 (Scott-Denton et al. 2006). Microbial biomass carbon was quantified by combustion (Apollo 9000 TOC Analyzer, Teledyne Tekmar) and calculated as the difference between the fumigated and unfumigated extractions adjusted for an extraction efficiency of 0.45 (Brookes et al. 1985). Soil moisture was measured for each soil sample by drying soils at 105 °C to constant weight. A 2-g subsample of soils was frozen at −20 °C for measurement of extracellular phosphatase activity as a metric of AM fungal function; enzyme activity was quantified fluorometrically following Allison et al. (2007). Statistical variation in soil biogeochemical parameters in field soils are presented in Table S1.

AM fungal community characterization

From the field vegetation types, DNA was extracted from adult plant roots picked from all soil cores and identified morphologically as belonging to the dominant tree species at three dates (September 2012, February 2013, and September 2013) (4 vegetation types × 4 blocks × 3 dates = 48 samples). From the feedback pot experiment, DNA was extracted from roots of each host species from a subset of replicates: we focused on the experimental treatments where each host plant was paired with AM fungi from conspecific tree stands and the mature forest (3 plant hosts × 2 AM fungal communities/host × 3 soil types × 4 replicates = 72 samples). We chose these treatments to compare the drivers of AM fungal composition under adult trees in the experimental field plots and seedlings in the shadehouse pots.

Root DNA was extracted from two 0.25 g subsamples per replicate with MoBio PowerPlant Pro DNA extraction kits (MoBio, Carlsbad, CA, USA) and quantified fluorometrically (Qubit Fluorometer, Life Technologies, Carlsbad, CA, USA). Duplicate extractions were combined and standardized to ~10 ng μl^{-1}. A ~350 b region of 28S AM fungal DNA was amplified

with primers that consisted of Illumina TruSeq V3 indices (Illumina, San Diego, CA, USA) ligated to 454 barcodes (Roche, Basel, Switzerland) linked to AM-fungal specific FLR3-FLR4 primers (Gollotte et al. 2004). Each reaction contained: 21.5 μl of Platinum PCR Supermix (Invitrogen, Carlsbad, CA, USA), 1.25 μl of each primer (10 μM), 0.5 μl of BSA (20 mg ml^{-1}), and 2 μl (~20 ng) of DNA. PCR reactions were run in triplicate with an initial denaturing step of 93 °C for 5 min, 35 cycles of 93 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, followed by a final extension step of 72 °C for 10 min. Triplicate reactions were combined, cleaned with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), and quantitated fluorometrically (Qubit Fluorometer, Life Technologies, Carlsbad, CA, USA). Samples were then pooled in equal amounts into eight libraries (four for the field experiment and four from the pot experiment) and sequenced as 2 × 300 b reads to an average depth of 25,000 reads/sample on 1/8th of a flowcell of an Illumina MiSeq v3 sequencer at the University of Texas Genome Sequencing and Analysis Facility.

Contigs of forward and reverse sequences (478,633 in total) were created using the mothur v. 1.33.3 pipeline (Schloss et al. 2009). All sequences were quality filtered using the default settings in the QIIME pipeline v. 1.8.0–20,140,103 (Caporaso et al. 2010) and discarded if they were less than 300 bases in length, had more than six ambiguous bases, contained any ambiguous bases in the barcode, or were chimeric based on UCHIME with default parameters (Edgar et al. 2011). This excluded 48% of the run leaving 231,600 sequences. Sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity cutoff using UCLUST with default parameters (Edgar 2010). OTUs that only occurred once in the entire dataset and OTUs that only occurred in one sample were discarded as probable sequencing artifacts (Dickie 2010). This removed ~30% of the OTUs, leaving 680. The remaining OTUs were taxonomically classified using the Ribosomal Database Project (RDP) classifier against the RDP 28S fungal database. Non-AM fungal OTUs were discarded when they did not match known Glomeromycotina with 97% or greater confidence, leaving 649 full-length AM fungal OTUs. This method may have excluded some novel tropical AM fungi.

We then used the DeSeq2 algorithm (Love et al. 2014) to transform the relative abundance of each OTU. This algorithm provides the most sensitivity to detect treatment differences across a range of sample sizes (McMurdie and Holmes 2014). An alignment and maximum likelihood phylogeny were created with one representative sequence from each of the 649 AM fungal OTUs using the Practical Alignment using SATé and TrAnsitivity (PASTA) algorithm (Mirarab et al. 2015) with default settings. To ensure that OTU assignments were accurate, we re-defined OTUs using this alignment, which collapsed 7 OTUs. Sequences were deposited in the NCBI Sequence Read Archive (SRA) under accession number (SUB1665085).

Statistics

Composition of AM fungi in experimental field plots

AM fungal diversity and composition were assessed both phylogenetically and taxonomically. We first calculated alpha diversity for each sample as richness based on the number of unique OTUs that occurred in each replicate. We calculated Faith's PD of AM fungal communities in each replicate as the sum of branch lengths connecting all AM fungal species in an ultrametric phylogeny using the Picante package (v. 1.6–2, Kembel et al. 2010) in R (v. 3.1.1, R Development Core Team 2009). Community-wide phylogenetic signals were calculated as both mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) using the Picante package. MPD assesses phylogenetic structure at the base of the phylogeny, whereas MNTD focuses on phylogenetic relatedness near the tips of the phylogeny.

To understand how vegetation type, date, block, and soil abiotic factors affected AM fungal community composition in adult trees in the field, we performed a PERMANOVA with vegetation type, block, and date as well as the two-way interactions of vegetation type with block and date. We also included continuous abiotic soil variables (ammonium, nitrate, phosphorus, DOC, and moisture) in the model. PERMANOVA was run using the Adonis function in the vegan package in R (R Core Team 2009). To obtain Type III sums of squares, rather than the default Type I, we repeated ran the full model with each variable added last. This did not change the model outcome compared to the typical Adonis output with Type I sums of squares (data not presented). We visualized the shifts in AM fungal community composition among vegetation types using non-metric multidimensional scaling (NMS) ordination in the metaMDS function in the vegan package in R (R

Core Team 2009). Axis 1 of this ordination explained 51% of the variance in AM fungal composition and axis 2 explained 29% of the variance in AM fungal composition. The mean scores for each vegetation type from each of the NMS axes were used to represent AM fungal composition in the initial inoculum for the pot experiment. All community composition statistics were run in the vegan package in R (Oksanen et al. 2007).

Feedback pot experiment

The field-based vegetation types were used to represent the conditioning phase of the plant-soil feedback experiment. Therefore, for each AM fungal inoculum source and soil source treatment we calculated feedback to the plant as ln (conspecific treatment/heterospecific treatment), where conspecific AM fungi and soil source refer to growth of a focal plant species on AM fungal inoculum or soil from field plots where the tree species matched the pot focal plant and heterospecific refers to growth of a focal plant species on AM fungal inocula and soil from all other tree species that did not match, as well as the sterile control (Bever 2003; Brinkman et al. 2010). For each plant species, we calculated both an overall feedback effect of the combined feedback of all treatments. In addition, feedback was calculated separately for each inoculum (including sterile controls) and soil source to understand variation among conditioning plant species. We used total biomass of roots and shoots for each feedback calculation. For each plant species, student's T test was used to examine if the overall plant-soil feedback effect was different from zero. For each plant species, we used two-way ANOVA to examine how feedback was affected by AM fungal community source, soil source, and their interactions. All factors were treated as fixed effects.

Drivers of feedback

To examine the contributions of abiotic and biotic mechanisms to overall observed feedback, we created three separate multiple regression models for each plant species including (1) all variables, (2) abiotic variables only, and (3) biotic variables only. The abiotic variables were soil moisture, ammonium, nitrate, phosphate, and DOC in all of the greenhouse pots. The biotic variables were microbial biomass carbon, phosphatase activity, intraradical colonization by aseptate hyphae, septate hyphae, arbuscules, and vesicles, extra-radical aseptate and septate hyphal lengths in all of the greenhouse pots, and AM fungal community parameters from the field vegetation types: AM fungal species richness, AM fungal community composition, and AM fungal PD, MPD, and MNTD. The field AM fungal community composition was represented by the centroid for each vegetation type from NMS axes 1 and 2. NMS ordination centroids are non-metric, so correlations of plant-soil feedback with AM fungal composition should be interpreted as magnitude, agnostic of sign. The composition of AM fungi from the pot experiment was not included in these analyses as we only sequenced the AM fungal community from a subset of treatments. To meet assumptions of normality and homoscedasticity, phosphatase activities were log-transformed and fungal colonization rates were arcsine square root transformed for all multiple regression analyses.

For each species and all species together, separate models were created for plant total biomass, aboveground biomass, AM fungal feedback, and soil feedback. Regressions were run using the MASS package in R (R Core Team 2009) with model fit optimized using AIC selection. None of the biotic or abiotic factors covaried by more than 75% when compared with Pearson correlations and thus all independent variables were retained in the analyses.

Composition of AM fungi in feedback pot experiment

We used PERMANOVA to analyze AM fungal community composition in the pot experiment as a function of plant species, AM fungal inoculum source, soil source, their interactions, and the continuous abiotic soil variables (ammonium, nitrate, phosphorus, labile carbon, and moisture). PERMANOVAs were performed in R as described for field AM fungi.

Comparing AM fungal composition in the field and feedback pot experiments

We compared the composition of AM fungal OTUs in the field and in pots with a PERMANOVA that included plant species, soil nutrients, and soil moisture. PERMANOVA was chosen because it is robust to the unbalanced design between the field and feedback pots. AM fungi were only sequenced from the three focal plant species in the greenhouse, whereas AM fungi in the field were sequenced from these three plant species as well as the primary forest. This design precluded

Procrustes analysis which is more common in community composition concordance studies (Jackson 1995). In addition, we characterized the overall and genus-specific percent overlap of AM fungal taxa in field and pots for each plant species. To understand the contributions of generalist and specialist AM fungi from the field to the pot AM fungal communities, we correlated the proportion of vegetation types from which each of the AM fungal OTUs were isolated with their abundance in the feedback pot experiment using Pearson correlations with Bonferroni correction for multiple comparisons (Bonferroni 1936). We also determined if the AM fungal community in the pots was a nested subset of the field AM fungal community using two approaches to consider both abundance and presence of taxa: (1) we examined relative abundance data following Rodriguez-Girones and Santamaria (2006) using the nestedtemp function in Vegan in R (Oksanen et al. 2007), and (2) we compared the nestedness ranks of pot versus field AM fungal communities with a Mann-Whitney U test (Mann and Whitney 1947).

Results

AM fungi in experimental field plots

Soils conditioned by the vegetation types in the field contained different AM fungal communities, except *Hyeronima* and *Virola* ($R^2 = 0.145$, $P < 0.001$; Fig. 1a, Tables S2, S3). There was also significant spatial variation in AM fungal composition both among blocks ($R^2 = 0.093$, $P = 0.023$; Fig. 1b) and based on the block interaction with vegetation type ($R^2 = 0.182$, $P = 0.011$). There was no significant effect of purely temporal variation in AM fungal community composition ($R^2 = 0.052$, $P = 0.152$; Fig. 1c), but the effect of vegetation type varied over time ($R^2 = 0.160$, $P = 0.006$). Abiotic soil parameters did not explain any additional variation in AM fungal composition (Table S2). AM fungal species richness, PD, MPD, and MNTD did not vary with vegetation type, block, date, or their interactions ($P > 0.05$).

Feedback in the pot experiment

Because we were able to sample mono-dominant vegetation types and a nearby primary forest, inoculum and soil source in our experiment represent the conditioning phase of a plant-soil feedback experiment. Each of the three plant species experienced different biomass feedback to the AM fungal inoculum sources compared to its own AM fungal community. Across all AM fungal inoculum sources, *Hyeronima* plants experienced positive feedback (Fig. 2a), *Pentaclethra* neutral feedback (Fig. 2b) and *Virola* negative feedback (Fig. 2c). When broken down by AM fungal inoculum source, *Hyeronima* had positive feedback when growth on conspecific AM fungi was compared to growth with AM fungi from *Pentaclethra* ($T = 5.968$, $P < 0.001$), primary forests ($T = 15.568$, $P < 0.001$), and in sterile controls ($T = 17.271$, $P < 0.001$), but neutral feedback when compared to growth on AM fungi from *Virola* ($P > 0.05$, Fig. 2). *Pentaclethra* experienced negative feedback when growth on conspecific AM fungal inoculum was compared to growth on primary forest AM fungi ($T = 2.814$, $P = 0.009$) and neutral feedback when growth was compared on all other AM fungal treatments ($P > 0.05$; Fig. 2). *Virola* had negative feedback when grown on its own versus *Pentaclethra* AM fungi ($T = 3.894$, $P < 0.001$), but neutral feedback when growth on its own AM fungi was compared to growth on *Hyeronima* AM fungi, primary forest AM fungi, and sterile controls ($P > 0.05$; Fig. 2).

The three plant species also experienced differential biomass feedback to soils originating from the different vegetation types in the field. When all soil sources were considered together, *Hyeronima* (Fig. 3a) and *Virola* (Fig. 3c) plants experienced positive feedback when growth on conspecific soils was compared to that on heterospecific soils, whereas *Pentaclethra* plants experienced negative feedback in the same comparison (Fig. 3b). When broken down by soil sources, *Hyeronima* plants had positive feedback when growth on its soils was compared to growth on soils conditioned by all other plant species (*Pentaclethra*: $T = 4.192$, $P < 0.001$; *Virola*: $T = 3.915$, $P < 0.001$; Fig. 3). *Pentaclethra* plants had negative feedback when growth on its soil was compared to growth on soil conditioned by *Hyeronima* ($T = 3.021$, $P = 0.004$) and neutral feedback compared to growth on soil conditioned by *Virola* ($P < 0.05$; Fig. 3). Finally, *Virola* experienced neutral feedback when growth on its own soil was compared to growth on all soil treatments ($P > 0.05$; Fig. 3). There were no interactions of AM fungal inoculum source and soil source for biomass feedback in *Hyeronima* ($F = 1.087$, $P = 0.390$; Table S4), *Pentaclethra* ($F = 0.422$, $P = 0.889$), or *Virola* ($F = 0.680$, $P = 0.706$) plants. Consistent with

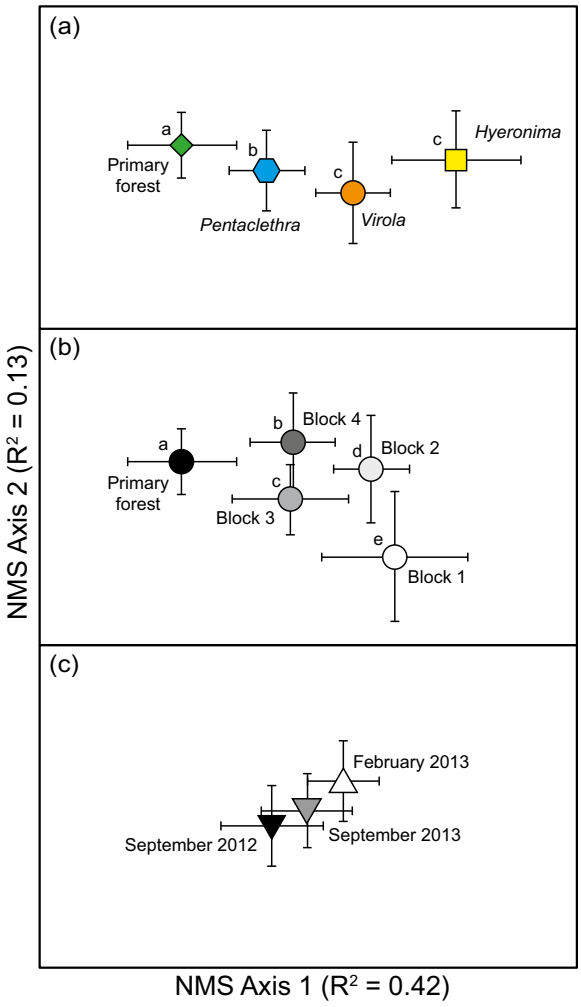

Fig. 1 AM fungal community composition in the field among (**a**) vegetation types, (**b**) blocks, and (**c**) dates. Points are averages of NMS scores (± 1 SE) for each treatment. Letters indicate treatments that were significantly different in posthoc tests

the feedback response, the total biomass of *Hyeronima* plants was only affected by AM fungal inoculation source ($F = 9.665$, $P < 0.001$). Biomass of *Pentaclethra* and *Virola* were not affected by AM fungal inoculation source or soil source ($P > 0.05$; Tables S4, S5).

Drivers of plant-soil feedback

Based on multiple regression models, the relationship between feedback and abiotic vs. biotic factors differed for each of the three plant species. The best-fit model for *Hyeronima* feedback to AM fungal inoculum source included only biotic factors: feedback was positively associated with AM fungal species richness in the field vegetation types and negatively associated with both AM fungal phylogenetic diversity in the field vegetation types and non-AM fungal hyphal colonization in the pots. Both *Pentaclethra* and *Virola* feedback to AM fungal inoculum source were best explained by abiotic factors alone, based only on positive associations with soil moisture in the pots. All plant species feedback to soil source was driven by the same variables as feedback to AM fungal inoculum source. However, regression models only explained between 15 and 39% of the observed variation in feedback to AM fungal and soil sources (Table 1). The drivers of plant biomass were similar to those for feedback for all three plant species, except the biomass of *Hyeronima* plants was also negatively associated with soil phosphatase activities in the pots (Table S6).

AM fungi in the pot experiment

The three plant species in the pot experiment were associated with different AM fungal communities ($R^2 = 0.060$ $P < 0.001$, Fig. 4a; Tables S7, S8). AM fungal community composition in the pots also varied by AM inoculation source ($R^2 = 0.069$, $P < 0.001$; Fig. 4b). There were no significant main or interaction effects of soil origin treatment on AM fungal communities in the pots (Fig. 4c; Table S6). However, AM fungal composition varied by AM inoculation sources differently across plant hosts (Table S7). Root colonization of AM fungi varied from ~4–31% depending on plant host, but did not vary among inoculated treatments. AM fungi were observed at low rates in sterile controls; abundance was consistently ~10 times lower compared to treatments inoculated with AM fungi (Table S8).

Comparing AM fungi in experimental field plots and pot experiment

The composition of AM fungi in the pots was weakly associated with AM fungal composition in conspecific field plots in the PERMANOVA analysis ($R^2 = 0.100$, $P < 0.001$; Fig. 5a). At the individual pot level, the pot AM fungal communities did not capture the AM fungal species richness of roots in the field (*Hyeronima* pots 43 ± 9%; *Pentaclethra* pots 25 ± 5%; *Virola* pots 48 ± 6% of field richness). This was also seen across treatments as only 24% of *Pentaclethra* AM fungi and only 44% of both *Hyeronima* and *Virola* AM fungi overlapped between the field vegetation types and the pot experiment

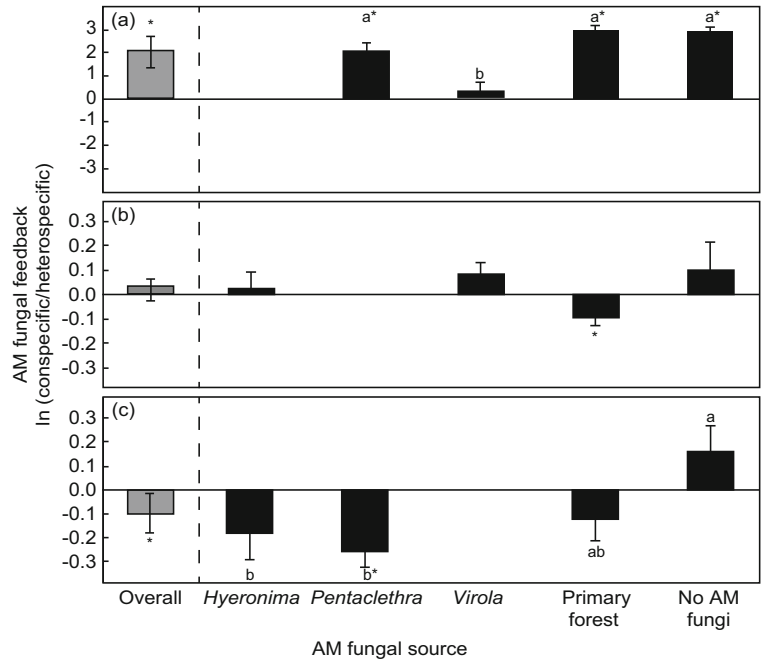

Fig. 2 Feedback of experimental pots to AM fungal inoculum for (**a**) *Hyeronima*, (**b**) *Pentaclethra*, and (**c**) *Virola* plants. Gray bars represent the overall feedback of each plant species. Black bars represent average feedback for each treatment (± 1 SE; $n = 16$). Letters indicate treatments that were significantly different in Sidak posthoc comparisons. Asterisks designate treatments in which feedback was significantly different from zero. Note the 10× difference in scale for *Hyeronima* feedback

(Fig. 5b). When the fungi were partitioned by observed genera, the experimental field vegetation plots and pots shared 3–8% of *Scutellospora*, 14–15% of *Rhizophagus*, and 6–15% *Glomus* AM fungal taxa, with the proportions of shared taxa in each genus varying slightly by vegetation type. There was also one *Acaulospora* AM fungal taxon found in the field vegetation types, but never in the pots. The overall

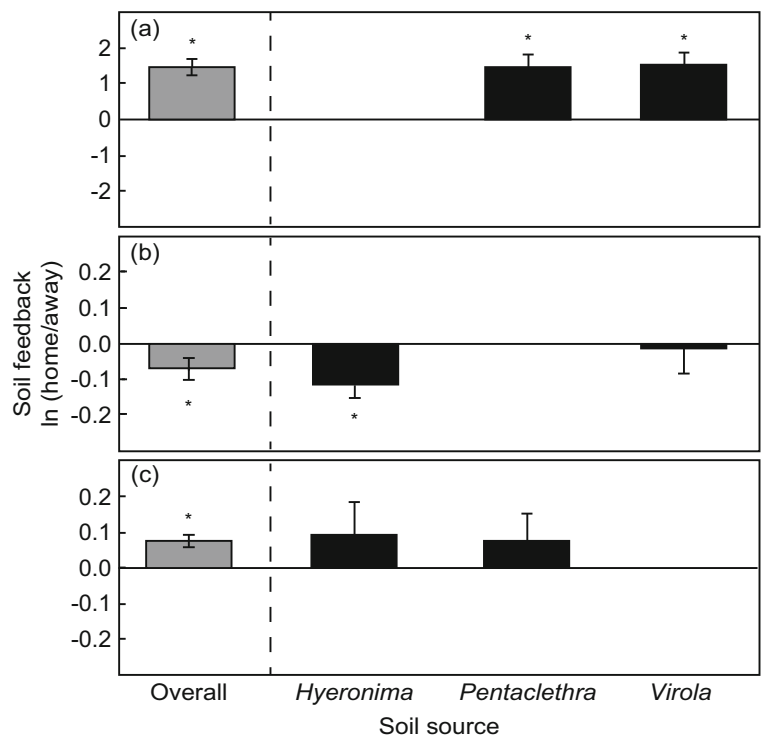

Fig. 3 Feedback of experimental pots to soil source for (**a**) *Hyeronima*, (**b**) *Pentaclethra*, and (**c**) *Virola* plants. Gray bars represent the overall feedback of each plant species. Black bars represent average feedback for each treatment (± 1 SE; $n = 24$). Asterisks designate treatments that were significantly different from zero. Note the 10× difference in scale for *Hyeronima* feedback

Table 1 Multiple regression of the biomass feedback response to AM fungal inoculation and soil source as a function of abiotic and biotic factors for all three host species

	Soil moisture	Non-AM fungal hyphal colonization	AM fungal species richness	AM fungal PD	Total model fit***		
AM fungal feedback*	r	r	r	r	R^2	P	AIC
Hyeronima	–	−0.301	0.455	−0.470	0.380	<0.001	37.164
Pentaclethra	0.406	–	–	–	0.150	<0.001	−143.28
Virola	0.407	–	–	–	0.277	<0.001	−123.37
Soil feedback**							
Hyeronima	–	−0.322	0.454	−0.479	0.389	<0.001	34.289
Pentaclethra	0.406	–	–	–	0.150	<0.001	−143.28
Virola	0.407	–	–	–	0.277	<0.001	−123.37

Because multiple regression models either highlighted only biotic factors (*Hyeronima*) or abiotic factors (*Pentaclethra* and *Virola*) as determinants of feedback, we only present the full model results

*Calculated as ln (conspecific/heterospecific)

**Calculated as ln (home soil/away soil)

***Only significant factors are listed

abundance of AM fungi in the feedback pot experiment was positively, but weakly correlated with their prevalence among vegetation types in the field ($R^2 = 0.121$, $P < 0.001$, Fig. 5c) and this was true regardless of plant host in the pot (Table S9). While the entire AM fungal community in the study was nested ($T = 6.818$, $P < 0.001$), the AM fungal community in the pots was not a nested subset of the AM fungal community in the field for any plant species ($P > 0.05$; Fig. 5d).

When all plant hosts were considered simultaneously, the main effects of plant host and soil nutrients explained some of the variation in AM fungal composition in the field ($R^2_{host} = 0.145$; $R^2_{soils} = 0.040$) and in the pots ($R^2_{host} = 0.060$; $R^2_{soils} = 0.035$). However, AM fungal communities on individual plant species in the pot experiment varied with respect to host-specific AM fungal inoculum isolated from the field. The composition of AM fungi colonizing *Pentaclethra* ($R^2 = 0.055$, $P = 0.029$) and *Virola* ($R^2 = 0.137$, $P < 0.001$) plants varied among AM fungal inoculation sources, but AM fungi colonizing *Hyeronima* plants did not ($R^2 = 0.033$, $P = 0.512$).

Discussion

In the field, spatial and temporal heterogeneity of AM fungal communities among plots was on par with differences among plant hosts, and interactions between them were dominant. Evidence from this study site (Kivlin and Hawkes 2016a, b) and other tropical rainforests (Waring et al. 2016) suggests that microbial communities are highly spatially variable in tropical forests. If so, then relating plant-soil feedback to plant abundance in the tropics (Mangan et al. 2010b) may overestimate the role of biotic interactions in predicting plant coexistence in these ecosystems. Moreover, in a recent survey of AM fungi from the same forest plots as the Mangan et al. (2010b) study, soil phosphorus, and not plant host, was the main driver of AM fungal composition (Schappe et al. 2017), suggesting that plant-soil feedback may be spatially variable or not rely on AM fungi in tropical ecosystems. In the current study, heterogeneity of AM fungal composition may explain why feedback based on field conditioning of AM fungal inocula and soils was affected by AM fungal inoculum source in only one plant species (*Hyeronima*) in the greenhouse plant-soil feedback experiment.

The degree of mycorrhizal dependence likely varies among tropical forest plant hosts (Janos 1980). We found that *Hyeronima* plant biomass was highly correlated with shifts in AM fungal diversity and non-AM fungal abundance and for the most part experienced positive feedback; *Hyeronima* plants performed better on their own versus foreign inoculum. *Hyeronima* plants also experienced the largest shifts in plant-soil feedback among inoculation sources. Conversely, the other two plants in our experiment, *Pentaclethra* and *Virola*,

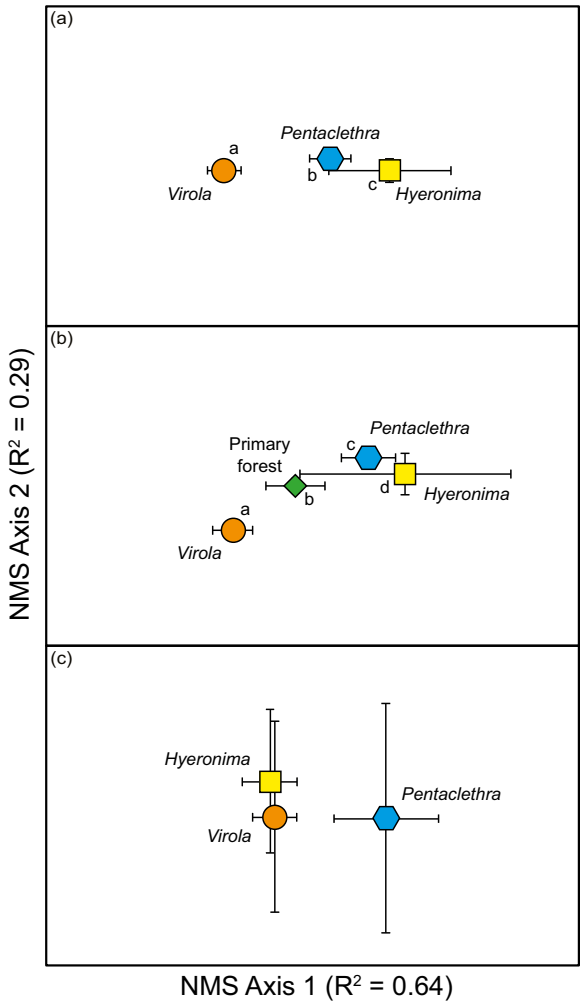

Fig. 4 AM fungal community composition in the shadehouse pots among (**a**) plant species, (**b**) AM fungal inoculum sources, and (**c**) soil types. Points are averages of NMS scores (± 1 SE) for each treatment. Letters indicate treatments that were significantly different in posthoc tests

responded more to abiotic shifts in soil moisture and had feedbacks that were 1/10th in magnitude compared to *Hyeronima*. Recent evidence from surveys of AM fungi in Puerto Rico supports a changing functional role of AM fungi as forests age, with AM fungi negatively affecting early successional trees, but positively affecting late successional (Bachelot et al. 2018) and rare tree species (Bachelot et al. 2017). Here, the rarest species in our study, *Hyeronima*, a tree-fall gap colonizing species, may rely on AM fungal networks to successfully establish and acquire limiting soil nutrients, but then quickly be out-competed or accumulate pathogens as trees age. Conversely, *Pentaclethra* and *Virola* are later successional species and therefore may benefit from a wide range of AM fungi or be more able to withstand soil pathogens (Bagchi et al. 2014). In addition, *Pentaclethra* is a nitrogen-fixer and thus may rely on these symbionts more than AM fungi (Nasto et al. 2014), although we did not observe any nodules in our experiment.

The AM fungi in our field vegetation plots varied among vegetation types, with significant spatial and temporal heterogeneity. Across an approximately 400 × 800 m area of tropical rainforest, spatial and temporal variation in AM fungal composition was similar in magnitude to other soil microbial guilds (Kivlin and Hawkes 2016a, b), although with different patterns among vegetation types. This suggests that underlying variation in soil nutrients (Vandecar et al. 2011; Waring et al. 2016; Schappe et al. 2017), topography (Powers et al. 1997), and understory vegetation (Slik et al. 2013) in tropical forests influence a range of microbial taxa, in varying ways. Moreover, the temporal variation of AM fungal communities among vegetation types was substantial, despite the aseasonality of rainfall in this region. While plant community composition is correlated with belowground mycorrhizal communities in other tropical forests (Peay et al. 2013), fungi can also respond to temporal variation in precipitation, even across a plant diversity gradient (McGuire et al. 2012), highlighting the lack of generality in soil microbial drivers identified within and among tropical ecosystems.

Given the high variability of AM fungi in the field plots, it is not surprising that plant-soil feedback observed in response to AM fungal inoculum in the feedback pot experiment was also variable. Recent studies have indicated that plant-soil feedback in the greenhouse is not consistent with plant-soil interactions in the field and therefore cannot explain patterns of coexistence (Lankau and Lankau 2014; Stanescu and Maherali 2017). Several factors could explain these findings. Plant coexistence may not rely on soil microorganisms if environmental filtering (Lavorel and Garnier 2002) or plant-plant competition (Tilman 1994) structures communities, and this may especially be true in forest ecosystems with long-lived tree species. Even if AM fungi do affect plant coexistence, when AM fungal communities differ with plant ontogeny (e.g., Husband et al. 2002a, b), feedback experiments of seedling and juvenile species in pots may never match the feedback that adult plants experience in natural ecosystems (Hawkes et al. 2013; Kardol et al. 2013). For

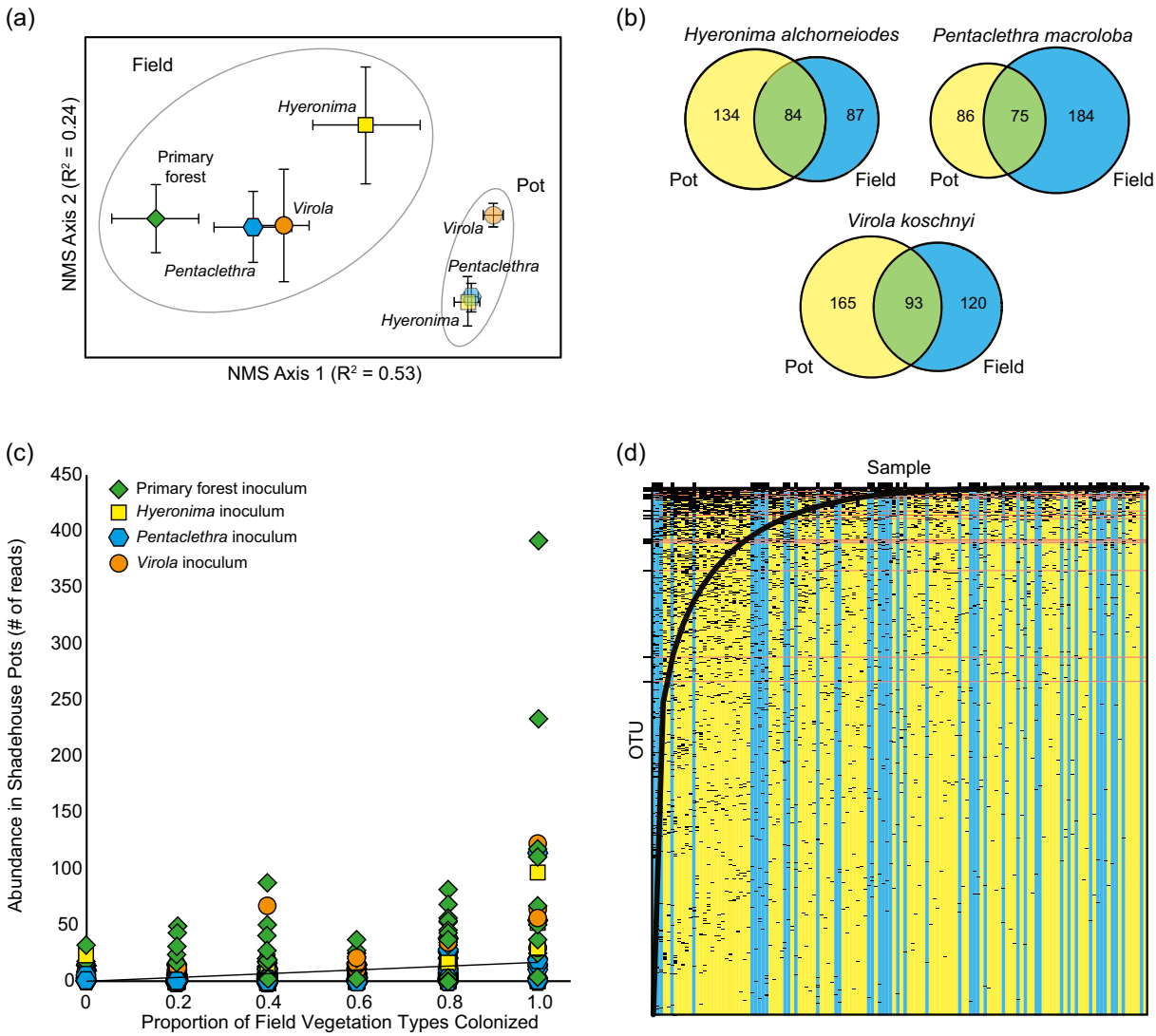

Fig. 5 **a** AM fungal community composition in field tree roots and in roots of feedback pots (lighter shaded symbols) plotted as average NMS scores (± 1 SE). **b** Overlap of AM fungal OTUs in the pot versus field samples for each host species visualized in Venn diagrams. **c** The abundance of AM fungal taxa in the feedback pot experiment as a function of the proportion of vegetation types colonized in the field. **d** Nestedness plot of AM fungal OTUs that occurred in each sample (black rectangles). The curve (black line) indicates the isocline of perfect nestedness. Blue columns are AM fungal communities from the field vegetation types and yellow columns are AM fungal communities from the feedback pot experiment. Orange rows noted by black hashes on the left side of the panel indicate generalist AM fungal OTUs that occurred in all five field vegetation types

example, in the only studies to track variation in AM fungi across plants over time in tropical forests, the abundance of individual AM fungal taxa varied 8-fold after 1 year (Husband et al. 2002a) and up to 55-fold over 5 years (Husband et al. 2002a). Therefore, it is likely that the current study and all other greenhouse-based plant-soil feedback studies also exhibit some degree of discordance owing to different plant life stages. In addition, differences in AM fungal composition in plant-soil feedback pots compared to field communities may be one mechanism contributing to the lack of transferability of feedback patterns, especially when plants are highly responsive to AM fungi. These differences may arise because AM fungal inoculum in greenhouse experiments is often added as spores, not a mycorrhizal network (e.g., the current study, Mangan et al. 2010a), AM fungi are added in isolation without other animal and microbial taxa that may modify their

composition (Klironomos and Ursic 1998), or the fact that plant-soil feedback experiments usually only grow one plant host in isolation, which can affect AM fungal communities (Burrows and Pfleger 2002; Kivlin and Hawkes 2011) and create misleading coexistence predictions when plant-soil feedback is non-additive (Hawkes et al. 2013).

Perhaps because of these inherent differences in greenhouse versus field conditions, greenhouse roots contained vastly different, and in some cases more diverse AM fungal communities compared to field-collected analogs. However, despite the broad differences in AM fungal communities between field and pot experiments, AM fungi that were more widespread in the field vegetation types were also more abundant in the pots, suggesting that some generalists were at an advantage. At the global scale, some AM fungi tend to have widespread distributions across climates and plant communities (Kivlin et al. 2011; Davison et al. 2015), suggesting that this long-lived lineage can be environmentally plastic. However, local adaptation with respect to soil nutrients (Johnson et al. 2010) and climate (Antunes et al. 2011) supports the existence of many specialist, low abundance AM fungal taxa, which were the majority of AM taxa in the current experiment. Greenhouse plant-soil feedback experiments may have larger transferability to environmental plant-soil feedback when AM fungi drive feedback outcomes in a plant-specific manner, as in Mangan et al. (2010a, b). However, in more diverse and heterogeneous AM fungal communities, environmental interactions with specialist, low abundance AM fungal species may moderate plant-soil feedback. Such context-dependent feedback will be difficult to predict when AM fungal community composition differs along environmental gradients in underlying resources and climate. Current efforts to describe large-scale surveys of conditions favoring local adaptation of AM fungal taxa (e.g., Rua et al. 2016), and their relative effects on plant-soil feedback along environmental gradients (e.g., Smith-Ramesh and Reynolds 2017) are a strong way forward to creating an inclusive and generalizable framework of plant-soil feedback.

Our study has several caveats that could preclude broad applicability of these patterns to other tropical forests. First, our experimental design only allowed us to address plant-soil feedback in three species. While feedback varied among the three plant hosts, it is difficult to extrapolate these findings to entire primary forests. Furthermore, many of the biotic and abiotic drivers of plant-soil feedback were weak and this experiment only captured the AM fungal component of feedback. Future studies disentangling mycorrhizal versus decomposer and pathogen-driven feedback in isolation and combination are necessary to elucidate the belowground portion of feedback dynamics (Klironomos 2002). In addition, the control pots in our experiment only received water, and not sterilized AM fungal spores, which may have reduced their nutrient content given that spores are rich in lipids (Beilby 1980; Beilby and Kidby 1980). However, because the pots used in this experiment were so large, the inoculum only added ~8% more spores than would have been found in the sterilized background soil. There was also a low level of AM fungal and non-AM fungal colonization in our control pots, most likely via airborne dispersal or rain splash within the relatively open shadehouse environment. Control pots consistently contained <1/10th of the AM fungal hyphal lengths in soil compared to inoculated treatments and exhibited minimal intraradical root colonization, whereas colonization rates and extraradical hyphal lengths of AM fungi in inoculated pots in the greenhouse mimicked those of tree roots and soils in the field (Kivlin and Hawkes 2016b, Kivlin and Hawkes, unpublished data). The low level of non-AM fungal colonization observed in the greenhouse may have affected the outcome of plant-soil feedback, but to a minor extent. Non-AM fungal colonization explained less than 10% of the variation in plant-soil feedback in *Hyeronima* plants and none of the variation in feedback in the other two plant species. Nevertheless, these caveats do not undermine our finding that high spatial and temporally heterogeneity of AM fungal communities within vegetation types in tropical forests constrains the potential generality of plant-soil feedback derived from pot experiments.

Conclusions

Our study provides evidence that AM fungal communities in a Neotropical forest can be spatially and temporally heterogeneous and this heterogeneity may be a reason that observed greenhouse plant-soil feedback is not consistently influenced by AM fungi. Instead, plant growth in natural systems may be a function of AM fungal communities as well as a diverse array of biotic and environmental conditions. More expansive, field-based plant-soil feedback studies that occur across spatial and temporal scales are necessary to determine when

feedback is consistent within a plant host and when it is environmentally context-dependent. Characterizing when and where soil microbial communities are spatially and temporally variable across ecosystems is the first step to understanding context-dependent plant-soil feedback. However, to capture truly long-term trends, natural experiments, such as plant invasions or climate change-induced range expansions may provide the most tractable platform, especially when time since establishment is known, allowing a temporally explicit look at the buildup of plant-soil feedback. Ultimately, approaches that compare the strength of plant-soil feedback to other biotic and abiotic processes (e.g., competition or herbivory; van der Putten et al. 2016) will provide a more expansive and predictive framework for understanding the role of plant-soil feedback in structuring natural plant communities.

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