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Taxa–area relationship and neutral dynamics influence the diversity of fungal communities on senesced tree leaves

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Summary

This study utilized individual senesced sugar maple and beech leaves as natural sampling units within which to quantify saprotrophic fungal diversity. Quantifying communities in individual leaves allowed us to determine if fungi display a classic taxa–area relationship (species richness increasing with area). We found a significant taxa–area relationship for sugar maple leaves, but not beech leaves, consistent with Wright's species-energy theory. This suggests that energy availability as affected plant biochemistry is a key factor regulating the scaling relationships of fungal diversity. We also compared taxa rank abundance distributions to models associated with niche or neutral theories of community assembly, and tested the influence of leaf type as an environmental niche factor controlling fungal community composition. Among rank abundance distribution models, the zero-sum model derived from neutral theory showed the best fit to our data. Leaf type explained only 5% of the variability in community composition. Habitat (vernal pool, upland or riparian forest floor) and site of collection explained > 40%, but could be attributed to either niche or neutral processes. Hence, although niche dynamics may regulate fungal communities at the habitat scale, our evidence points towards neutral assembly of saprotrophic fungi on individual leaves, with energy availability constraining the taxa–area relationship.

Introduction

Fungal community composition has been shown to be an important factor regulating decomposition (Balser and Firestone, 2005; Waldrop and Firestone, 2006). Therefore, our ability to predict decomposition rates and responses to environmental changes may be enhanced with increased understanding of the processes that regulate fungal distributions. Variation in composition of ecological communities is commonly divided into two components: alpha diversity (number and evenness of taxa within a sampling unit) and beta diversity (taxa turnover among areas) (Gaston and Blackburn, 2000).

For macroorganisms, it has been frequently observed that there is a correlation between the size of habitat patches or survey areas and the most fundamental measure of alpha diversity, number of taxa detected (Rosenzweig, 1995; Connor and McCoy, 2001; Lomolino, 2001; Drakare *et al*., 2006). The 'taxa–area relationship' (TAR) refers to the shape of the increase in number of taxa with increasing area, and has been most often modelled as a power law ($S = cA^z$) where S is number of species, A is area, c is the intercept in log-log space, and z is a constant related to the rate of species turnover across space (Arrhenius, 1921; Gleason, 1922). Taxa–area relationships have been used to extrapolate species richness (Colwell and Coddington, 1994; He and Legendre, 1996; Plotkin *et al*., 2000), estimate regional diversity inventories (Chong and Stohlgren, 2007), and compare species abundance distributions (May, 1975; Harte *et al*., 1999; Pueyo, 2006). TARs are also used as an important tool in conservation efforts to protect species from habitat fragmentation and destruction (Faith *et al*., 2008). Quantifying fungal TARs may help us understand processes regulating fungal community assembly and determine how sampling design affects our ability to detect fungal diversity.

Communities in discrete habitat patches (e.g. islands) have proven useful in studying TAR patterns because such communities have well-defined boundaries (MacArthur and Wilson, 1967; Schoener, 1976; Bell *et al*., 2005). Although a few studies have utilized leaves as discrete habitats for microbial communities (Kinkel *et al*., 1987; Jacques *et al*., 1995; Newell and Fell, 1997), most previous molecular studies examining forest floor fungal communities have utilized homogenized mixed 'grab' samples of leaves (O'Brien *et al*., 2005; Neubert *et al*., 2006; Blackwood *et al*., 2007a; Keeler *et al*., 2009; Redford *et al*., 2010). However, early after leaf fall, individual leaf boundaries must limit the size of fungal

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communities because the leaves are each colonized individually in the canopy, during leaf fall, and on top of the existing forest floor. At later successional stages it is unclear to what extent the scale of communities coincides with individual leaves because some fungi will colonize leaves from existing mycelia in the forest floor. However, even if individual fungi extend beyond the boundaries of a single leaf, individual leaves are still a natural sampling unit because they represent unit resources with differing characteristics depending on plant species of origin, and in which a highly diverse assemblage of fungal taxa can be found (Cooke and Rayner, 1984). In other words, fungi interact with leaves as discrete units, unlike, for example, soil cores or vegetation quadrats, which are arbitrary subsamples of a larger area. Hence, taking advantage of the individual nature of forest floor leaves may provide important insight into fungal community organization.

Fungal alpha and beta diversity may be influenced by a variety of environmental and biological mechanisms that act on the organism niche. The biochemical makeup of leaves (e.g. proportion of soluble compounds, hemicelluloses, cellulose and lignin) represents the carbon and energy resources available to saprotrophic fungi. Some compounds (e.g. solubles) are easily metabolized and support a wide range of organisms; in contrast, lignin requires oxidative enzymes predominantly produced by white-rot *Basidiomycetes*. Because biochemical composition varies among tree species (Hobbie *et al*., 2006), leaf species type may act as an 'environmental filter', favouring microbial taxa that are able to exploit the resources present (Weand *et al*., 2010; Wu *et al*., 2011). In addition, competitive interactions may influence diversity of fungi that share similar niches. Fungal diversity may also be affected by environmental variability in abiotic conditions such as soil moisture. In general, saprotrophic fungal species richness is lower in aquatic habitats than terrestrial habitats (Nikolcheva *et al*., 2005; Fischer *et al*., 2009; Gessner *et al*., 2010). This could be mediated by lower spatial and temporal variation in aquatic habitats, as well as enhanced water-dependent dispersal among microsites, leading to greater dominance by competitively superior taxa. Under these conditions, we might also expect a shallower TAR (i.e. a lower *z*-value) because of lower beta diversity among habitat patches.

Alternatively, neutral theory seeks to explain community composition strictly by immigration dynamics, ignoring species niches (Hubbell, 2001; Maurer and McGill, 2004). Here, we use two methods of testing the importance of niche determinism versus neutral dynamics in structuring communities. The first method is examination of rank abundance distributions. One prediction of neutral theory is that rank abundance distributions will be consistent with the zero sum model (Hubbell, 2001). In contrast, niche-based theory has been used to derive rank abundance distribution models including the pre-emption (geometric) and broken stick models (Motomura, 1932; MacArthur, 1957). The lognormal and Zipf-Mandlebrot are more flexible models that have also been related to niche theory (McGill *et al*., 2007). The second method is to explain variation in composition among communities (i.e. beta diversity). If variation in community composition is due to environmental filtering, we would expect community composition to be related to environmental or resource gradients (Jongman *et al*., 1995; Chase *et al*., 2004), whereas this would not be the case under neutral dynamics (Hubbell, 2001).

Our goals in this study were to (i) characterize fungal TARs utilizing the discrete nature of leaf habitat, (ii) test the importance of leaf type and habitat in determining fungal alpha and beta diversity, and (iii) determine whether taxa rank abundances correspond with niche-based and/or neutral models of community assembly. To determine the effects of leaf biochemistry and habitat soil moisture, we collected two leaf types of differing recalcitrance from the forest floor of three habitats providing variable levels of moisture, vegetation type and soil conditions: upland forest, riparian forest and vernal pools. While upland forest floor remains dry other than during rainfall or snowmelt, vernal pools are saturated with water in late winter, spring and periodically over the rest of the year, and riparian is occasionally flooded. Fungal diversity and community composition were determined by PCR amplification and sequencing of the fungal internal transcribed spacer (ITS) region, a common fungal taxonomic marker (Martin and Rygiewicz, 2005).

Results

Sequence analysis

Leaves were collected from two forest floor sites per habitat. At each site, sequences were obtained separately from the largest and smallest beech and maple leaves. A total of 2701 sequences met our selection criteria (containing both primers or longer than the longest sequence containing both primers), and these were clustered into 416 distinct operational taxonomic units (OTUs) at 97% sequence similarity. The majority of OTUs (278) were singletons, and these comprised 11.1% of total OTU relative abundance. We classified 41 OTUs as 'dominant' ($\geq$ 5% relative abundance on at least one leaf), comprising 61.2% of total OTU relative abundance. Each leaf had 1–6 dominant OTUs (Fig. S1). This dominance could be extreme; the most dominant OTU had greater than 80% relative abundance on two leaves. Nearest neighbours of dominant OTUs in GenBank included 27 genera; 6 in the *Basidiomycetes* and 21 in *Ascomycetes* (Table 1). The OTUs with dominant relative abundance on the most

Table 1. BLAST results and percentage of variance explained by habitat ($P = 0.001$) or site nested within habitat ($P = 0.001$) for dominant OTUs (> 5% relative abundance on at least one leaf).

OTU ID No.	Accession No.	Score	E value	Phylum	Order	Family	Genus	% variance explained by		Habitat
								Habitat	Site	
1	FJ553305.1	239	8e-60	*Basidiomycota*	*Agaricales*	*Tricholomataceae*	*Mycena*	25.93	43.08	Δ◇
2	EU040223.1	507	2e-140	*Ascomycota*	*Xylariales*	NA	*Phlogicylindrium*	5.29	23.00	Δ∞◇
3	EF596821.1	571	7e-160	*Ascomycota*	*Helotiales*	*Sclerotiniacae*	*Phialea*	39.02	9.26	Δ◇
4	EF029239.1	569	4e-159	*Ascomycota*	*Helotiales*	*Helotiaceae*	*Helicodendron*	17.36	39.54	∞◇
5	GQ303287.1	468	1e-128	*Ascomycota*	NA	NA	*Polyscytalum*	34.39	9.64	Δ◇
6	GQ303287.1	592	6e-166	*Ascomycota*	NA	NA	*Polyscytalum*	3.60	15.40	Δ∞◇
7	FJ554434.1	440	2e-120	*Basidiomycota*	*Agaricales*	*Hydnangiaceae*	*Laccaria*	0.28	14.25	Δ◇
8	AF346545.1	667	0.0	*Ascomycota*	*Xylariales*	*Amphisphaeriaceae*	*Amphisphaeria*	8.70	13.04	∞
9	DQ273371.1	431	1e-117	*Basidiomycota*	*Cantharellales*	*Cantharellaceae*	*Cantherellus*	15.49	23.24	◇
10	AM901701.1	381	1e-102	*Ascomycota*	*Helotiales*	*Sclerotiniacae*	*Sclerotinia*	37.29	7.27	Δ ◇
11	AF141164.1	420	3e-114	*Ascomycota*	*Helotiales*	*Dermateacea*	*Dermea*	8.70	13.04	Δ
12	EU726288.1	459	7e-126	*Ascomycota*	*Helotiales*	NA	NA	4.81	13.60	Δ◇
13	AF444526.1	475	6e-131	*Basidiomycota*	*Filobasidiales*	*Filobasidiaceae*	*Rhodotorula*	26.98	40.47	Δ
14	DQ914671.1	407	3e-110	*Ascomycota*	*Helotiales*	NA	*Cystodendron*	18.17	0.02	Δ
15	AF141189.1	466	4e-128	*Ascomycota*	*Helotiales*	*Dermateacea*	*Neofrabraea*	17.97	26.95	◇
16	FJ553668.1	414	1e-112	*Ascomycota*	*Helotiales*	*Dermateacea*	*Pezicula*	8.70	13.04	∞
17	EU998923.1	590	2e-165	*Ascomycota*	*Helotiales*	*Hypogastruroidea*	*Articulospora*	17.30	25.95	Δ
18	EF596821.1	472	8e-130	*Ascomycota*	NA	NA	*Phialea*	17.73	26.60	∞
19	EU935520.1	542	7e-151	*Basidiomycota*	*Agaricales*	*Marasmiacea*	*Marasmius*	8.70	13.04	◇
20	EU040235.1	424	2e-115	*Ascomycota*	NA	NA	*Parapleurotheciopsis*	8.70	13.04	∞
21	AY204588.1	435	1e-118	*Ascomycota*	*Acarosporales*	*Altosporidea*	*Altospora*	18.17	0.02	∞
22	FJ839639.1	385	1e-103	*Ascomycota*	*Mycosphaerellales*	*Dothidiomycetes*	*Xenostigmina*	18.15	27.23	∞
23	FJ839639.1	412	4e-112	*Ascomycota*	*Mycosphaerellales*	*Dothidiomycetes*	*Xenostigmina*	8.70	13.04	∞
24	EU552142.1	329	5e-87	*Ascomycota*	*Pleosporales*	*Lophiostomataceae*	*Massarina*	18.10	27.15	∞
25	AF141168.1	507	2e-140	*Ascomycota*	*Helotiales*	*Dermateacea*	*Scleropezicula*	8.70	13.04	Δ
26	FJ554419.1	357	2e-95	*Ascomycota*	*Helotiales*	*Dermateacea*	NA	8.70	13.04	∞
27	FJ554126.1	409	7e-111	*Ascomycota*	*Pezizales*	*Lecanoromycetidae*	NA	8.70	13.04	◇
28	FJ553703.1	435	1e-118	*Ascomycota*	*Eruotiales*	*Trichocomaceae*	*Pennicillum*	8.70	13.04	Δ
29	AJ301937.1	671	0.0	*Ascomycota*	*Phyllachorales*	*Phyllachoracea*	*Colletotrichum*	8.70	13.04	∞
30	FJ554126.1	501	5e-138	*Ascomycota*	*Pezizales*	*Lecanoromycetidae*	NA	8.70	13.04	∞
31	FJ553305.1	398	2e-107	*Basidiomycota*	*Agaricales*	*Tricholomataceae*	*Mycena*	8.70	13.04	◇
34	EF029241.1	326	6e-86	*Ascomycota*	*Chaetosphaeriales*	*Chaetosphaeriacea*	*Dictyochaeta*	8.70	13.04	∞
36	FJ553648.1	610	2e-171	*Basidiomycota*	*Agaricales*	*Tricholomataceae*	*Mycena*	8.70	13.04	◇
37	AM901701.1	381	1e-102	*Ascomycota*	*Helotiales*	*Helotiaceae*	*Sclerotinia*	8.70	13.04	∞
38	AB126047.1	222	9e-55	*Basidiomycota*	*Ustilaginales*	*Ustilaginaceae*	*Sporobolomyces*	8.70	13.04	Δ
40	GQ509692.1	425	8e-116	*Non-cult. clone*	NA	NA	NA	8.70	13.04	Δ
41	FJ554323.1	436	4e-119	*Basidiomycota*	*Agaricales*	*Hydnangiaceae*	*Laccaria*	8.70	13.04	∞
44	DQ646542.1	230	7e-57	*Ascomycota*	*Pezizales*	*Pezizacea*	*Peziza*	8.70	13.04	∞
47	EU040223.1	464	1e-127	*Ascomycota*	*Xylariales*	NA	*Phlogicylindrium*	8.70	13.04	∞
51	FJ185160.1	778	0.0	*Basidiomycota*	*Agaricales*	*Agaricacea*	*Coprinellus*	8.70	13.04	∞
54	AJ301970.1	584	9e-164	*Ascomycota*	*Phyllachorales*	*Phyllachoracea*	*Glomerella*	8.70	13.04	Δ
57	FJ554064.1	339	1e-89	*Basidiomycota*	*Polyporales*	*Atheliaceae*	NA	8.70	13.04	Δ
64	AY706329.1	494	2e-136	*Ascomycota*	*Pezizales*	*Leotiomycetes*	*Leohumicola*	8.70	13.04	∞
70	AF096215.1	213	5e-52	*Ascomycota*	*Pezizales*	*Lecaneromycetes*	*Unbilicaria*	8.70	13.04	Δ

Symbols indicate the habitat that dominant OTUs were associated with (Δ = upland, ∞ = vernal pool, ◇ = riparian).
NA, not assigned.

leaves (7 out of 24 leaves) were OTU 2 (*Phlogicylindrium* as nearest neighbour) and OTUs 5 and 6 (*Polyscytalum*; Table 1). OTU 1 (*Mycena*) was the most dominant overall OTU with a 53% average relative abundance. Dominant OTU distributions for each leaf are shown in Fig. S1.

Alpha diversity and taxa–area relationship

Fifty-nine to 158 sequences per leaf met our analysis criteria. Rarefaction was used to reduce bias associated with unequal sample sizes from different communities by simulating diversity index values corresponding to the number of individuals in the smallest sample (Magurran, 2004). The rarefaction curves for H' and $1/D$ reached an asymptote by 59 sequences, although S continued to rise (Fig. S2). Therefore, we use S obtained from rarefaction as an indicator for comparative purposes.

For all diversity indices (S, H', $1/D$, $E_{1/D}$ and Chao), there were no significant main effects of habitat, leaf species or leaf size. However, the interaction between leaf species and leaf size was significant or marginally significant for S ($P = 0.04$), H' ($P = 0.01$), $1/D$ ($P = 0.10$) and $E_{1/D}$ ($P = 0.08$). Separate analyses of leaf species showed that the interaction between leaf species and size was due to a significant effect of leaf size in maple leaves ($P = 0.053$ for S and $P < 0.05$ for H', $1/D$ and $E_{1/D}$), but no effect of leaf size in beech leaves ($P > 0.1$ for all indices, Fig. 1).

Akaike Information Criterion corrected for small sample size (AICc) was used as a model selection criterion to determine the influence of ln(leaf area), as well as habitat, on ln(S) in maple leaves. AICc was minimized by a model including ln(leaf area) and habitat as predictors, without interactions or random effects [i.e. a model in which habitat alters the intercept of the relationship between ln(area) and ln(S), but not the slope; Fig. 2A]. The z-value of the TAR for fungal taxa on sugar maple leaves was estimated to be 0.22 ($\pm$ standard error of 0.07; Fig. 2A). Using the model selection procedure on beech leaves resulted in a model containing only a habitat effect, and no effect of leaf area (Fig. 2B).

Beta diversity and community composition

A high degree of taxa turnover was indicated by an average Jaccard similarity index of 0.082. Redundancy analysis (RDA) of Hellinger distances (relative abundance data) indicated that habitat explained 20.7% of the variability in OTU relative abundance ($P = 0.001$). Sites nested within habitat type explained an additional 20.6% of the variability in OTU relative abundance ($P = 0.001$). Leaf type explained 5% of variation in OTU abundance ($P = 0.047$), while leaf size was not significant. Redundancy analysis on Sorenson distances (presence/absence data) indicated that habitat explained 15.5% of the variance ($P = 0.001$) and sites nested within habitat type explained 15.4% ($P = 0.003$), while leaf species and leaf size were not significant.

Habitat explained the majority of variability in five dominant OTUs (Table 1). OTUs 3 (matching sequences in GenBank from the genus *Phialea*; Table 1), 5 (*Polyscytalum*) and 14 (*Cystidendron*) were dominant on multiple leaves at both upland field sites, while OTUs 10 (*Sclerotinia*) and 21 (*Altospora*) were dominant on leaves at both vernal pool sites. OTUs 3, 5 and 10 were also dominant on leaves at one of the two riparian sites. Hence, the upland forest and vernal pool fungal communities were never dominated by the same OTUs (Fig. S1). Riparian forest leaves at one site (site J2) shared dominant OTUs with both upland and vernal pool communities. This is reflected in the RDA ordination in Fig. 3 by the intermediate position of this site on the second ordination axis. Leaves from the other riparian site (site Q1) were mostly dominated by OTU 1 (*Mycena*), resulting in a fungal community dramatically different from all other sites. Hence, a major pattern in Fig. 3 is separation of site Q1 from all other sites on the first ordination axis.

Species rank abundance distribution models

In a comparison of different rank abundance distribution models, AIC indicated that the zero-sum model was the closest fit with our data for all 24 leaves (Table S1 and Fig. S3), consistent with neutral theory dynamics. The minimum difference in AIC between the zero-sum model and any other model was 32 (Table S1), indicating no support for any model other than the zero-sum model (Burnham and Anderson, 2002). This trend was consistent across all habitats, leaf types and leaf sizes.

Discussion

Alpha diversity and taxa–area relationship

We have shown that the diversity of saprotrophic fungal communities living on sugar maple leaves fits the classic power law TAR. However, this pattern was not observed on beech leaves, in which z was not significantly different from zero, although it is possible that further sampling of beech leaves may reveal a shallower or more noisy TAR. There are several mechanisms proposed in the literature to account for TARs. The ‘passive sampling hypothesis’ (Connor and McCoy, 1979) proposes that larger areas support a greater variety of organisms than smaller areas because they receive more colonists than smaller areas. The ‘habitat diversity hypothesis’ (Williams, 1964) proposes instead that larger areas offer a greater variety of habitats, while the ‘area per se hypothesis’ (Simberloff, 1976) states that larger areas support higher populations

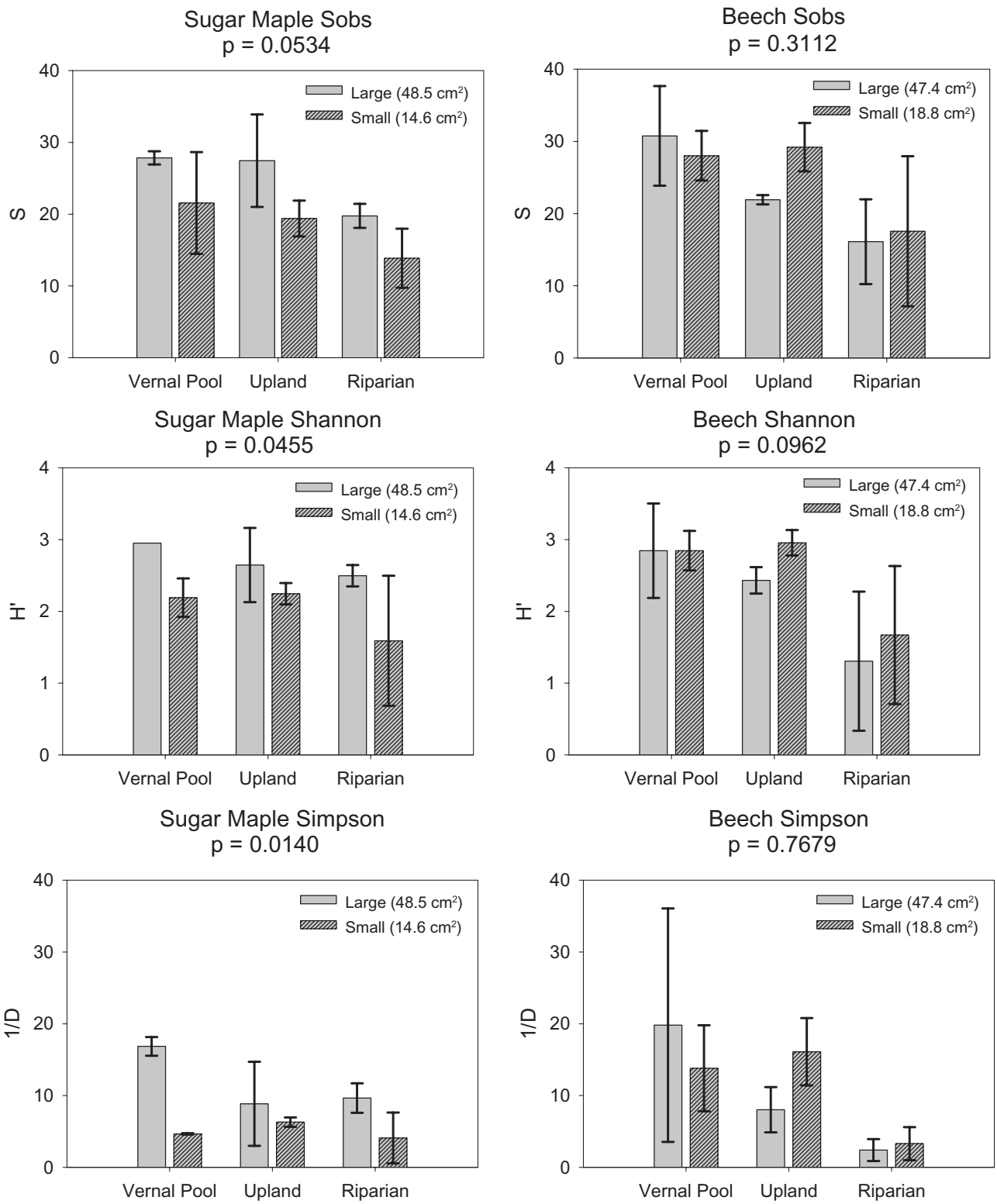

Fig. 1. Mean OTU richness (*S*), Shannon diversity (*H'*) and Simpson diversity (*1/D*) values for large versus small sugar maple and beech leaves gathered from 3 temperate forest habitats ($n = 2$ leaves per bar). Analysis of sugar maple and beech leaves together resulted in a significant species × size interaction (see text). *P*-values shown with each chart indicate significance of leaf size within either sugar maple or beech (size × habitat interactions were never significant).

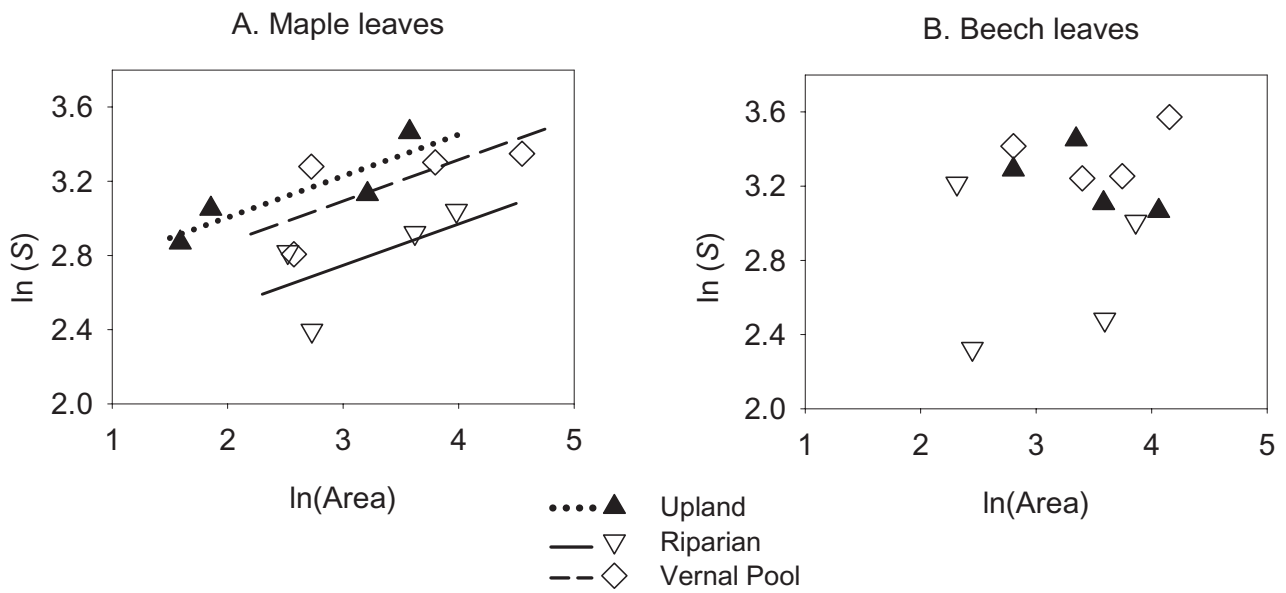

Fig. 2. Fungal taxa–area relationship detected on maple leaves ($P < 0.05$) but not beech. Regression lines are shown for maple upland, vernal pool and riparian habitat TARs. For maple leaves, model selection using AICc resulted in a model where habitat TARs have identical *z*-values (slope; 0.22 ± 0.07) and different *c*-values (*Y*-axis intercepts).

of each species, decreasing the probability of any particular species going extinct. The above hypotheses do not seem adequate to explain the different TAR patterns we found in maple and beech. In contrast, Wright's species-energy theory (Wright, 1983) can be directly related to known differences between maple and beech leaves. Wright proposed that area is actually an indicator for available energy, or another resource limiting energy use, available in a habitat patch. According to Wright (1983), the proximate causes of increased species richness in larger areas are (i) the larger population sizes supported by increased resource production rates, and (ii) a correlation between resource quantity and resource heterogeneity that can support a greater diversity of species niches. In this case, one would expect stronger TARs for habitats in which a change in area causes a greater change in energy supply than in other habitats.

Energy is obtained by saprotrophic fungi from biochemicals present in plant tissue (Berg and McClaugherty, 2003). In Jennings Woods, where our leaves were sampled, sugar maple leaves contain approximately 16% acid-insoluble material, whereas beech leaves contain

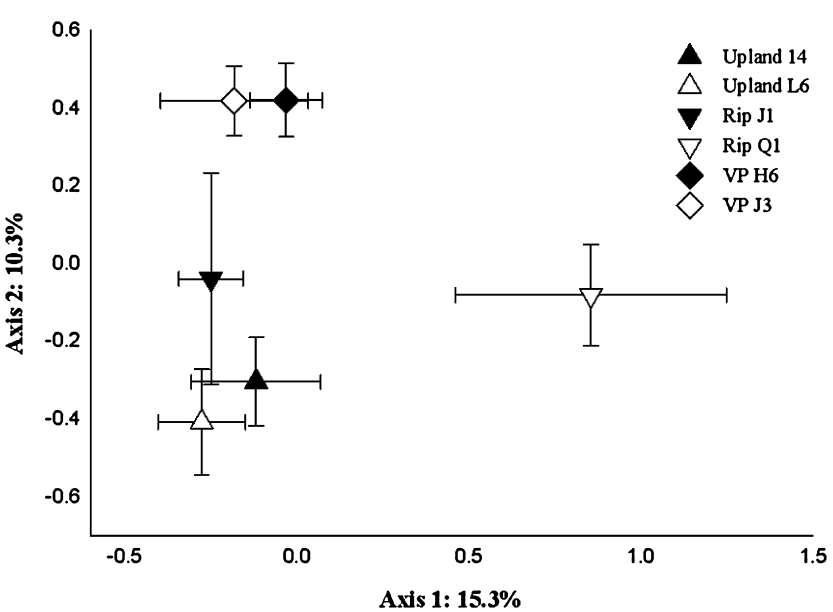

Fig. 3. Redundancy analysis ordination of fungal community composition at the six field sites. Habitat is indicated by symbol shape, with site names shown in legend. Because leaf type explained little variation in community composition (5%), each symbol represents the centroid of the four leaves sampled at each site.

approximately 29% (L.M. Feinstein and C.B. Blackwood, unpubl. data). Microbial growth on lignin, the primary component of acid-insoluble plant compounds, is inefficient, requiring secretion of costly oxidative extracellular enzymes that release aromatic monomers that are not readily metabolized (Berg and McClaugherty, 2003). Lignin also forms a protective coating over labile plant cell wall polysaccharides, further reducing the rate of resource acquisition possible by microbial saprotrophs. Hence, substantially less energy per unit area is made available for saprotrophs growing on beech leaves in a given period of time. Variation in leaf size therefore translates into less variation in available energy on beech leaves than on maple leaves, and this smaller variation in energy availability is hypothesized to result in a shallower TAR, making detection of a significant relationship more difficult to achieve statistically.

The TAR rate of increase we found ($z = 0.22$) is very close to the canonical value of 0.26 (Preston, 1962; Drakare *et al*., 2006) and similar to that found by ITS sequencing for ectomycorrhizal fungi associated with tree 'islands' (0.2–0.23; Peay *et al*., 2007). Green and Bohannan (2006) report *z*-values for microorganisms ranging from 0.02 to 0.47, but these values are difficult to compare due to inclusion of a wide variety of habitats, taxa and sampling formats (contiguous, non-contiguous and island). Furthermore, there is debate over whether different molecular methods result in comparable measures of community diversity (Woodcock *et al*., 2006; Blackwood *et al*., 2007b). Horner-Devine and colleagues (2004) used 16S ribosomal DNA sequencing (which is similar to the method we employed here) to characterize bacterial diversity in marsh sediments and found a much lower *z*-value (0.02–0.04) than found for fungi. Microorganism distribution is thought to be less limited by dispersal than macroorganism distribution, leading to fewer local endemics and a lower *z*-value (Finlay *et al*., 1998; Hillebrand *et al*., 2001; Drakare *et al*., 2006). Fungal *z*-values being closer to those of plants than bacteria may suggest that dispersal patterns and mechanisms of community assembly are more similar between plants and fungi.

Support for neutral dynamics from rank abundance

The zero-sum model best fit our rank abundance distributions for fungal communities in individual leaves. Neutral theory has been supported using the zero-sum model in a number of empirical community assemblages including arbuscular mycorrhizal fungi (Dumbrell *et al*., 2010), marine intertidal invertebrates (Wootton, 2005), South American and European birds (Ricklefs, 2006), subtropical/temperate plants (Forster and Warton, 2007) and pacific corals (Connolly *et al*., 2009). In actuality, metacommunity species distribution patterns may be a result of a complicated mix of more than one factor (Leibold *et al*., 2010). Our analyses of beta diversity found a relatively minor effect of the potential environmental filter leaf type (5% of variance explained); however, leaf type does explain a somewhat larger portion of variance for some taxa (OTU 5, 16.3%; OTU 7, 14.0%), indicating niche-based processes may be involved in some species distributions. Habitat and site (nested within habitat) explained much more community composition variance (20.7% and 20.6% respectively), but these habitats differ dramatically in environmental characteristics, and we do not have enough sampling sites to attribute the habitat and site effects strictly to dispersal limitation or environmental filtering.

Although neutral theory predicts that all species are equally competitive and differences in community composition at a given location are primarily due to colonization order and priority effects (Hubbell, 2001), competition has been shown to alter species abundances in some cases (Connell, 1983; Schoener, 1983). Competition for resources has been described as a primary mechanism driving fungal establishment and succession (Boddy and Rayner, 1983; Peiris *et al*., 2008). In our study, many of the OTUs that were dominant on one leaf were also found in lower abundance on other leaves. For example, OTU 1 was found with relative abundance values of 0.7–3.9% at all sites except at site Q1 where its mean relative abundance across four leaves was 81.7%. Neutral theory would predict that dominance of OTU 1 is due to preemptive colonization. However, the possibility of competitive displacement of other fungi by OTU 1 could be empirically tested by evaluating its competitive ability against OTUs that were dominant at the five sites where OTU 1 was found, but not dominant.

We determined fungal relative abundances in this study using clone library sequences, a process that is potentially affected by biases in DNA extraction and PCR amplification (Wintzingerode *et al*., 1997). Although quantification of taxa richness from clone libraries has generally been found to be robust (Avis *et al*., 2010; Tedersoo *et al*., 2010), it is possible that the procedural biases favoured rank abundance distributions that are best fit by the zero-sum model. However, one general form of PCR bias is for a flattened rank abundance distribution (Kanagawa, 2003), which would bias against the zero-sum model, rather than for it. In contrast, preferential amplification of particular taxa is also a common PCR artefact, and this could favour the zero-sum model. In our data, fungal taxa common to multiple samples were not consistently dominant or rare, indicating that preferential amplification of certain taxa was not particularly strong. Our primers amplified the ITS1 region, which is less variable in length than the ITS2 or full ITS regions, reducing preferential amplification and chimera formation (Bellemain

et al., 2010; Tedersoo *et al*., 2010). We also took other steps to reduce preferential amplification through pooling multiple PCR replicates, using as few PCR cycles as possible, and diluting samples to reduce the concentration of environmental PCR inhibitors (Kanagawa, 2003; Tedersoo *et al*., 2010). Finally, several studies indicate DNA extraction bias is not a particular problem for fungi in environmental samples (Oberkofler and Peintner, 2008; Feinstein *et al*., 2009).

Conclusions

Taking advantage of leaves as naturally occurring resource patches, we found a significant TAR on sugar maple leaves, but not beech leaves. We hypothesize that Wright's species-energy relationship may explain this difference in TAR patterns between leaf types. This suggests that energy availability as affected by plant biochemistry may provide a heretofore-undescribed mechanism that influences fungal diversity over a variety of spatial scales. Fungal beta diversity was high, indicating a large degree of turnover between habitat patches. Individual leaf rank abundance distributions are consistent with zero-sum dynamics, providing support for a neutral theory of metacommunity organization for forest floor saprotrophic fungi. Further research is necessary to elucidate mechanisms driving the patterns we have described, including further investigation of the link between fungal community resource pool quality, habitat size and taxa richness.

Experimental procedures

Field site and sample collection

All leaves were collected from Jennings Woods, a 30-hectare deciduous forest in north-eastern Ohio. The two most dominant tree species are sugar maple (*Acer saccharum*, 22% relative abundance of all forest trees) and beech (*Fagus grandifolia*, 19% relative abundance). Two sampling locations were selected within each of three habitats: upland forest, riparian forest (within 30–50 m of the West Branch of the Mahoning River), and seasonally saturated vernal pools. The vernal pools were embedded within the riparian forest and ~ 15 m from the riparian forest sampling locations.

A total of 30 leaves were randomly selected from 26.7 × 31.8 cm areas in each habitat during June 2008. Among the 30 leaves collected at each sampling location, the largest and smallest sugar maple and beech leaves were used in this study, resulting in four leaves used per site. Leaves were photographed in the field, collected with sterile implements, and placed into individual, sterile, pre-weighed containers in coolers containing dry ice. Leaf surface area was calculated from digital images using the software WinRhizo (2007 version, Regent Instruments, Canada) with settings adapted to leaves. Samples were stored at −80°C. Leaves were lyophilized (VirTis Genesis 25EL, Biopharma Process Systems Ltd, Winchester, NH, USA) and dry leaf weight recorded.

DNA extraction and PCR amplification

Leaves were ground in sterile collection vials in a Geno-grinder 2000 (SPEX CertiPrep, Metuchen, NJ, USA). DNA was extracted as in Wu and colleagues (2011). The fungal ITS1 region was amplified using primers NSI1F (5′-GATT GAATGGCTTAGTGAGG) and 5.8SR (5′-GCTGCGTTC TTCATCGA) (Martin and Rygiewicz, 2005). PCRs were performed with a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad, Hercules, CA, USA) using 0.025 U μl^{-1} Taq DNA polymerase, 3 mM $MgCl_2$, 1× ammonium polymerase buffer (B-Bridge International, Mountain View, CA, USA), 0.2 μM each primer (Integrated DNA Technologies, Coralville, IA, USA), 0.16 mM each dNTP, and 0.1 $\mu g\ \mu l^{-1}$ bovine serum albumin (New England Biolabs, Ipswich, MA, USA). PCR reaction conditions were: initial denaturation for 3 min at 95°C, 30–35 cycles of denaturation for 30 s at 94°C, primer annealing for 30 s at 60°C and extension for 90 s at 72°C, and a final extension for 7 min at 72°C. Negative controls were included in each PCR run. PCR products were run on a 1.5% agarose gel to confirm successful amplification. Cycle number was varied for each sample to obtain a strong band without non-specific amplification. Three replicate PCRs were performed for each sample, and these were pooled prior to the cloning ligation reaction.

Clone libraries

PCR product was purified using the UltraClean PCR Clean-up kit (MoBio, Carlsbad, CA, USA). Amplicons were then ligated into a pGEM-T vector with overnight incubation and used to transform competent *E. coli* cells following the manufacturer's instructions (Promega, Madison, WI, USA). One hundred and ninety-two white colonies per sample were used to inoculate wells of LB broth with 10% glycerol. Cultures were grown overnight and stored at −80°C. Sequencing was performed at the Genome Sequencing Center at Washington University in St Louis, MO, USA using M13 primers.

Plasmid sequence was removed and amplicon sequences were trimmed for quality in Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) using default settings. Clustering of sequences into OTUs was performed using BlastClust (Altschul *et al*., 1990) implemented in the MPI Bioinformatics Toolkit (Biegert *et al*., 2006). Sequences were only used if they contained both forward and reverse primer sequences or contained one primer and were longer than the longest sequence containing both primers (i.e. longer than 390 bp). This criterion ensures that BLAST score variation is not affected by length variation of partially sequenced amplicons. A per cent identity threshold of 97% was used to define OTUs. For OTUs with ≥ 5% relative abundance on a leaf, nearest neighbours in GenBank were identified using BLAST searches on representative sequences (Altschul *et al*., 1997). Sequences generated in this study have been submitted to GenBank under accession numbers JN394655–JN397349.

Statistical analysis

The software EstimateS 8.2 (Colwell, 2009) was used to calculate sample-based rarefaction curves and alpha diversity indices [observed taxa richness (*S*), expected richness (Chao 2), Shannon diversity (*H'*), Simpson diversity (*1/D*)] for each leaf (Gotelli and Colwell, 2001; Colwell *et al*., 2004; Mao *et al*., 2005). The Simpson evenness index ($E_{1/D}$ *sensu* Smith and Wilson, 1996) was calculated from *S* and *1/D*-values obtained by rarefaction. Diversity indices standardized to a common clone library size by rarefaction were compared with mixed model analysis of variance using SAS Proc Mixed (SAS Institute, Cary, NC, USA). The effects of leaf size, leaf species and habitat type were examined as fixed effects in a three-way ANOVA with all potential interactions. Collection site within habitat type was designated a random effect (2 sites per habitat). Where leaf size was significant, we calculated *z* by regression of log-transformed *S* (obtained by rarefaction) against log-transformed surface area. Other terms initially included in the regression model [i.e. habitat as a fixed factor, habitat × ln(area) interaction, and site within habitat as a random factor] were eliminated stepwise to find the model minimizing the AICc (Burnham and Anderson, 2002).

To quantify beta diversity, taxa turnover was measured with the Jaccard similarity index generated in the software R (version 2.10.1) using the vegan package (Oksanen, 2010). Redundancy analysis was used to assess the effects of habitat, site nested within habitat, leaf type and leaf size on OTU composition. RDA was performed on (i) Hellinger distances, an abundance-based metric that excludes joint absences and downweights the most abundant OTUs and effects of differences in sampling effort (Legendre and Gallagher, 2001); and (ii) Sorenson similarity indices calculated from presence/absence of OTUs. Statistical significance of RDA results was determined with the software Canoco (Microcomputer Power, Ithaca, NY, USA) using 999 random permutations of sample identity.

The OTU rank abundances for each leaf were fit to broken stick, pre-emption, log-normal, Zipf and Zipf-Mandelbrot rank abundance models using the command 'radfit' found in the R package vegan (Oksanen, 2010), and to the zero-sum model using TeTame (Jabot *et al*., 2008). The AIC values were compared to determine which model provided the best fit to the empirical data (Burnham and Anderson, 2002). The comparison of AIC values generated by radfit and TeTame is biased against the zero-sum model (F. Jabot, pers. comm., and as fully described in Table S1). However, because the zero-sum model is unambiguously supported using this conservative test (see *Results*), this bias only strengthens our conclusions.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. A. Relative abundance charts show proportion of community occupied by dominant (multi-coloured) and non-dominant (black) OTUs located on vernal pool leaves (sites H6 and J1).
B. Relative abundance charts show proportion of community occupied by dominant (multi-coloured) and non-dominant (black) OTUs located on upland leaves (sites 14 and L6).
C. Relative abundance charts show proportion of community occupied by dominant (multi-coloured) and non-dominant (black) OTUs located on riparian leaves (sites J1 and Q1).
Fig. S2. A. Rarefaction curves for diversity indices for individual leaves located at Upland Sites L6 and 14.
B. Rarefaction curves for diversity indices for individual leaves located at Riparian Sites J1 and Q1.
C. Rarefaction curves for diversity indices for individual leaves located at Vernal Pool Sites J3 and H6.

Fig. S3. Visual fit of the Broken stick, Pre-emption, Log-Normal, Zipf and Zero Sum models against the observed data (red circles) of four representative fungal community habitats. All models are could be plotted directly as continuous functions except for the Zero Sum model. Zero Sum model realizations ($n = 1000$) were generated using the R package untb after fitting using TeTame. An average value of these realizations was then calculated which is consistent with the expectation of the Zero Sum model.

Table S1. AIC values for 6 rank abundance distribution models. Lowest AIC value for each sample represents the best fit model.

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