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Plant and root endophyte assembly history: interactive effects on native and exotic plants

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Abstract. Differences in the arrival timing of plants and soil biota may result in different plant communities through priority effects, potentially affecting the success of native vs. exotic plants, but experimental evidence is largely lacking. We conducted a greenhouse experiment to investigate whether the assembly history of plants and fungal root endophytes could interact to influence plant emergence and biomass. We introduced a grass species and eight fungal species from one of three land-use types (undisturbed, disturbed, or pasture sites in a Florida scrubland) in factorial combinations. We then introduced all plants and fungi from the other land-use types 2 weeks later. Plant emergence was monitored for 6 months, and final plant biomass and fungal species composition assessed. The emergence and growth of the exotic *Melinis repens* and the native *Schizachyrium niveum* were affected negatively when introduced early with their "home" fungi, but early introduction of a different plant species or fungi from a different site type eliminated these negative effects, providing evidence for interactive priority effects. Interactive effects of plant and fungal arrival history may be an overlooked determinant of plant community structure and may provide an effective management tool to inhibit biological invasion and aid ecosystem restoration.

Key words: assembly history; Florida rosemary scrub; fungal root endophytes; invasion; priority effects; restoration.

Introduction

The history of community assembly is an increasingly recognized, yet still poorly understood factor that can dictate the structure of ecological communities (Fukami 2015). Assembly history affects community structure via priority effects, where the order of species arrival determines how species affect one another. Most experiments investigating the effect of assembly history on community structure have manipulated introduction order within a single trophic level (Schröder et al. 2005, Fukami et al. 2010). A limited number of multi-trophic studies used microbial microcosm experiments involving producers (e.g., algae) and herbivores (e.g., zooplankton) (Robinson and Dickerson 1987; Drake 1991, Fukami and Morin 2003) or intraguild predators and prey (Price and Morin 2004; Louette and De Meester 2007, Olito and Fukami 2009). To our knowledge, however, introduction order has not been independently manipulated at multiple trophic levels in a factorial fashion. Experiments with such designs are more complex, but necessary to evaluate whether assembly history within different trophic levels has an interactive effect on species abundance and community structure.

One potential driver of interactive priority effects in terrestrial systems is the biotic feedback between plants and soil fungi. Previous work has shown that the order and timing of plant species arrival can shift plant community composition, productivity, and the relative abundance of native vs. exotic species (Fargione et al. 2003, Fukami et al. 2005, Ejrnæs et al. 2006, Körner et al. 2008, Collinge and Ray 2009, Martin and Wilsey 2012, Plückers et al. 2013, von Gillhaussen et al. 2014). Simultaneously, stochastic dispersal in soil fungi (Peay and Bruns 2014) can produce variation in fungal assembly history among local sites, which can then alter the species composition of the fungi as well as the plants that have symbiotic associations with the fungi. Mycorrhizal fungi, for example, form symbioses with the majority of vascular plants and exhibit priority effects, with early-arriving fungi controlling the ability of late-arriving fungi to establish and benefit plant hosts (Kennedy et al. 2009, Mummey et al. 2009, Werner and Kiers 2014). Changing the order of host plant arrival, including native and exotic grasses, can also alter the community composition of symbiotic fungi (Hausmann and Hawkes 2010). However, few previous experiments manipulated the

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order of plant and fungal species arrival in a factorial fashion to test for multi-trophic interactive priority effects.

In this paper, we test the hypothesis that the assembly history of plants and their root-inhabiting endophytic fungi interact to alter the success of exotic vs. native plant species by manipulating the introduction order of grasses and fungal root endophytes in a factorial design. A pot experiment was conducted in a greenhouse using plants and fungi sampled in a Florida shrubland (Abrahamson et al. 1984, Weekley et al. 2008). In this shrubland, several introduced grasses have become invasive, potentially displacing native plants, particularly at disturbed sites. The species composition of soil fungi differed among undisturbed shrubland sites, disturbed shrubland sites, and former shrubland sites converted to pasture (Glinka and Hawkes 2014), raising the possibility that these fungi may partly explain the observed differences in the success of exotic plants among different sites. In this study, we focus on fungal root endophytes because they have a broad spectrum of host effects, from negative to positive (Mandyam and Jumpponen 2005), and because dispersal differences via hyphal fragmentation or conidia (Rodriguez et al. 2009) are likely to cause a large degree of spatial and temporal variation in arrival timing in the field. In addition to plant emergence and biomass, we quantified fungal community composition to better explain the effect of plant and fungal assembly history on plant performance.

Materials and Methods

Plants

Grass seeds were isolated from sites at Archbold Biological Station in Florida (27°10′50″ N, 81°21′0″ W). We focused on three C_4 grass species: *Aristida gyrans* Chapm., *Schizachyrium niveum* (Swallen) Gould, and *Melinis repens* (Willd.) Zizka. *Aristida gyrans* and *S. niveum* are native to Florida scrub, occurring in undisturbed oak and scrubby flatwoods, as well as rosemary scrub, sites dominated by the shrub *Ceratiola ericoides* Michaux (Quintana-Ascencio and Menges 1996, Weekley et al. 2006, Menges et al. 2008). *Schizachyrium niveum* is endemic to central Florida and is listed as state endangered. *Aristida gyrans* is also present in disturbed sites, although less commonly. *Melinis repens* is native to South Africa and was introduced to Florida as a pasture grass in the 1960s. It is a category I invasive plant in Florida (FLEPPC 2013) and is abundant in disturbed shrubland sites and roadsides and occurs alongside other introduced, rhizomatous grasses in pasture sites within our study area (David and Menges 2011, B. A. Sikes, *personal observation*). Seeds of these species were collected from multiple sites throughout 2010.

Fungi

Fungal endophytes were isolated from undisturbed Florida rosemary shrublands (scrub), disturbed scrub (disturbed largely through roller chopping), and scrub sites previously converted to pasture (in and around the 1960s) at Archbold Biological Station (Glinka and Hawkes 2014). Fungal isolation was from the dominant C_4 grass and shrub species in each environment, but plant species were not identical, because plant species composition did not completely overlap among the three sites, particularly between pasture and undisturbed sites. The source location and environment for each fungal isolate is listed in Appendix S4. Field observations and culturing from 14 other plant species indicated that the isolated fungal endophytes used in our experiment were widespread among plant species. Although we were not able to use the same plant host for culturing across all sites, the ability of these fungi to be continuously cultured also likely functions as a filter against host-specific fungi. Roots were refrigerated and surface sterilized within 3 h after field collection. Roots were washed of any sand, then surface sterilized by immersion in 95% ethanol for 5 s, 0.5% NaOCl (bleach) for 2 min, 70% ethanol for 2 min, and sterile water for 1 min. Roots were placed in sterile Whirl-Pak bags (Nasco, Salida, Colorado, USA) and transported with cold packs (to reduce fungal growth and degradation) to the University of Texas at Austin. To culture fungal endophytes in the laboratory, surface-sterilized roots were placed on potato dextrose agar plates (Mandyam et al. 2010) supplemented with ampicillin (100 μg/mL) to retard bacterial growth. Individual fungal endophytes were isolated when multiple species were cultured from a single root. Control tests where roots were only touched to plates and then removed indicated that isolated fungi were true endophytes and had not merely avoided the surface sterilization process.

Cultured fungi were identified using molecular methods. Fungal DNA was extracted using a CTAB phenol-chloroform extraction with bead beating (Griffiths et al. 2000). The 28S gene region of rDNA was amplified using the fungal primers NL1 and NL4 (O'Donnell 1993) and the following PCR protocol: an initial 2 min denaturation step at 95°C, followed by 30 s at 95°C, 30 s at 55°C, and 2 min at 72°C for 30 cycles, then a final 5 min extension step at 72°C. PCR products were directly sequenced on an ABI 3730XL DNA analyzer (Foster City, CA, USA) at the University of Texas DNA Sequencing Facility. Although the ITS region is commonly used for fungal barcoding, the large ribosomal subunit (28S) region more effectively differentiates clades of Ascomycetes likely to be present as endophytes (Schoch et al. 2012), allows better resolution of deep phylogenetic relationships (Liu et al. 2011), and does not include large size variations that can bias next-generation

Table 1. Fungal root endophytes used in assembly experiment.

Site type	ID	Putative BLAST ID	Max score†	Putative genus based on ML phylogeny	Accession no.
Undisturbed	U1	*Sordariomycetidae* sp. TG17	970	*Coniochaeta* sp.	KM657057
Undisturbed	U2[t]	*Chaetomium cupreum*	981	*Chaetomiaceae* sp. 1	KM657058
Undisturbed	U3	*Ophiostomataceae* sp. JCM 16786	815	*Ceratocystiopsis* sp.	KM657059
Undisturbed	U4	*Trimmatostroma salicis*	926	*Vibrissea* sp.	KM657060
Undisturbed	U5	*Neosartorya glabra*	996	*Aspergillus* sp.1	KM657061
Undisturbed	U6	*Myrmecridium schulzeri*	989	*Sordariomycete* sp.; *Myrmecridium sister*	KM657062
Undisturbed	U7	*Nemania* sp. 5192	966	*Xylaria* sp.	KM657063
Undisturbed	U8	*Talaromyces rugulosus*	972	*Trichomaceae* sp.2	KM657064
Disturbed	D1	*Leotiomycetes* sp. OTU30	992	*Leotiomycetes* sp.2	KM657045
Disturbed	D2	*Pseudophialophora eragrostis*	946	*Magnaporthaceae* sp.	KM657046
Disturbed	D3	*Chaetomium cuyabenoensis*	933	*Chaetomium* sp.	KM657047
Disturbed	D4	*Sordariomycetes* sp.	928	*Xylariaceae* sp.2	KM657048
Disturbed	D5	*Penicillium javanicum*	994	*Eupencillium* sp.	KM657049
Disturbed	D6	*Chaetothyriales* sp.	861	*Herpotrichiellaceae* sp.	KM657050
Disturbed	D7	*Echria gigantospora*	946	*Lasiosphaeriaceae* sp.	KM657051
Disturbed	D8[t]	*Chaetomium cupreum*	989	*Chaetomiaceae* sp. 2	KM657052
Pasture	P1	*Paraphaeosphaeria neglecta*	979	*Paraphaeosphaeria sp*	KM657053
Pasture	P2	*Curvularia papendorfii*	1009	*Pleosporaceae* sp.1	KM657054
Pasture	P3	*Curvularia tuberculata*	994	*Pleosporaceae* sp.2	KF036304
Pasture	P4	*Nigrospora* sp.	983	*Nigrospora* sp.	KM657055
Pasture	P5[s]	*Fusarium oxysporum*	994	*Gibberella* sp.1	KM657056
Pasture	P6[s]	*Fusarium oxysporum*	959	*Gibberella* sp.2	KF036302
Pasture	P7	*Trichoderma koningiopsis*	966	*Hypocrea* sp.	KF036303
Pasture	P8	*Leotiomycetes* sp.	876	*Leotiomycetes* sp.1	KF036305

Note: Isolates with the same superscripted letter (t, s) BLAS ed to identical records and clustered close enough in the ML phylogeny to be in the same genus.

†Max Score represents the normalized score of the aligned sequence (using substitution matrix and gap penalty).

sequencing (Roche Technical Bulletin T2011-001 2011). Each sequence was cut to the primers, edited to insure ambiguous bases were called correctly, and blasted against GenBank to obtain putative identities. Because endophyte diversity differed among site types, eight isolates were chosen from each site type so that additions represented differences in origin alone, rather than also differences in diversity. Pasture sites had only eight morphologically unique isolates, all of which were used in the experiment (Experimental Design). Of 28 and 12 unique isolates obtained from undisturbed and disturbed scrub sites, respectively, eight were randomly selected for experimental use from each of these two site types. Genbank accession numbers and putative identities (Blast and Maximum Likelihood Phylogeny) for the 24 isolates are listed in Table 1. Alignment and construction of the phylogenetic tree was carried out in SATè (Liu et al. 2012; see details in Appendix S1).

The endophytic nature of all fungi was confirmed and, based on their identities and on previous research, most of the fungi used in this experiment were Class 2 or Class 4 fungal endophytes (Rodriguez et al. 2009). We do not know the specific functional roles of these fungi, which can be highly context dependent (Johnson 2010, Giauque and Hawkes 2013). Here we use the endophytes to demonstrate the assembly process in a greenhouse environment, but we acknowledge that the observed functions of these fungi may not carry over to other conditions.

Experimental design

The overall design of the greenhouse experiment was a full factorial of fungal community origin (undisturbed, disturbed, pasture) and plant species (*A. gyrans, S. niveum, M. repens*). Each fungal community was composed of the eight fungal isolates selected from a specific site type. All treatments consisted of an initial introduction of one plant species paired with one fungal community, followed by an introduction of all remaining plants and fungi 2 weeks later. Treatments were replicated five times for a total of 45 pots.

Pasture soil was chosen for the pot experiment because the sites were being used for experimental restoration of native plants using fungal endophytes (B. A. Sikes, *unpublished data*), and thus our results could have direct relevance to that effort. To account for spatial variability in nutrient pools among sites within a vegetation type (Hamman and Hawkes 2013),

soils were collected from four different pasture sites adjacent to undisturbed sites. Soils were combined, thoroughly homogenized, sieved to 4 mm, and sterilized by autoclaving three times (one 30-min wet cycle followed by two 30-min dry cycles) with 24 h rest periods in between cycles. Soils were then combined 1:1 with sterile silica sand, distributed to experimental pots (~6 kg soil in each pot of 15.875 cm diameter and 15.875 cm depth), and saturated with sterile water.

To produce fungal inoculum, fungal isolates were grown independently for 10 d in 45 mL of potato dextrose broth. To create the fungal treatments, the eight individual isolates from each site type were combined 4 h prior to inoculation by blending twice for 30 s using a bleach-sterilized blender (Magic Bullet, Homeland Housewares, Los Angeles, California, USA).

Assembly history was experimentally varied in pots by adding fungal inoculum from one site type and seeds of one plant species, allowing them to grow for 2 weeks, and then adding the seeds and fungi of the remaining species (see Appendix S3 for a full list of all assembly treatments). The 2-week interval was used for a conservative test of priority effects. Theory suggests that longer intervals lead to stronger priority effects (reviewed in Fukami 2015). A priority effect observed 6 months after assembling experimental communities with inoculation order manipulated using just a 2-week interval would indicate that priority effects could be larger with a longer introduction interval. Twenty milliliters of inoculum for one of the three fungal communities (undisturbed, disturbed, or pasture) was added and then covered with 250 mL (~2 mm) of sterile soil. Thirty seeds of the initial plant species were then added and again covered with 250 mL of soil. After 2 weeks, we added 30 seeds of each of the other plant species and 20 mL of each fungal inoculum (simulating dispersal or addition treatments in the field), which were then covered with 100 mL (~1 mm) of sterilized soil and washed flat with 100 mL of water. Seedlings of the initial plant species were often present at this point and the added soil was thin enough to cover the new seeds without overtopping the existing seedlings. This way, fungi and plant seeds were layered near the soil surface in the same region so that only introduction order varied.

Each pot was watered with 6 mm of water every 3 d, which is similar to the daily averages for the local May/June transition from dry to wet season (Weekley et al. 2007), when plants are likely to germinate. Experimental communities were grown for 6 months after the second plant and fungal introduction.

Plant measurements

Throughout the experiment, the number of emergent individuals of each plant species was recorded every 3 d. At the end of the experiment, the number of each plant species was recorded, and all plants harvested. Plants were shaken free of soil, and roots washed thoroughly. For each pot, root and shoot biomass of individual plant species was measured, and total biomass by species, root : total biomass ratios, and total biomass by pot were calculated. For each pot, approximately 100-mg subsamples of roots from each plant species (still attached to the plant to confirm identity) were placed in 70% ethanol. The subsamples were surface sterilized and used for fungal community analysis as detailed in the next section.

Fungal measurements

To characterize the composition of fungal endophyte communities, DNA was extracted from each root sample using a MoBio Power Soil Kit (Carlsbad, California, USA). The soil kit has the identical components as the MoBio plant kit, but with an additional washing step to remove soil compounds that can inhibit downstream applications like PCR. In ongoing work at these sites, the soil kit has been more effective for root DNA extraction and producing low inhibition in PCR. We extracted DNA from a total of 92 different samples representing roots of every plant species present in each of the 45 pots (Appendix S5). For each pot, roots from a given species were pooled from up to three individual plants. Each sample was PCR amplified in triplicate using the NL1–NL4 primers, but the total number of PCR cycles was reduced to 25 in an attempt to minimize PCR bias that could interfere with detecting rare sequences downstream.

PCR amplicons were combined with unique adaptors to develop libraries of fungal 28S rDNA for each sample that were checked for quality, then analyzed using 454 pyrosequencing. Pyrosequencing data were error checked using Acacia (Bragg et al. 2012) and the QIIME bioinformatics pipeline (Caporaso et al. 2010). Detailed methods for the DNA processing as well as bioinformatic steps are specified in Appendix S2. Command line steps for the bioinformatic analyses are available by request.

Pyrosequencing produced 599,406 sequences in total. After error checking, trimming short sequences, and splitting, 229,777 sequences contained (archived in NCBI Sequence Read Archive: SRP047491) the forward primer and 169,354 contained the reverse primer. The bioinformatic pipeline including sample rarefaction reduced this dataset significantly, producing a fungal data set that contained the forward primer with 500 sequences for each of 84 total samples that passed this threshold (41,873 sequences in total).

Statistical methods

For each plant species, we tested if emergence and biomass were affected by the identity of the first plant species introduced, the first set of fungi introduced,

and their interaction. Our experimental design enabled assembly history effects to be evaluated by testing for effects associated with the identity of the first species introduced (see Fukami et al. [2010] and Dickie et al. [2012] for similar experimental designs and statistical analyses). For emergence, we tested for differences using a generalized linear model with a quasi-Poisson distribution (for count data) and chi-squared tests. The number of emergent individuals of an individual species was the dependent variable and the independent variables were first plant, first fungi, or their interaction. Analysis of variance (ANOVA) was used to determine if biomass was affected by the order of introduction (first or later) of plants, that of fungi, and their interaction. For each plant species, growth response variables were assessed independently and included total biomass, root and shoot biomass, and the ratio of root : total biomass. Biomass was log-transformed to improve normality. For all significant treatments, Tukey HSD tests were used to identify specific differences among groups of the significant factor.

Fungal communities of each treatment were visualized using nonmetric multidimensional scaling (NMDS). Differences among treatments were analyzed using Bray-Curtis distance metrics and sum of squares variance partitioning through the adonis function in the vegan package in R (Oksanen et al. 2013), which is often referred to as a nonparametric MANOVA. Samples were stratified by pot, since roots of multiple plant species came from the same pot. In addition to the identity of the initial plant and fungal community factors, plant host species from which the fungal endophyte community was extracted was also included as an independent variable. These analyses were carried out on derivations of every data set (see Appendix S2), including use of forward or reverse primers, at 97% or 99% sequencing similarity for OTU clustering, and at the minimum saturated sampling based on rarefaction curves that included all samples or at double this number (e.g., 500 vs. 1000 for forward primer).

Results

Plant and fungal introduction order altered the emergence and growth of individual plant species, but these effects varied by species, sometimes showing strong interactive effects of plant and fungal assembly histories. For example, the native *S. niveum* emerged poorly when introduced first with fungi isolated from undisturbed scrub (plant × fungi, χ^2 df = 4, 44, $P < 0.0005$, Fig. 1A), but this negative effect of early *S. niveum* introduction was not observed when fungi from other sites were introduced first (Fig. 1B, C). Differences in *S. niveum* biomass among treatments were statistically significant, but emergent plants often died or rarely grew large enough to provide reliable biological inferences about the effect of introduction order on *S. niveum* growth (Fig. 2A–C).

Strong interactive effects were also observed in the emergence of the nonnative *M. repens*, whereas its biomass was affected by plant and fungal introduction orders independently. Specifically, *M. repens* seeds emerged well when it was not the first plant species introduced or was introduced with fungal endophytes from undisturbed scrub sites. However, if the exotic plant was introduced first with fungal endophytes from either disturbed or pasture sites, 84–90% fewer seeds emerged than with undisturbed scrub fungi (plant × fungi, χ^2 df = 4, $P < 0.005$, Fig. 1G–I). The biomass of *M. repens* was independently determined by introduction order of both the plant (first plant, $F_{2,36} = 16.87$, $P < 0.0001$) and fungi (first fungi, $F_{2,36} = 3.44$, $P < 0.05$, Fig. 2G–I), producing significantly less biomass when introduced first ($P < 0.05$ for all pairwise comparisons) or when fungi from pastures were introduced first ($P < 0.05$ all pairwise).

Emergence and biomass of the native *A. gyrans* was affected by plant introduction order independent of fungal introduction order (Figs. 1D–F and 2D–F). When introduced first, fewer *A. gyrans* seeds emerged (first plant, χ^2 df = 2, 42, $P > 0.001$; Fig 1, top row) and plants were smaller (first plant, $F_{2,36} = 8.95$, $P < 0.001$; Fig. 2, top row) than when introduced after another plant species. Initially introducing fungal endophytes isolated from pasture sites resulted in larger *A. gyrans* plants than with fungi from disturbed sites (first fungi, $F_{2,36} = 3.72$, $P < 0.05$), but fungal introduction order had no significant effects on *A. gyrans* emergence.

For the fungal community inhabiting roots in the pots, clustering at 97% sequence similarity produced 327 total OTUs among all the samples. The first three NMDS axes together explained 80.3% of the variation among fungal communities; the first two axes explained 63.7% of the variation (Fig. 3). Composition of the fungal community was unaffected by plant introduction order (first plant, $R^2 = 0.04$, $P = 0.13$), fungal introduction order (first fungus, $R^2 = 0.03$, $P = 0.35$), or host plant (species, $R^2 = 0.05$, $P = 0.07$). Regardless of sequence processing approach (including forward vs. reverse primers, sequence clustering method, and doubling thresholds for sequence numbers), the treatments never explained more than 7% of the variance in fungal community composition. Within the final endophyte communities, only a single isolate of the original 24 added was detectable even before exclusion of singleton OTUs.

Discussion

Taken together, our results indicate that the species arrival history of plants and endophytic fungi can interactively affect seedling emergence and plant size. For example, the exotic *M. repens* rarely emerged when introduced first with endophytes from sites it occupies (disturbed sites), but these negative effects of early arrival were completely eliminated by prior introduction with endophytes from undisturbed sites or when native

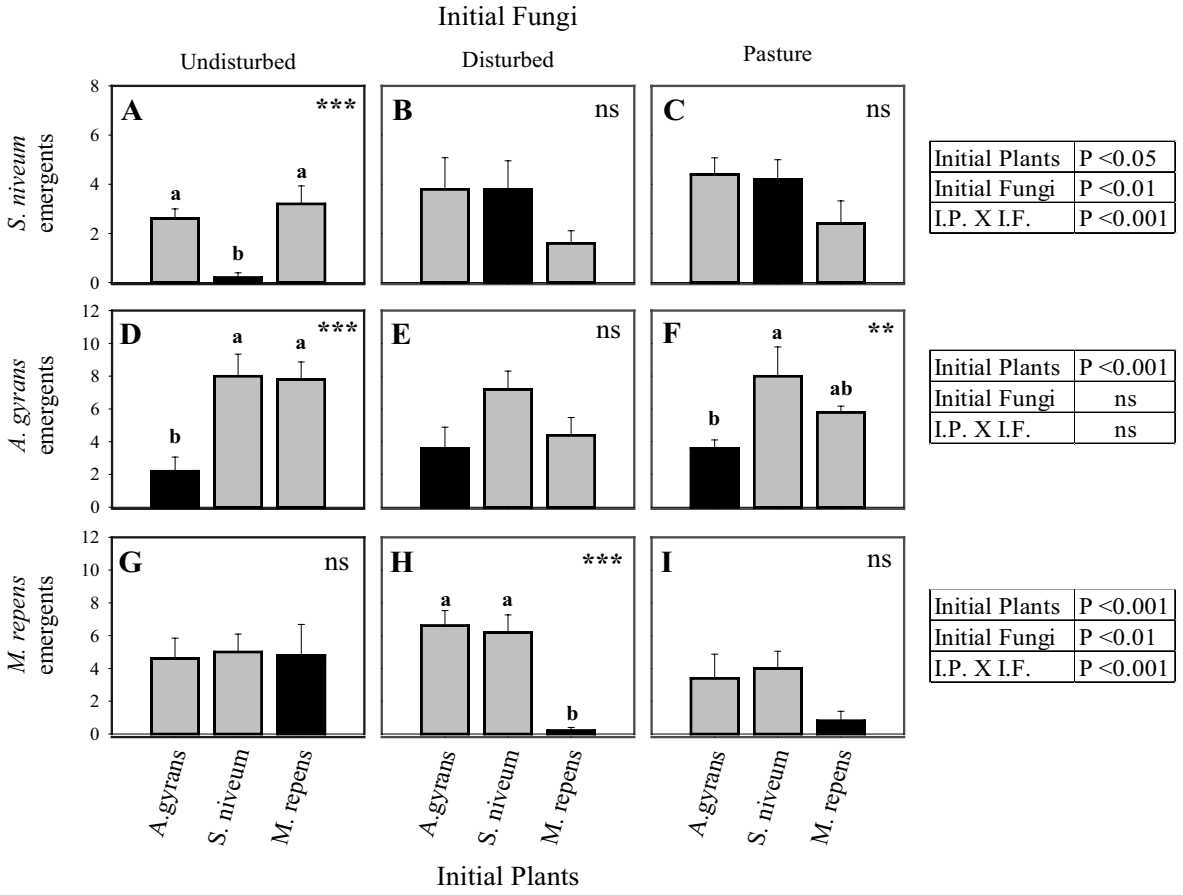

FIG. 1. Effect of plant and fungal assembly order on the seedling emergence of individual plant species. Initial plant species introduced is on the *x*-axis (*Aristida gyrans*, *Schizachyrium niveum*, *Melinis repens*). The origin of initial fungi introduced are in columns. Error bars are standard errors. Each row of the matrix is the emergence of an individual plant species. Statistical significance for treatment effects conducted for each species once across all nine treatments (three initial plants × three initial fungi) is indicated to the right of each row. Additionally, in each panel, statistical significance for ANOVAs separately conducted for the data presented within each panel is indicated. To account for multiple comparisons (i.e., nine comparisons for three initial fungi × three plant species as the response variable), we indicate significance (at $\alpha = 0.05$) after Bonferroni (B) corrections (Dunn 1961), Holm-Bonferroni (HB) corrections (Holm 1979), and False Discovery Rates (FDR) corrections (Verhoeven et al. 2005, Pike 2011). In each panel, ns means nonsignificant, * means $P < 0.05$ after FDR corrections, but not after B or HB corrections, ** means $P < 0.05$ after FDR and HB corrections, but not after B corrections, and *** means $P < 0.05$ after FDR, HB, and B corrections. Results of Tukey HSD test corresponding to these ANOVAs are indicated by different letters.

grasses were instead introduced just 2 weeks beforehand. The native grass *S. niveum* showed similar negative effects when introduced first with undisturbed fungi, which were eliminated when preceded by other fungi or if *A. gyrans* or *M. repens* was instead introduced first. The mechanisms responsible for this difference remain unclear, but one potential explanation is that a fungus that parasitizes seeds or germinants of these species changes its life-history strategy when another plant establishes first, restricting later parasitism. Fungal life-history switches by endophytes along the endophyte–pathogen–saprotroph continuum appear common in similar species (Arnold 2007, Promputtha et al. 2007). Different effects of arrival timing on emergence compared to total biomass also show that priority effects can differ between plant life-history stages. For example, *M. repens* plants that were given a head start did not suffer emergence effects when started with fungi from undisturbed sites, but still had significantly lower biomass across all head start fungal communities. In all cases, early-arriving plants paired with fungi from sites where they are absent emerged as well as, or better than, they did when they were paired with fungi from sites they normally occupy.

In other recent experiments in which fungal assembly history (but not plant assembly history) was varied, small differences in early introductions had large, lasting effects on fungal species composition (Fukami et al. 2010, Dickie et al. 2012, Peay et al. 2012). In contrast, fungal species composition at the end of the present study did not differ significantly among introduction order treatments, which could have

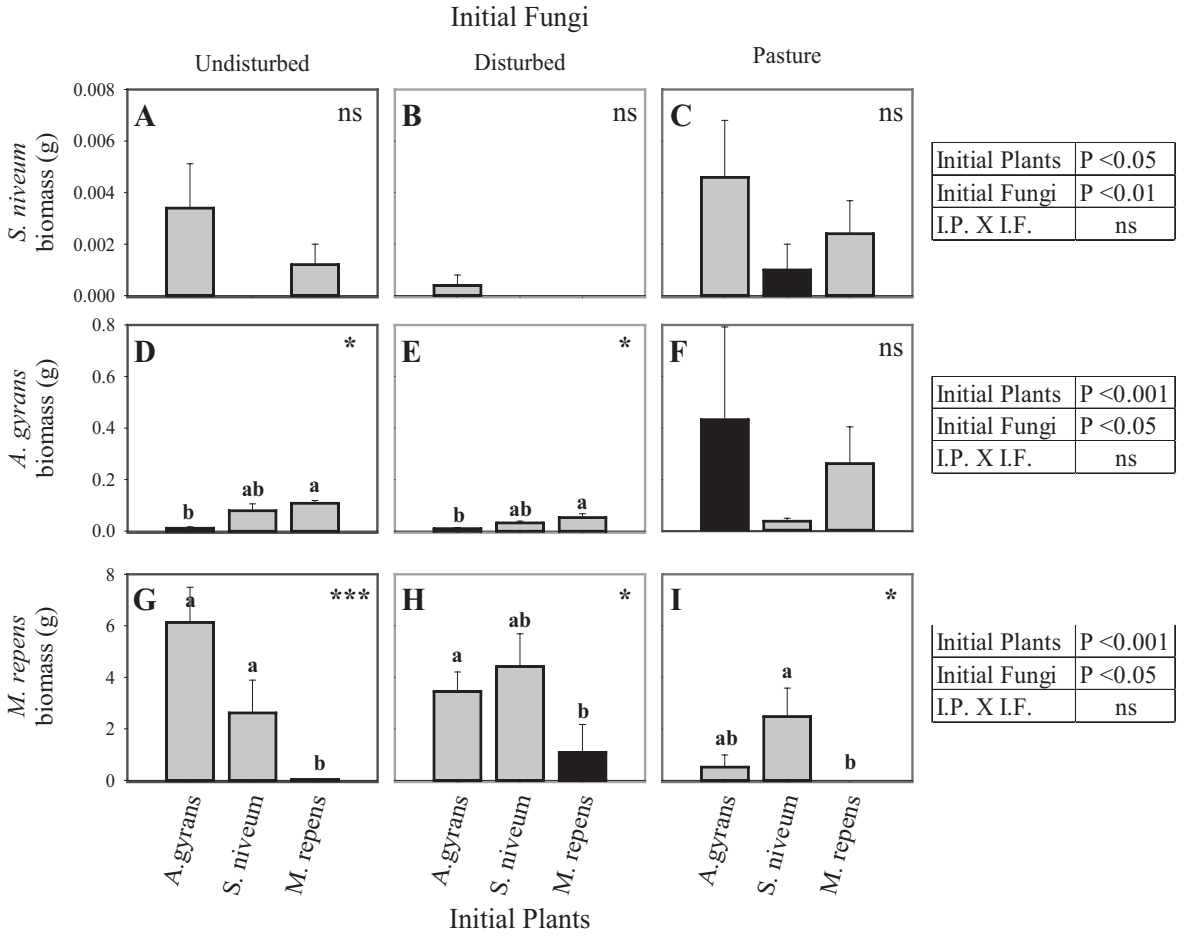

FIG. 2. Effect of plant and fungal assembly order on the final total biomass (above and belowground) of each plant species. Treatments, colors, and symbols are as in Fig. 1.

resulted from growth in a greenhouse environment where constant dispersal eventually overwhelmed starting fungal communities. At the experiment's end, soil colonization by septate fungi (which would include these fungi) similarly showed no response to assembly history (Appendix S6). Despite high variability within site types, the community composition based on head starts from pasture to disturbed to undisturbed fungi produced a consistent pattern for a given starting plant species, with the exception of *S. niveum* started with fungi from undisturbed sites (Fig. 3). This treatment combination resulted in almost no *S. niveum* emergence of biomass, which may be the reason behind this break in the pattern. We did not collect data to determine whether the original fungal treatments even established on the plants early in the experiment (as collecting such data would have required sacrificing plants). Nonetheless, it is interesting that, despite the absence of the introduced fungi at the end of the experiment, fungal introduction order still had significant effects on plants. Based on our results, we speculate that the fungal community underwent complete turnover during the course of the experiment, and that some of the introduced fungi have acted as catalytic species (sensu Warren et al. 2003), ghost species (sensu Miller et al. 2009), or springboard species (sensu Olito and Fukami 2009) to cause priority effects on plant performance. In other words, we suspect that the fungi affected plant performance without themselves persisting until the end of the experiment. Such transient dynamics correspond to facilitation in the Connell-Slatyer model of succession (Connell and Slatyer 1977), although the impact of arrival order, the hallmark of priority effects, was not explicitly considered by Connell and Slatyer (1977).

Although results of greenhouse experiments should not be uncritically extrapolated, findings from this study suggest that the arrival order of plants and fungi relative to each other may have implications for plant invasion and restoration of native Florida scrub habitats. For example, endophytes from sites long invaded may be a promising tool to limit the spread and establishment of invasive plants. Inoculating soils at invasion fronts with

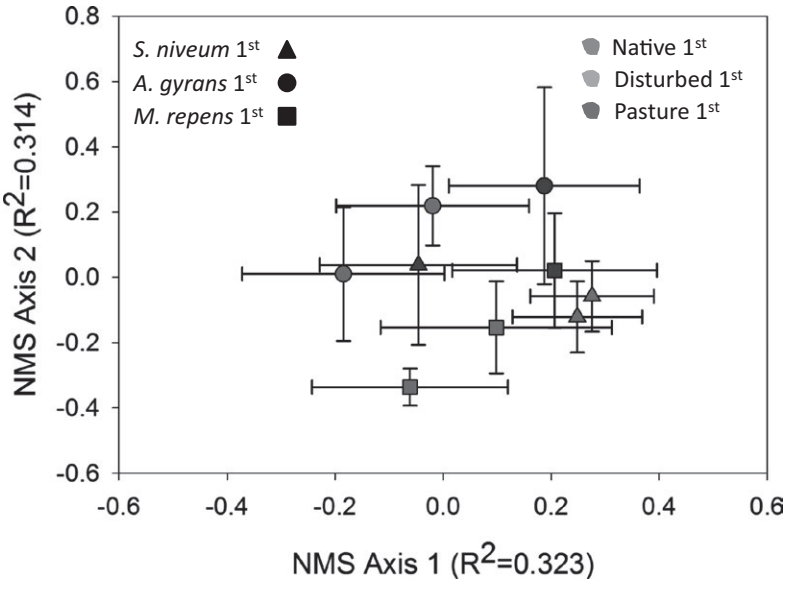

FIG. 3. Nonmetric multidimensional scaling of final endophyte communities within roots based on different plant and fungal assembly orders. Treatments and colors are as in Fig. 1. Fungal communities where different plants were given a head start are denoted with shapes: circle, *A. gyrans*; triangle, *S. niveum*; square, *M. repens*.

these endophytes may help limit the spread of new plants in Florida sites (Levine et al. 2006). However, the release from negative effects with only a short shift in introduction timing may indicate that native plantings may rapidly degrade the effectiveness of inoculations, so that the timing of native plants relative to fungal inoculation is critical for successful restoration. Thus, assembly timing should be considered to increase the effectiveness of native plant restoration (Kardol and Wardle 2010). For example, based on our data, we could recommend introducing *S. niveum* first in disturbed or pasture sites (where the fungi we used are likely already present) and then introducing *A. gyrans* second, which appears to limit its own establishment when introduced first. More broadly, reintroducing native plants with their native fungi (Harris 2009) may impede restoration if negative effects from co-introduction limit plants.

The small number of plants and fungi used in this experiment may have limited its relevance to understanding patterns present in the field. There are 122 plant species listed as characteristic of Florida scrub, many of which are shrub or sub-shrub species (Menges and Hawkes 1998). These dominant plants could exhibit priority effects (with and without fungal endophytes) that were not present in our experimental communities consisting only of grasses. For example, several endemic herbaceous species in undisturbed Florida scrub communities are found only in microhabitats created by these shrubs (Menges 1992, Hawkes and Menges 1995, Menges and Kimmich 1996, Menges et al. 1999). All three grasses we used are abundant in their respective habitats and their trait overlap may make it more likely that plant assembly history affected community composition through competitive interactions. The experiment also used only easily culturable endophytes added at similar richness among site types. Other soil fungi, such as mycorrhizal fungi or root pathogens, may have different impacts on plant assembly and may swamp out effects observed here (Kardol et al. 2007). Future studies should examine assembly history effects with more species of plants and fungi.

In summary, our results suggest that soil fungal communities should be considered when assessing the role of assembly history in plant community structuring and the success of native and exotic species. Small differences in early arrival history during the first 2 weeks of community assembly were sufficient to affect plant performance for 6 months. Differences of this magnitude in introduction timing are likely to occur in the practice of restoration or biological control simply because of logistical constraints (e.g., seeds shipping late or weather related). Actively controlling the introduction timing of plants relative to their soil biota may be a powerful, yet unrealized management tool. Improved knowledge of the role of assembly history in plant-soil interactions should inform both basic and applied community ecology.

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