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Finding partners in a habitat mosaic: Patch history and size mediate host colonization by arbuscular mycorrhizal fungi

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Abstract. Restoration of glades over the past 30 years, involving removal of woody cover and re-establishment of herbaceous plant communities, has created an archipelago of habitat patches varying in age, size, and isolation. Within these glades, habitat varies in quality from edge to core. We investigated impacts of within- and among-glade variation on the frequency of root colonization by arbuscular mycorrhizal fungi (AMF) for two host species, *Schizachyrium scoparium* and *Rudbeckia missouriensis*, and the plant community at large. We also conducted a soil analysis to explore the role of nutrient resources in mediating putative habitat effects. We used Akaike's information criterion (AIC) to evaluate a set of a priori models that assessed effects of glade size, isolation, age, and edge effects on AMF colonization. In community samples, AMF colonization increased while variation in AMF colonization decreased with restoration age. Edge effects also reduced plant community AMF colonization. AMF colonization was higher in *R. missouriensis* than in *S. scoparium*, but increased for both species in core habitat. Glade size had no direct effect on AMF at the plant scale, but indirectly affected overall AMF occupancy at the landscape scale via its geometric relationship to glade edge:core ratio. Hosts in small glades that are dominated by edge exhibit lower occupancy rates. The first two axes in a principal components analysis (PCA) combining soil nutrient variables explained 74% of sample variance: PC1 correlated positively with organic matter (OM), total nitrogen (N), calcium (Ca), magnesium (Mg), and potassium (K) and decreased from the edge to the core of older glades. PC2, which correlated positively with pH and negatively with available P, increased with time since restoration. While results are consistent with a role of soil properties in explaining edge and age effects, the two PCA dimensions themselves are weakly correlated with AMF colonization. Overall, our findings suggest that environmental filters associated with woody encroachment limit the colonization of otherwise suitable herbaceous hosts and create a landscape mosaic with larger, older glades serving as hotspots or reservoirs of AMF root colonization and smaller, newly restored glades as cold spots or potential sinks.

Key words: arbuscular mycorrhizal fungi; edge effect; glades; grasslands; restoration history.

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INTRODUCTION

Habitat patch characteristics may indirectly alter the distribution of organisms through impacts on their symbiotic partners, especially when the relationships are specialized (Schultz 1998, Schultz and Crone 2001) and/or obligate (Carvalho et al. 2003). In a fragmented landscape, species diversity, richness, and abundance are largely determined by habitat characteristics such as patch size (Hill et al. 1996, Honnay et al. 1999, Wahlberg et al. 2002), successional age (Honnay et al. 1999, Hastings 2003, Dickie et al. 2013), isolation (Fahrig and Jonsen 1998,

Michalski and Peres 2007, Peay et al. 2010), and edge effects (Matlack 1994, Murcia 1995, Dickie et al. 2005). Effects of patch characteristics on population and community dynamics depend on traits of the organism (Marsh and Pearman 1997, Collins et al. 2009) as well as distributions of its mutualists (Uezu et al. 2005, Dickie et al. 2013) and enemies (Hovel and Lipcius 2001). For example, the Fender's blue butterfly changes behavior and flight pattern depending on the proximity of habitat patches that contain its host plant (Schultz 1998), and the microbial community of flower nectar depends on how patch connectivity affects pollinators (Belisle et al. 2012).

Arbuscular mycorrhizal fungi (AMF) are drivers of plant community structure, aboveground diversity, and productivity in grassland ecosystems (van der Heijden et al. 1998, Hartnett and Wilson 1999, Klironomos et al. 2000, O'Connor et al. 2002). AMF are obligate symbionts, relying completely on host plant provisioning for carbon resources while providing plants with myriad benefits including nutrients and water in return. Habitat patch characteristics that influence the distribution of plant hosts may also influence dispersal, recruitment, and distribution of AMF both directly and indirectly (Smith and Read 2008). Recruitment of mycorrhizal fungi into newly opened habitat patches is beneficial for the establishment of the plant community, yet mycorrhizal fungal recruitment depends reciprocally on the presence of plant hosts (Janos 1980, van der Heijden 2004, Zhang et al. 2011). AMF disperse via spores or extraradical hyphae through the soil (Warner et al. 1987, Friese and Allen 1991, Staddon et al. 2003). Grilli et al. (2012) found that AMF root colonization, spore diversity, and spore abundance increased in larger forest patches within an agricultural matrix. Although AMF spores vary in size and dispersal ability, their generally large mass suggests movement by wind is ineffective (Warner et al. 1987, Egan et al. 2014) and, instead, that animals are the main dispersal agents (Trappe and Maser 1976, Lekberg et al. 2011). Habitat patch size, isolation, and restoration stage may influence disperser abundance and mobility as well as availability of plant hosts, thus influencing AMF–plant interactions in a fragmented landscape.

Arbuscular mycorrhizal fungi root colonization may also vary in frequency within and among habitats in fragmented landscapes due to environmental factors affecting fungal costs and benefits to host plants (Lekberg et al. 2007, Dumbrell et al. 2010, Dickie et al. 2013). Variation in light (Hayman 1974, Heinemeyer et al. 2004, Johnson et al. 2015), soil nutrient levels (Johnson et al. 2015), and soil pH (Lekberg et al. 2011) influence AMF–host interactions and thus endosymbiont population size. As habitat patches age, successional changes alter their quality for colonizing species (Wahlberg et al. 2002, Ranius et al. 2008). For example, in grassland systems, soil C and N increase and light intensity decreases with woody cover (Zavaleta and Kettley 2006, Liao et al. 2008). Such resource transitions alter AMF–plant interaction frequency and identity (Janos 1980, Kardol et al. 2006, Dickie et al. 2013). Environmental variation might also decrease through succession. For example, insect populations varied more in early successional compared to later successional forest stands (Niemala et al. 1996). Successional trajectories can vary spatially (Turner et al. 1998), and variation in the rate of change in woody cover and soil nutrients among sites could lead to more variable AMF–plant interaction frequencies early in succession.

Glades are patchily distributed habitats in the Ozark Mountains of the south-central USA (Templeton et al. 1990, Ryberg and Chase 2007, Miller-Struttmann 2013). Spatial patterns of AMF within- and among-glade habitats are not well understood. Variation in amount and chemistry of leaf litter (Read and Perez-Moreno 2003, Becklin et al. 2012), soil nutrients (Averill et al. 2014, Johnson et al. 2015), and canopy cover (Hayman 1974, Heinemeyer et al. 2004, Johnson et al. 2015) may directly or indirectly promote heterogeneity among glades in mycorrhizal interactions. Glade persistence depends on fire disturbance regime. Fire suppression beginning in the 18th century allowed *Juniperus virginiana* (eastern red cedar) and other woodland species to invade glade openings (Nelson 2010). Restoration of Missouri glades, which began over 30 years ago, has involved removing *J. virginiana* and reintroducing frequent fire. Removal of *J. virginiana* generally returns the glade to grassland over time (Appendix S1: Fig. S1). *J. virginiana*, like the majority of glade plant species, associates with AMF (Wang and Qiu 2006). However, changes in environmental conditions during invasion of

J. virginiana and other woody vegetation may alter costs and benefits of AMF interactions in re-colonizing glade plant species. Furthermore, while glades early in the restoration process may vary in openness, woody cover, and soil characteristics, conditions in later successional sites are more likely to converge on open glade-like environments. We hypothesize that this gradual replacement of dominant plant species, concomitant shifts in resources for plant growth, and approach to a narrower range of resource states should influence prevalence and variance in plant–AMF interactions. Here we address these ideas using glade habitat patches restored over the past 30 years in the Missouri Ozarks.

As fragmented habitats, glades exhibit a range of connectivity that may alter the dispersal potential of AMF and their host plants (Miller et al. 2015). Glades also vary in size, from small forest openings (ca. 200 m^2) to large expansive hillsides (over 50,000 m^2). AMF colonization may depend on habitat patch isolation and size given dispersal limitation. For example, if fungal spores disperse more frequently into closer and larger patches as expected with isolation by distance and random movement, then AMF should be more abundant in large contiguous glades than smaller, more isolated ones. However, larger habitats may also contain more AMF because they have proportionately lower edge:core ratio, diminishing potential negative impacts of woody edge species (Hayman 1974, Heinemeyer et al. 2004, Johnson et al. 2015). Here we sample AMF in edge and core microhabitats within glades to determine whether larger glades contain more AMF and whether this trend reflects dispersal limitation or edge effects on habitat quality.

We predict that inter-glade variation in age, size, and isolation will explain patterns of AMF colonization. Specifically, we test for decreasing frequency of AMF in younger, smaller, and more isolated glades and decreasing variation in AMF frequency through successional time. Second, we predict that if costs of hosting AMF decrease with distance from woody vegetation and benefits in nutrient economy of plants hosting AMF increase, then plants in core glade habitat will host more AMF than those at the edge. Last, we explore the potential role of soil properties in mediating variation in AMF colonization at both local and landscape scales.

Methods

Study system

The dry, thin soil of glades, although not necessarily nutrient poor (Crabtree et al. 2010), is consistent with conditions where mycorrhizal fungi are beneficial for plant growth and survival. Many glade plant species also occur in prairies (Erickson et al. 1942, Baskin and Baskin 2000, Van Zandt et al. 2005) where they rely on AMF for nutrients (Wilson and Hartnett 1997, Wang and Qiu 2006). Sites used in this study contain soils of fairly neutral pH (6.6–7.5) and high levels of bases (e.g., calcium (Ca), ranging from 1342 to 2980 mg/kg) due to the exposed calcareous substrate (Pallardy et al. 1988, Crabtree et al. 2010).

We selected 26 glade sites in central and south-central Missouri (USA; Fig. 1a) that vary in restoration age (0–30 yr) and size (258–54,710 m^2; Appendix S2: Table S1). Glades that have not yet been restored were denoted by age zero, while glades denoted by age 30 were restored at least 30 years ago and at that time were not completely degraded. Glade size was estimated by measuring total area using shape files over an ArcGIS 2010 built-in basemap layer (ESRI 2010). We also used ArcGIS 2010 to enumerate the number of neighboring glades penetrating into a 1 km radius surrounding each focal glade (ESRI 2010) as an index of isolation that is highly correlated with the proximity index used by Miller et al. (2015) (Pearson's $r = 0.85$, $n = 8$, $P = 0.01$). We used Pearson's correlation coefficients to test for linear correlations among landscape variables.

In each glade, we collected root samples from two widespread host species, *Rudbeckia missouriensis* (*Asteraceae*) and *Schizachyrium scoparium* (*Poaceae*), to examine effects of landscape features and habitat quality on AMF colonization, without potentially confounding variation in host identity. *R. missouriensis* and *S. scoparium* are dominant plant species in calcareous glades (Baskin and Baskin 2000). In tallgrass prairie, *S. scoparium* depends almost completely on AMF for survival (Anderson et al. 1994, Wilson and Hartnett 1998, Wang and Qiu 2006). Although AMF of *R. missouriensis* have not yet been studied, other *Rudbeckia* form AMF partnerships (Wilson and Hartnett 1998, Klironomos 2003). Species-specific collections were supplemented with rhizosphere

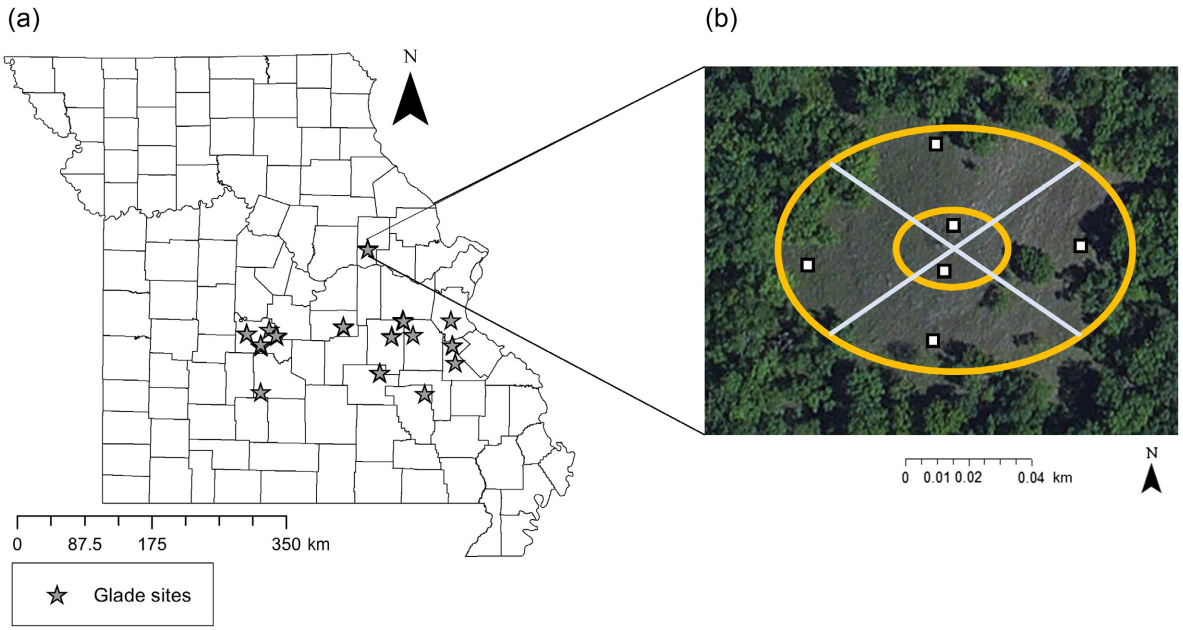


Fig. 1. (a) Locations (indicated by stars) of 26 glades sampled in this study. *Schizachyrium scoparium* and *Rudbeckia missouriensis* were found, respectively, in 21 and 23 of these sites. (b) Sampling locations within glades for soil and root collections. Core samples were collected in the center 10% as indicated by the smaller yellow oval; edge samples were collected about 4 m inside of the perimeter of each glade at points (indicated by boxes) aligned with the four cardinal directions.

samples taken at random points from the plant community, because glades filled with *J. virginiana* do not contain either *S. scoparium* or *R. missouriensis* and two new restoration sites did not contain *S. scoparium*.

AMF enumeration

In late June, July, and early August of 2011, we collected root samples at random points from the plant community and from individual plants of *S. scoparium* and *R. missouriensis* in the center 10% of the glade (core habitat) and within 4 m of the glade/woodland transition boundary (edge habitat). Sampling design differed between habitat zones because edge is more extensive than core. More points ($n = 4$ vs. 2 per glade) spaced further apart (aligned with each cardinal direction from the glade center vs. 3 m part at the center; Fig. 1b) were sampled, respectively, in edge than in core habitat.

At each sampling point (Fig. 1b), we collected all roots in a soil block approximately 10 cm deep by 5 cm in diameter at the center of a 1-m^2 plot. These community samples were collected as deep as possible given the underlying substrate, which was typically less than 10 cm. We also collected the closest *S. scoparium* and *R. missouriensis* plants to each community sample, excavating above- and belowground biomass. In total, we collected samples at six points per site totaling 156 community samples, 144 *R. missouriensis* samples (absent from two *J. virginiana*-filled glades), and 132 *S. scoparium* samples (absent from two *J. virginiana*-filled glades and two new restoration sites).

Ten randomly chosen one cm length root sections were collected from community samples as well as each plant of *S. scoparium* and *R. missouriensis*. Root sections were stained with trypan blue solution (Gange et al. 1999) and examined at 400× magnification under a light microscope to visualize AMF colonization. Positive colonization events were scored when any of the following were observed: arbuscules, vesicles, or coils. We calculated proportion of root length colonized by AMF as the number of fields of view with colonizing structures divided by 100 views per sample.

Soil analysis

Glade soil is shallow and sparse, so it is important to limit soil removal when excavating plants in these sites. We worked carefully with local conservation agencies to ensure little disturbance. Because of this, we limited our soil samples for nutrient analysis to 12 sites. For each of the 12 glades varying in restoration age, we sampled soil at two points in the core habitat and two points in the edge. Soil cores from each habitat/site were pooled before analysis. Because damage to these protected sites was a concern, soil collections were restricted, and we used all available samples over 2 years (2011–2012) for this study. Five glades were sampled in 2011, six in 2012, and two in both years. We measured available phosphorous (P) (Bray I method), potassium (K), calcium (Ca), magnesium (Mg), and percentage of organic matter (OM). The Bray I method for measuring available soil P is generally used for soils under a pH of 7.4 (Sawyer and Mallarino 1999). Eight of the 37 samples tested had pH slightly above the recommended range for the Bray I analysis and thus were also tested using Mehlich III analysis for P. As results were equivalent, we report P based on the Bray I test. Total N was determined by combustion at 950°C using a LECO TruSpec CN Analyzer (Leco Corp., St. Joseph, Michigan, USA). The University of Missouri Soil and Plant Testing Laboratory conducted all analyses besides total N.

Statistical analysis

Variation among and within glades in AMF colonization.—When there are numerous variables and combinations of variables, Akaike's information criterion (AIC) allows testing of multiple models with different combinations of variables that address multiple hypotheses (Burnham and Anderson 2002). To explore how glade size, restoration age, isolation, and edge effects influence AMF colonization, we developed a set of a priori linear mixed effect models in the nlme package in R and compared them using AIC (Burnham and Anderson 2002, Pinheiro et al. 2015; Appendix S3). We also calculated the Akaike weight (ω_i) to determine the relative importance of each variable (Burnham and Anderson 2002). By calculating ω_i for each variable, we were able to compare the relative importance of each variable and interaction term (Burnham and Anderson 2002). While collection date was a significant ($P < 0.01$) predictor of colonization in the plant community samples, it did not predict colonization on *R. missouriensis* or *S. scoparium* ($P > 0.10$ for both), and including collection date did not change the main trends from the analysis of community samples. Accordingly and for ease of comparison among models, we did not include collection date in the analysis of community AMF colonization.

Community AMF colonization was analyzed using a different set of a priori linear mixed effect models than species-specific counts. Candidate models for AMF colonization in community root samples included biologically relevant sets of fixed effects and interactions between these fixed effects (Table 1). The structure of each candidate model is explained in Appendix S3. Here and elsewhere, site was included as a random effect in all models. We also calculated coefficient of variation (CV) in community AMF colonization for each site and assessed the change in CV across restoration age using linear regression.

For the analysis of AMF colonization in roots of *R. missouriensis* and *S. scoparium*, we included species identity and its interactions with other effects in the model to assess whether AMF colonization varied between host species independently or due to species interactions with habitat type, glade restoration age, glade size, and glade isolation (Table 1). AMF colonization was logit-transformed ($\log(y/[1 - y])$) to meet the assumptions of normality (Warton and Hui 2011). The structure of candidate models is explained in Appendix S3.

Model fit for all linear mixed effect models was calculated by regressing the values predicted by the model on the original data (R^2), using the lm function in R (R Core Team 2013). To calculate variance among sites, we subtracted the marginal R^2 from the conditional R^2. Marginal R^2 is the variance explained by the fixed effects only, while the conditional R^2 is the variance explained by both fixed and random effects. The conditional and marginal R^2 were calculated using the r.squaredGLMM function in the R package MUMIn (Barton 2015). We considered supported models as those with a $\Delta AIC_c < 4.0$.

Variation in AMF abundance with glade size at the landscape scale.—Although we recognize that AMF root colonization is only one way to measure abundance of AMF, and that AMF species differ in allocation patterns among spore production,

Table 1. Akaike's information criterion (AIC) model selection for analysis of variation within and among glades in arbuscular mycorrhizal fungi (AMF) colonization.

Model	AIC_c	ΔAIC_c	df	ω_i
Community AMF colonization				
Age + HT + (1|site)	−37.8	0.0	5	0.340
Age + HT + (Age × HT) + (1|site)	−37.1	0.7	6	0.234
Age + (1|site)	−36.7	1.1	4	0.196
HT + (1|site)	−34.6	3.2	4	0.068
Iso + (1|site)	−34.6	3.2	4	0.067
Null model: 1|site	−33.5	4.3	3	0.039
Iso + Size + (1|site)	−32.8	5.0	5	0.028
Size + (1|site)	−31.8	6.1	4	0.016
Iso + Size + (Iso × Size) + (1|site)	−30.7	7.2	6	0.010
Iso + Size + Age + (Iso × Size) + (Iso × Age) + (Size × Age) + (Iso × Size × Age)	−27.9	9.9	10	0.002
Full model	−22.2	15.6	17	<0.001
Plant species-specific AMF colonization				
Species + HT + (1|site)	643.2	0.0	5	0.522
Species + HT + (Species × HT) + (1|site)	645.2	2.1	6	0.185
Species + (1|site)	645.7	2.6	4	0.144
Species + Size + (1|site)	647.3	4.1	5	0.066
Species + Iso + (Species × Iso) + (1|site)	649.1	6.0	6	0.026
Species + Size + (Species × Size) + (1|site)	649.4	6.2	6	0.023
Species + Age + (Species × Age) + (1|site)	649.4	6.2	6	0.023
Species + Age + HT + (Species × Age) + (Species × HT) + (Age × HT) + (Species × Age × HT) + (1|site)	652.1	8.9	10	0.006
Species + Iso + Size + (Species × Iso) + (Species × Size) + (Iso × Size) + (Species × Iso × Size) + (1|site)	653.5	10.3	10	0.003
Full model	665.0	21.9	17	<0.001
HT + (1|site)	770.4	127.2	4	<0.001
Null Model: (1|site)	771.2	128.1	3	<0.001
Size + (1|site)	773.0	129.8	4	<0.001
Iso + (1|site)	773.2	130.0	4	<0.001
Age + (1|site)	773.2	130.1	4	<0.001
HT + Age + (HT × Age) + (1|site)	774.4	131.3	6	<0.001

Note: Full model for AMF community includes age (restoration age), size (glade area), iso (isolation, glades in radium of 1 km), HT (habitat type, cover vs. edge), all two- and three-way interactions, and site as a random variable. Full model for plant species-specific AMF colonization includes age, size, iso, species (plant species identity), all two- and three-way interactions, and site as a random variable.

extraradical hyphae, and internal root colonization (Hart and Reader 2002, Hempel et al. 2007), we postulated that all else being equal, if AMF root colonization is sensitive to reductions in habitat quality from core to edge (see *Results*, Figs. 3 and 4), then AMF abundance at the landscape scale would be sensitive to glade size as larger glades have proportionately less edge than smaller glades (Fig. 2). In other words, at the landscape scale, the abundance of AMF is dictated by the amount of a given habitat as well as the suitability of that habitat for AMF. To explore the magnitude of these landscape scale impacts, we created a simple model where we assume that the pool of AMF within the glade community or AMF glade occupancy (O) is directly related to the quality of that rhizosphere habitat such that:

$$O = (P_e C_e) + (P_c C_c)$$

where P_e represents the proportion of the glade containing edge habitat, C_e represents the edge-specific AMF colonization, and the product $P_e C_e$ thus describes the contribution of edge habitat to the glade's AMF pool. Similarly, P_c represents the proportion of the glade containing core habitat, C_c represents the core-specific AMF colonization, and the product $P_c C_c$ describes the contribution of core habitat to the glade's AMF pool.

We used the shape files used in the initial analysis of glade size (area) in ArcGIS 2010 to further

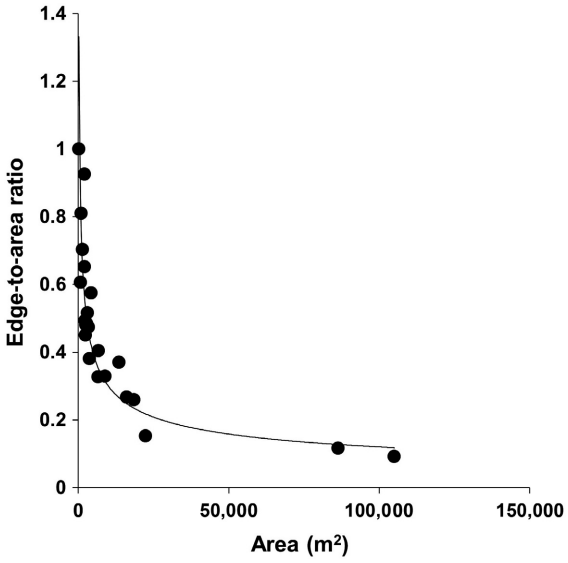


Fig. 2. Edge-to-area ratio decreases exponentially as total glade area increases.

calculate edge and core area. A second shape file was created using the buffer tool to border the core habitat, 4 meters into the glade from the edge. The area inside this shape was considered core habitat, and we calculated edge habitat by subtracting core habitat from the total area. We then used this to create an edge area to total area ratio (P_e) and core area to total area ratio (P_c) for each site. We then created average edge AMF colonization (C_e) and average core AMF colonization (C_c) for each site. These numbers were used in our equation for AMF abundance at a glade scale ($O = (P_eC_e) + (P_cC_c)$). We examined how edge:area ratio impacted AMF occupancy (O) for community, *R. missouriensis*, and *S. scoparium* root systems using separate linear models in R (R Core Team 2013).

Variation in soil composition.—Soil properties from the two sites sampled in both 2010 and 2011 were not significantly different between collection years ($P > 0.05$) and were averaged across years prior to the analysis. Principal components analysis (PCA) was used to identify axes of correlated variation in soil pH, percentage of OM, total N, available P, Mg, Ca, and K (Gauch 1982, Belsky et al. 1989; Appendix S4: Fig. S1). The analysis identified two PCA dimensions with eigenvalues greater than 1.00 (Table 3; Appendix S4: Table S1). We used linear mixed effect models from Type III ANOVA, and the least square means function in Proc Mixed (SAS 9.4) to test for variation in PC1 and PC2 with restoration age and habitat type, with site as a random effect. For one glade sampled in 2012, precise time of restoration was unknown, although we were able to assess that it had been restored within the last 10 years. There was a six-year gap in restoration age (between 11 and 16 yr since restoration) for the sites used for the soil analysis. Because of this age gap, lack of information for precise restoration age in one site, and because our sample size was restricted due to management concerns, we used a categorical analysis that allowed us to include all possible sites: Glades less than 15 years old ($n = 5$) were considered "newer restorations", and glades more than 15 years old ($n = 7$) were considered "older restorations." Scores on PC1 were square-root-transformed to fit the assumptions of normality. Post hoc least square means tests were used to test for differences in soil PCA dimensions between edge and core habitats of new and old glades. PCA was conducted in the R package FactoMineR (Husson et al. 2015).

Correlation of soil properties with AMF abundance.—We used Spearman rank correlation coefficients (due to non-normal distributions) to test for associations of community AMF colonization with PC1 and PC2. We pooled species-specific AMF colonization for this analysis. Pooling was carried out to increase sample size and power of the correlation analyses. Variation due to host taxon was removed statistically using residuals from a linear model that accounted for effects of host taxa on AMF colonization (Table 1). We then calculated Spearman correlation coefficients between these residuals and each soil dimension (PC1 and PC2). Correlation coefficients were calculated in the cor function, and all P-values were obtained using the cor.test function in R (R Core Team 2013).

Results

Variation among and within glades in AMF colonization

Root colonization by AMF varied at two spatial scales: among glades and between edge and core habitats within them. The proportion of root length colonized by AMF in the plant

Table 2. Weight for variables in Akaike's information criterion models.

Fixed effect	ω_i
Community	
Age	0.77
HT	0.64
HT × Age	0.23
Iso	0.11
Size	0.06
Iso × Size	0.01
Iso × Age	<0.01
Size × Age	<0.01
Iso × Age × Size	<0.01
HT × Size	<0.001
HT × Iso	<0.001
Iso × Age × HT	<0.001
Iso × Size × HT	<0.001
Age × Size × HT	<0.001
Species specific	
Species	1.00
HT	0.71
Species × HT	0.19
Size	0.09
Iso	0.03
Age	0.03
Species × Iso	0.03
Species × Size	0.03
HT × Age	0.02
Species × Age	0.02
Species × HT × Age	<0.01
Iso × Size	<0.01
Species × Iso × Size	<0.01
HT × Iso	<0.001
HT × Size	<0.001
Iso × Age	<0.001
Age × Size	<0.001
Species × HT × Size	<0.001
Species × HT × Iso	<0.001
Species × Age × Iso	<0.001
Species × Age × Size	<0.001

Note: For explanation of variable names, see Table 1.

community averaged 0.50 ± 0.22 (SD). The best supported model explaining among- and within-glade variation in community AMF colonization contained habitat type and restoration age ($AIC_c = 0$, $\omega_i = 0.37$, $R^2 = 0.34$, Table 1). The next best supported model included restoration age, habitat type, and their interaction ($\Delta AIC = 0.7$, $\Delta AIC_c = 0.4$, $\omega_i = 0.23$, $R^2 = 0.32$, Table 1). Restoration age explained most of the variation in community AMF colonization ($\omega_i = 0.77$) followed by habitat type ($\omega_i = 0.64$, Table 2). AMF colonization was higher in core vs. edge habitat (Fig. 3a) and increased with restoration age (Fig. 3b). Among-site differences explained 11–21% of the variance in AMF colonization depending on the model. Variation in colonization also decreased significantly with restoration age (Fig. 3b, for the change in CV; $R^2 = 0.4$, $F = 17.4$, $P < 0.001$).

The three most supported models for species-specific AMF colonization (ΔAIC_c of less than 4.0) contained host species, habitat type, and their interaction. The most supported model contained only host species and habitat type ($\Delta AIC_c = 0$, $\omega_i = 0.52$, $R^2 = 0.52$, Table 1). Mean (±SD) proportion of root length colonized by AMF was higher for *R. missouriensis* (0.76 ± 0.17 SD) than for *S. scoparium* (0.51 ± 0.16; Fig. 4a). AMF colonization for both host species was higher in core than in edge habitat (Fig. 4b). Among-site variation explained 12–13% of the variance in AMF colonization depending on the model.

Variation in AMF occupancy with glade size at the landscape scale

Fig. 5 shows how glade occupancy by AMF (*O*) varies with edge:area ratio and thus glade size for *S. scoparium*, *R. missouriensis,* and the plant community. As the edge:area ratio increases in small glades, *O*, or glade occupancy by AMF, decreases at the landscape scale (Fig. 5). Thus, the relationship of glade size to AMF abundance is an indirect outcome of deleterious edge effects on AMF colonization.

Variation in soil composition

Principal components analysis identified two factors with eigenvalues greater than 1.00. PCA dimension 1 (PC1) explained 51.3% of the variance and was positively correlated with total N, percentage of OM, available Ca, K, and Mg (Table 3). PC2 explained an additional 22.4% of the variance and was positively correlated with pH and negatively correlated with available P (Table 3).

For PC1, we found a significant interaction effect between habitat type and restoration age ($F_{1,11} = 7.7$, $P = 0.02$). Soil nutrients associated with PC1 did not vary significantly between edge and core habitat in newly restored glades, but did in older glades (Table 4, Fig. 6a) where nutrient concentrations were lower in core than in edge habitat (Fig. 6a). PC2 (increasing pH and decreasing available P) also varied significantly with glade

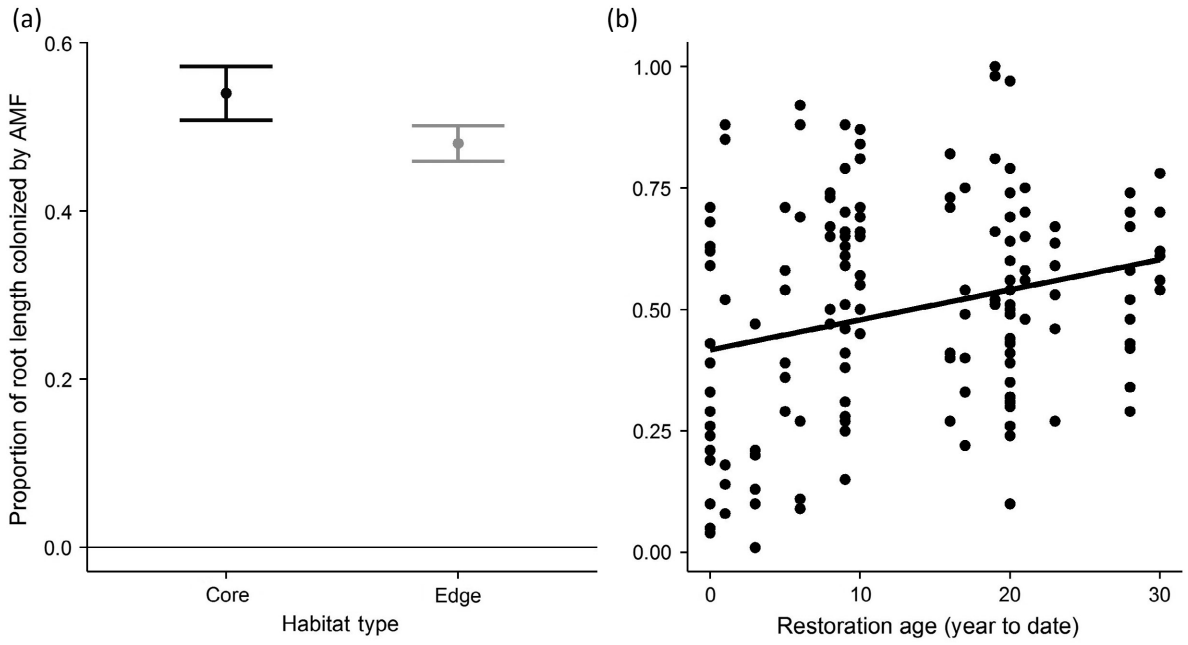


Fig. 3. Proportion of root length colonized by arbuscular mycorrhizal fungi (AMF) for roots of unknown species in the plant community. (a) Mean (±SE) colonization in core and edge habitats ($P = 0.07$, $\omega_i = 0.64$), and (b) relationship of AMF colonization to restoration age ($R^2 = 0.32$, $P = 0.02$, $\omega_i = 0.77$). *P*-values for restoration age and habitat type were obtained from the most highly supported Akaike's information criterion model (colonization = age + HT + (1|site)).

age ($F_{1,11} = 15.0$, $P = 0.003$), indicating that older sites have less available phosphorous and higher pH than newer ones (Table 4, Fig. 6b). AMF colonization in roots from the plant community did not correlate with PC1 ($\rho = 0.31$, $P = 0.12$, $n = 26$) or PC2 ($\rho = 0.30$, $P = 0.14$, $n = 26$). Similarly, AMF colonization of species-specific root samples showed no significant correlation with either axis of soil variation ($P > 0.1$, $n = 50$ for both).

Discussion

Our study indicates that the frequency of interactions between AMF and their host plants shifts across restoration history and from core to edge habitat, recognizing that other sources of environmental complexity may influence AMF distributions in this system. Our results contrast with research in forest fragments, where AMF colonization and patch size are positively correlated (Grilli et al. 2012), but agree with findings of Bahram et al. (2015), suggesting that AMF communities are limited more strongly by environmental conditions rather than dispersal. Although many glade plant species are rare to absent in surrounding woodlands, some woody species (e.g., *Juniperus virginiana*) and understory species host AMF (Wang and Qiu 2006, Egan et al. 2014), potentially reducing the isolation effect.

We predicted that after removal of woody *J. virginiana*, herbaceous hosts should have a greater range of niche space for exploiting AMF networks, and AMF colonization of glade host species should increase accordingly. Although *J. virginiana* hosts AMF (Wang and Qiu 2006), it shows an increase in AMF biomass in winter months when herbaceous vegetation is dormant (Coppick 2009), suggesting *J. virginiana* may host an AMF community more conducive to its evergreen life history. The gradual increase in community AMF colonization samples with time since restoration is consistent with this view. However, AMF of the widespread glade generalist species *S. scoparium* and *R. missouriensis* are less sensitive to restoration age than the community at large. The discrepancy between AMF colonization in the community vs. in these

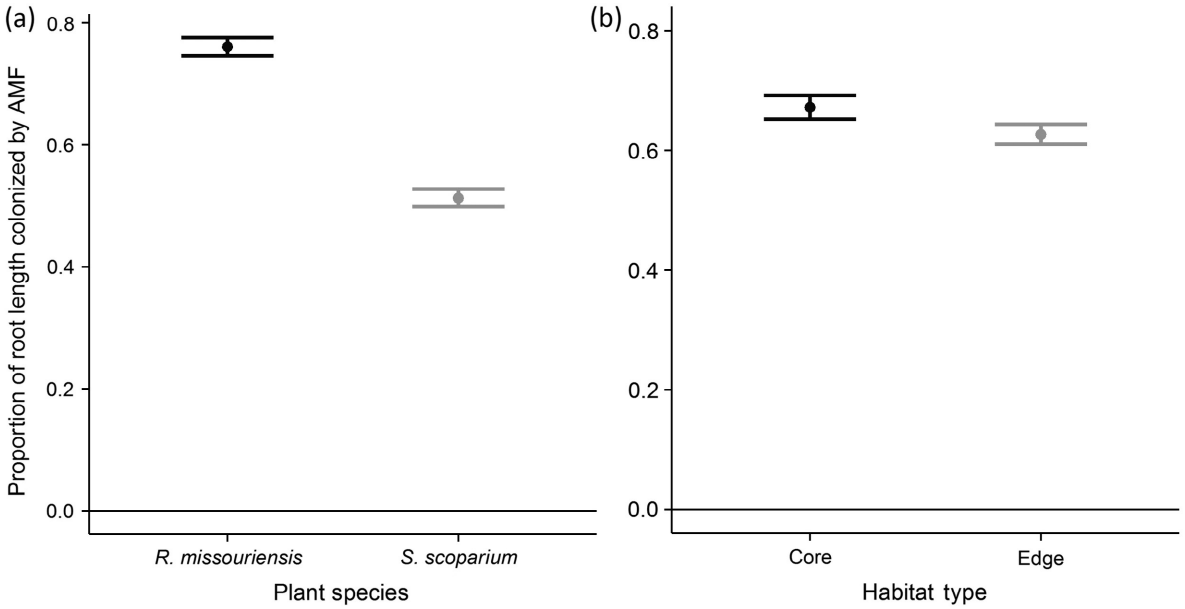


Fig. 4. (a) Proportion of root length colonized by arbuscular mycorrhizal fungi (AMF) for *Rudbeckia missouriensis* and *Schizachyrium scoparium* ($P < 0.001$, $\omega_i = 1.00$) and (b) variation in proportion of root length colonized by AMF between core and edge habitat for *R. missouriensis* and *S. scoparium* pooled ($P = 0.03$, $\omega_i = 0.71$). Logit transformation of colonization was used in the analysis; however, raw values are plotted for ease of interpretation. The *P*-values given are associated with the most highly supported Akaike's information criterion model (logit (colonization) = species + HT + (1 | site)).

generalist host species is consistent with the idea that vegetation change with succession is driving the increase in community-level colonization (Koziol and Bever 2015). Increasing community AMF colonization with restoration age is also consistent with the finding of stronger dependence on mycorrhizal fungi in more specialized, later successional grassland species (Janos 1980, Kardol et al. 2006, Middleton and Bever 2012, Koziol and Bever 2015).

Variation in AMF colonization decreased with restoration age (Fig. 3b), suggesting that glade plant communities and their interactions with AMF become more uniform through time. This could be due to consistently hotter, open environments later in restoration compared to patchy openings earlier in succession, when woody encroachment and canopy cover are more dominant and variable among sites.

Arbuscular mycorrhizal fungi colonization decreases from core to edge habitat in both the species-specific (Fig. 4b) and community samples (Fig. 3a). These results are consistent with earlier studies showing decreasing AMF root colonization with tree basal area for *S. scoparium* growing across a sand prairie forest gradient (Benjamin et al. 1989). Changes in the cost/benefits ratio of AMF endosymbionts for host plants between edge and core habitat likely drive this trend. AMF colonization decreases with shade and its inhibitory impact on carbon assimilation (Hayman 1974, Heinemeyer et al. 2004, Johnson et al. 2015). Increased canopy cover in edge habitat reduces light availability, thus potentially increasing costs of AMF to understory glade plants (Hayman 1974, Heinemeyer et al. 2004, Johnson et al. 2015). Increasing AMF in core habitat may also reflect release from abundant and/or persistent leaf litter of EMF hosts at the forest border (Read and Perez-Moreno 2003). For *R. missouriensis* and *S. scoparium*, the structure of the surrounding plant community could also contribute to decreasing AMF colonization over the core:edge transition gradient (Meadow and Zabinski 2012). Both woody encroachment and herbaceous neighborhood potentially drive spatial variation in AMF colonization within these Ozark glades, setting up a discontinuity in habitat

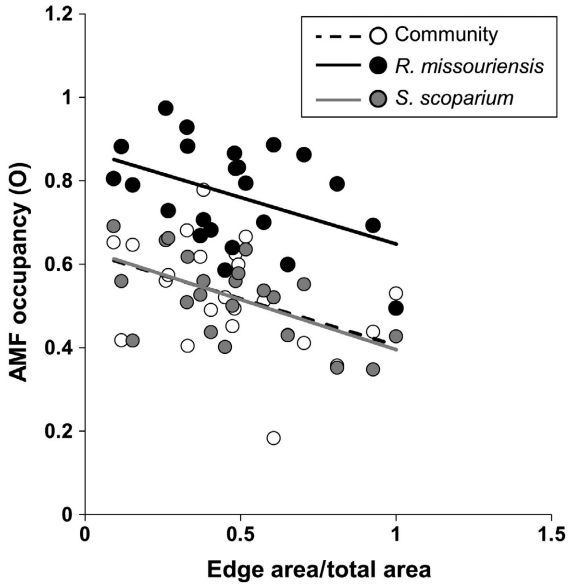


Fig. 5. Arbuscular mycorrhizal fungi (AMF) occupancy (*O*) within glades decreases as edge:area ratio increases. Open circles and dashed line represent occupancy on the plant community as a whole (R^2 = 0.14, F = 4.75, P = 0.04). Black circles and line represent occupancy on *Rudbeckia missouriensis* (R^2 = 0.16, F = 5.4, P = 0.03), and gray circles and line represent occupancy on *Schizachyrium scoparium* (R^2 = 0.31, F = 10.5, P < 0.01).

Table 3. Principal components analysis of soil properties.

Soil variable	PC1	PC2
pH	0.13	0.80
OM (%)	0.91	−0.08
P	0.13	−0.82
Ca	0.87	−0.09
Mg	0.78	0.41
K	0.78	0.06
Total N	0.86	−0.26
Eigenvalue	3.59	1.57
Percentage of variance	51.3	22.4

Notes: pH, potential of hydrogen; OM, organic matter; Ca, calcium; Mg, magnesium; K, potassium; N, total nitrogen. Character loadings (correlations) of soil nutrient values with two axes of soil variation (PC1 and PC2) accounting for 74% of the total variance.

quality that has implications for glade management. We also saw that overall AMF abundance increases with glade size (Fig. 5), due to decreases in the edge:area ratio. These results suggest that glade size, by mediating edge-to-core ratio, will dictate the population size of AMF within individual glades and their status as sources or sinks of AMF in this Ozark glade archipelago.

We hypothesized that environmental variation, specifically soil nutrient levels, contributes to increases in AMF from edge to core within habitat patches and across restoration age. Our soil analysis revealed lower N, Ca, Mg, K, and organic matter in core vs. edge habitat of older glades (Fig. 6a) and decreasing available P with restoration age (Fig. 6b). In other ecosystems, such changes in soil attributes influence plant–AMF interactions (Lekberg et al. 2011, Johnson et al. 2015). Although the small sample size for this analysis (12 glades total) could contribute to the non-significant result when testing for correlation between AMF colonization and soil nutrient properties, our results suggest that while underlying changes in the soil environment are substantial, they do not directly account for variation in AMF colonization. Other environmental factors associated with woody encroachment (e.g., shade, soil moisture) may play a more dominant role in restricting AMF of glade ecosystems.

The results from this study suggest that among- and within-habitat patch characteristics affect density of belowground interactions. Habitat patch quality and history combine to explain the frequency of AMF interactions in

Table 4. Analysis of variance in soil principal components (PC1 and PC2) due to restoration age (Age), habitat type (HT), and their interaction (Age × HT).

	Soil PCA dimension 1				Soil PCA dimension 2			
	Num df	Den df	*F*	*P*	Num df	Den df	*F*	*P*
Age	1	11	1.98	0.19	1	11	15.0	0.003**
HT	1	11	11.7	0.006**	1	11	3.14	0.1
HT × Age	1	11	7.73	0.018**	1	11	1.88	0.49

** P < 0.05.

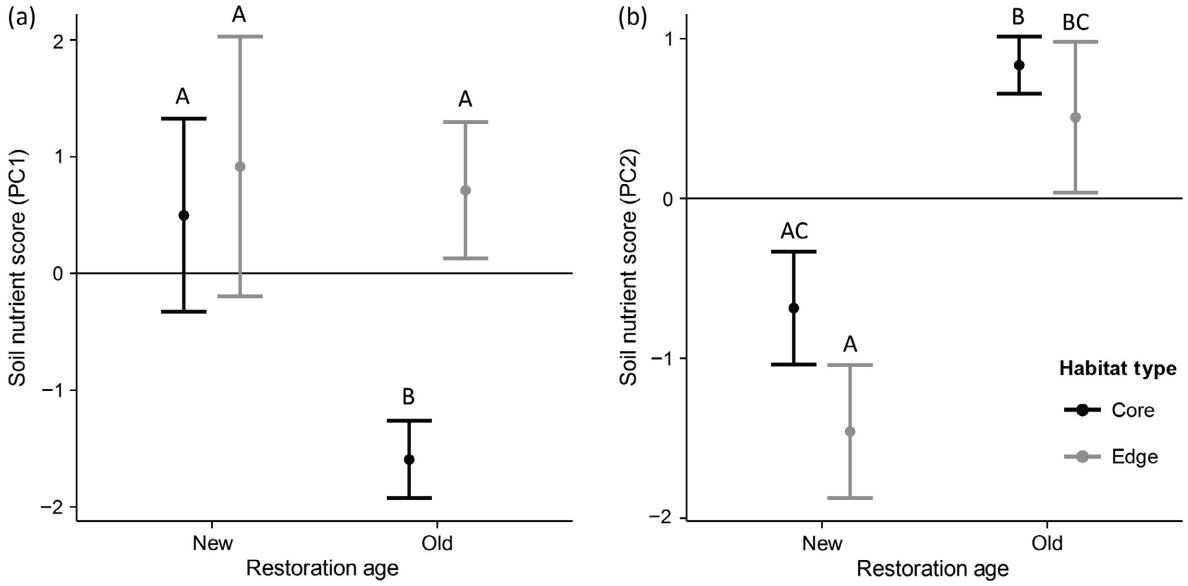


Fig. 6. Means for soil nutrient axes (a) PC1 and (b) PC2 (see Table 3 for character loadings) in edge and core habitats of newly restored and older glades. Black bars represent core, and gray bars represent edge habitat. Error bars indicate standard error. Letters above bars indicate significant differences between groups ($P < 0.05$) using least square means. Square root transformation of colonization was used in the PC1 analysis; however, raw values are plotted for ease of interpretation.

glades of the Ozarks, setting the stage for future work on the ecological mechanisms behind these patterns. Results suggest that environmental differences between edge and core habitats are as or more important to AMF colonization than isolation and elevate the role of large glades as hotspots of interaction intensity (Thompson 2005). Because large glades contain more core habitat, they disproportionately promote AMF frequency, which suggests that prioritizing restoration of large sites would be best to conserve AMF–plant interactions in glade systems. In this study, we did not consider changes in AMF community composition or distributions of particular AMF taxa across fragmented patches but focused on interaction frequency as a metric of conservation concern relevant for understanding how best to conserve both partner guilds (Soulé et al. 2005). Future work will examine how habitat patch age, size, isolation, and edge effects influence more conventional measures of biodiversity, including AMF community richness, diversity, and species distributions in the Ozark glade archipelago. Ultimately, understanding the relationship between interaction frequency and species richness will inform best practices for conservation of mutualists at the landscape scale.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1570/full