Superior dispersal ability can lead to persistent ecological dominance throughout succession

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Running head: Dispersal and dominance in microbial metacommunities

Abstract

A large number of descriptive surveys have shown that microbial communities experience successional changes over time, and that ecological dominance is common in the microbial world. However, direct evidence for the ecological processes mediating succession or causing ecological dominance remains rare. Different dispersal abilities among species may be a key mechanism. We surveyed fungal diversity within a metacommunity of pitchers of the model carnivorous plant *Sarracenia purpurea* and discovered that the yeast *Candida pseudoglaebosa* was ecologically dominant. Its frequency in the metacommunity increased during the growing season, and it was not replaced by other taxa. We next measured its competitive ability in a manipulative laboratory experiment and tracked its dispersal over time in nature. Despite its dominance, *C. pseudoglaebosa* is not a superior competitor. Instead, it is a superior disperser: it arrives in pitchers earlier, and disperses into more pitchers, than other fungi. Differential dispersal across the spatially structured metacommunity of individual pitchers emerges as a key driver of the continuous dominance of *C. pseudoglaebosa* during succession.

Importance

Microbial communities are ubiquitous and occupy nearly every imaginable habitat and resource, including human-influenced habitats (*e.g.*, fermenting food, hospital surfaces) and habitats with little human influence (*e.g.*, aquatic communities living in carnivorous plant pitchers). We studied yeast communities living in pitchers of the carnivorous purple pitcher plant to understand how and why microbial communities change over time. We

found that dispersal ability is not only important for fungal communities early in their existence, it can also determine the identity of a dominant species (here, the yeast *Candida pseudoglaebosa*) long after the species and its competitors have finished arriving. These results contrast with observations from many human-influenced habitats, in which a good competitor eventually outcompetes good dispersers, since humans often design these habitats to favor a specific competitor. This study will help microbiologists understand the qualities of microbial species that enable takeover of new habitats in both natural and human-influenced environments.

Introduction

Primary microbial succession occurs when a microbial community colonizes and develops on a newly available substrate (1). The advent of high-throughput sequencing has revolutionized observational studies of microbial succession, enabling researchers to describe the development of microbial communities in fine detail (2-5). A variety of successional patterns have been observed. For example, taxon diversity can increase, decrease, or randomly vary with successional time (4, 6, 7). However, researchers commonly observe replacement of early-successional taxa by late-successional taxa (2, 8). An ongoing challenge in microbial ecology is to connect observed ecological patterns to the ecological processes responsible for the patterns.

The development of ecological dominance by one or a few microbes over successional time is a particularly intriguing phenomenon (2, 9, 10), in part because of its obvious parallels to plant and animal ecology (11). Ecological dominance is apparent when one or a few species comprise most of the individuals or biomass in a community

(11). Environmental filtering, superior competitive ability of the dominant species, and ecosystem engineering can all cause dominance (8, 12-15). For example, *Saccharomyces cerevisiae* dominance in wine results from environmental changes, caused by *S. cerevisiae* itself, that make the fermentation environment hospitable to *S. cerevisiae* and inhospitable to other microbes (13, 16). As another example, algal dominance during bloom events can be the result of environmental filtering caused by eutrophication (17). However, the dynamics of microbial dominance have been primarily studied in domesticated systems, and the most frequently reported mechanisms may not be responsible for dominance in all, or even most, natural microbial communities. For example, environmental filtering and competition may be more important in systems where human beings have designed environments to favor domesticated microbes (18) and less important in natural environments with heterogeneous environmental conditions.

An overlooked mechanism driving ecological dominance in natural systems may be dispersal (*i.e.*, arrival in new habitats). When primary succession occurs on a sterile substrate, all members of the microbial community must first disperse onto the new substrate before establishing in the community. A good disperser may prevent the establishment of other community members through priority effects if it arrives in a habitat first, either by pre-empting or modifying available niches (19). Additionally, good dispersers in nearby communities can impact a microbial community during succession by producing propagules that then disperse into the developing community (20).

Dispersal may emerge as a key driver of ecological dominance in microbial metacommunities. Metacommunities are physically structured groups of communities in which individual communities are spatially isolated from one another but linked through

dispersal (21, 22). Community assembly in metacommunities is a function of dispersal among communities and ecological processes occurring inside each community. Ecological theory explains how dispersal can interact with intracommunity processes to maintain metacommunity diversity (21, 23). For example, populations occupying low-quality environmental patches can be maintained by dispersal from high-quality patches ("mass effects"), or fitness trade-offs between competitive ability and dispersal ability can mediate species diversity ("patch dynamics"). Theory predicts that dispersal and competition interact during succession in the individual component communities of a metacommunity and the result is a hump-shaped relationship between successional time and species richness (24, 25). Species richness is predicted to be low early in a component community's age, to increase as more species disperse into the community, and then to decrease as competitive interactions remove species from the community. Although rarely documented, it is also possible that a particularly good disperser will become dominant and remain dominant (and therefore decrease community diversity) in a metacommunity solely as a result of its dispersal ability.

We investigated the contribution of dispersal to ecological dominance over the course of natural fungal succession in pitchers of the carnivorous pitcher plant *Sarracenia purpurea*. *S. purpurea* is a perennial plant native to bogs and savannas in northern and eastern North America (26). Each *S. purpurea* plant produces modified vase-shaped leaves, or pitchers, annually (Figure 1a). At first, developing pitchers are entirely closed, sterile chambers (27). Once mature, the top portion of each pitcher opens, and open pitchers accumulate rainwater to form small pools of water (phytotelmata). Potential prey (ants and other small insects) are attracted to pitchers (28); some prey fall into pitchers

and drown, and are then shredded, decomposed, and mineralized by a food web of microorganisms and invertebrates (29). The pitcher microbial community includes bacteria, algae, and fungi, including culturable yeasts (30). Yeasts have long been recognized as components of pitcher plant food webs, and diverse fungi are readily detected by sequencing when eukaryote-specific primers are used (30, 31). Pitchers are individual communities within a metacommunity of other pitchers on the same and on different plants.

We followed fungal succession within individual pitchers in a *Sphagnum* bog in central Massachusetts, where the pitcher growing season lasts for about three months (although pitchers persist for longer and can overwinter) (32). We first documented that a single yeast species, *Candida pseudoglaebosa*, was numerically dominant in the fungal metacommunity throughout the growing season, and its frequency increased between early and later-successional stages of individual pitchers. We next investigated the ecological processes leading to *C. pseudoglaebosa* dominance. Unlike dominant yeasts in many other systems, *C. pseudoglaebosa* was not an especially good competitor against other tested yeasts. However, it was an especially good disperser. In the pitcher plant metacommunity, dispersal is defined as an organism's arrival or appearance in a pitcher previously unoccupied by that species. *C. pseudoglaebosa* is one of the first fungi to arrive in pitchers and it maintained high frequency in the pitcher plant metacommunity over the course of the season, even as its frequency within individual pitchers ranged from completely absent to over 90% of sequences. The apparently superior dispersal ability of *C. pseudoglaebosa* leads it to dominate the pitcher fungal metacommunity,

demonstrating that dispersal ability, like competitive ability, is an important contributor to ecological dominance in microbial communities.

Results

Fungal communities in pitchers change over successional time

To understand how fungal communities develop in pitchers, we first sequenced entire fungal communities in seventeen pitchers over the course of a growing season. Before selecting pitchers to track for sequencing, we identified 43 unopened pitchers on *Sphagnum* islands in Harvard Pond, located in Petersham, Massachusetts (Figure 1). We recorded the opening date of each pitcher and collected water from each pitcher at four days, seven days ("one week"), 34-42 days ("one month"), and 66-74 days ("two months") after opening. At the two month time point, insect herbivores, including moth larvae (33), had destroyed ten of the original 43 pitchers, and we could only sample water from 33 pitchers. In the sampled pitchers, the presence of fungal DNA was assayed using the ITS1F/ITS4 primer pair (34, 35). Fungi were detectable starting from the first measured timepoint at four days (in 33% of sampled plants), and were widespread after one week, one month, and two months (in 88%, 95%, and 73% of sampled plants, respectively). Fungi were detected in 100% of sampled pitchers at least once during the season, and seventeen of the pitchers contained detectable fungal DNA at every time point from one week to two months. We sequenced fungal DNA from these seventeen pitchers at all available time points, including four days if available, using 454 sequencing of PCR amplicons of the internal transcribed spacer (ITS) ribosomal region. We chose to sequence the ITS region because it is a common barcode used to discern

fungal species (36). While this region is generally able to distinguish between species, it is not useful for measuring intraspecific genetic diversity.

Fungal succession varied among pitchers. While community composition changed significantly with time (Figure 2A), only a small amount of variation in community composition was due to variation in time (distance-based redundancy analysis adjusted R^2 = 0.03, F = 2.00, df = 1,27, p = .012). This small influence of time on community composition was likely a result of high variation among pitchers. Succession followed two trajectories—five pitchers (hereafter referred to as "pitcher group 1") followed a different successional trajectory from the other twelve ("pitcher group 2")—and there was considerable variation among communities within each trajectory (Figure 2B). Distance among pitchers did not explain significant variation in community composition (partial mantel test of community composition on space controlling for time, mantel statistic r = -0.03, significance = .696).

Despite the observed variation in fungal community composition, diversity decreased, on average, in pitchers between four days and two months (Figure 3). To determine a sample's diversity, we calculated Hill numbers of orders 0 to 2 (^0D to ^2D) for each sample after rarefaction to 1143 sequences per sample. Hill numbers of different orders give community diversity with an emphasis on rare species (low orders) or common species (high orders) (37, 38). ^0D, ^1D, and ^2D are equal to operational taxonomic unit (OTU) richness, the exponent of Shannon diversity, and the inverse of Simpson's index, respectively. Diversity as indicated by all calculated Hill numbers decreased between early and late timepoints: on average, ^0D declined significantly from 43.3 within the first week (including four day and one week timepoints) to 23.9 after two months (t=-

3.5, df=27, p=.002); ^{1}D declined from 14.4 to 5.3 (t=-3.6, df=27, p=.001); and ^{2}D declined from 9.0 to 3.3 (t=-3.2, df=27, p=.004).

C. pseudoglaebosa *is the dominant fungal taxon in pitchers throughout succession*

C. pseudoglaebosa, in the class Saccharomycetes, was the numerically dominant taxon in the fungal metacommunity, but was not dominant in every pitcher (Figure 4). In the metacommunity, *C. pseudoglaebosa* was more frequent than any other taxon at every time point and its frequency increased between early- and late-successional timepoints. Its metacommunity frequency increased from 19% of total sequences at four days to 42% at two months (Figure 4A). However, its frequency did not increase over time in every pitcher. Depending on the pitcher, *C. pseudoglaebosa*'s within-pitcher frequency increased over the season, decreased over the season, peaked midway through the season, or dipped midway through the season (Figure 4B). We cannot say whether these increases or decreases in *C. pseudoglaebosa* sequence frequency reflect changes in the total cell numbers because we did not measure cell numbers or fungal biomass in pitchers. Pitcher group 1 never contained appreciable *C. pseudoglaebosa*: each pitcher in group 1 contained less than 1.6% *C. pseudoglaebosa* sequences regardless of the sampled time point (Figure 4B).

C. pseudoglaebosa *is not a superior competitor, but has complex interactions with other yeasts*

To better understand how interactions with other yeasts might influence the frequency of *C. pseudoglaebosa*, we grew *C. pseudoglaebosa* and other potentially

197 interacting pitcher yeasts in laboratory microcosms. We followed the strategy advocated
198 in (39), which suggested determining interacting species' effects on one another by
199 measuring organism performance as the number of interacting individuals increases. We
200 inoculated microcosms with all possible pairs of three culturable pitcher yeasts (*C.*
201 *pseudoglaebosa*, *Rhodotorula babjevae*, and *Moesziomyces aphidis*). *C. pseudoglaebosa*
202 represented 41%, *R. babjevae* represented 2%, and *M. aphidis* represented 0.06% of total
203 sequences in the sequencing dataset. Each low nutrient microcosm contained a focal
204 yeast, which was inoculated as a fixed number of cells, and an interactor, which was
205 inoculated as a varying number of cells. We then let the pairs of yeasts grow in the
206 microcosms and investigated the effects of interactors on each focal yeast using
207 regressions. We evaluated interaction qualities based on the direction (increasing or
208 decreasing focal yeast yield with more interacting cells) and shape (linear or polynomial)
209 of each regression, and we evaluated differences between interactor yeasts based on
210 whether adding interactor yeast identity to each regression improved its fit.
211 Interactions between pitcher plant yeasts ranged from facilitation to competition,
212 depending on the identities of the yeasts and the number of interactor cells present. Under
213 microcosm conditions, interactions between focal yeasts and interactors were polynomial
214 when the focal yeast was *C. pseudoglaebosa* or *M. aphidis* (Figures 5A, B, Tables 1-2):
215 both yeasts were facilitated by small numbers of co-inoculated cells, but their growth was
216 impeded by larger numbers of co-inoculated cells. Note that we detected facilitation of *C.*
217 *pseudoglaebosa* by *R. babjevae* when few *R. babjevae* cells were inoculated, but we did
218 not inoculate *M. aphidis* in small enough numbers to confirm *M. aphidis* facilitation of *C.*
219 *pseudoglaebosa* (Figure 5A). At high numbers of co-inoculated cells, *M. aphidis* had a

more detrimental impact on *C. pseudoglaebosa* than *R. babjevae* (F=6.79, df=1,50, p = 0.012, Figure 5, Table 2). In contrast, the two interactors of *M. aphidis* had similar effects on its yield: at low and intermediate inoculum sizes, both *R. babjevae* and *C. pseudoglaebosa* promoted *M. aphidis* growth, but at high inoculum sizes, both interactors inhibited *M. aphidis* growth (Figure 5B). Interactions between *R. babjevae* and interactor yeasts were linear (Figure 5C, Tables 1-2): *R. babjevae* yield was impeded by interactors regardless of the number of interactor cells present, and *C. pseudoglaebosa* had a more detrimental impact on *R. babjevae* than *M. aphidis* did (F=86.07, df=1,58, $p < 0.001$, Table 2). Additionally, microcosms with larger interactor inoculum sizes produced more interactor cells at the end of the experiment. In other words, the more of a species's cells we inoculated at the start of the experiment, the more cells we counted at the end of the experiment, regardless of whether the species was a focal or interactor species. The observation that *C. pseudoglaebosa* and *R. babjevae* are inhibited, while *M. aphidis* is facilitated, by intermediate numbers of competing cells was qualitatively supported by data from a three-way competition test, in which microcosms were inoculated with approximately 1000 cells of each species. In these microcosms, *M. aphidis* increased in frequency from 51% to 88% of all cells, while *C. pseudoglaebosa* and *R. babjevae* each decreased, from 26% and 22% to 4% and 8% of all cells, respectively.

C. pseudoglaebosa *is an early disperser in pitchers*

To investigate whether dispersal influences *C. pseudoglaebosa* dominance in pitchers, we observed the arrival times each of the three yeasts mentioned above in pitchers over the *S. purpurea* growth season in Harvard Pond. We surveyed the presence

243 or absence of each yeast in each of the 43 sampled pitchers using taxon-specific PCR
244 primers (Table 3) to determine when each yeast arrived in a pitcher and whether it
245 persisted throughout the season. We sought to amplify DNA of each of the three species
246 from all samples, including those in which fungi had not previously been detected using
247 the more general ITS1F/ITS4 primer pair. The three yeasts appeared in pitchers
248 sequentially (Figure 6): *C. pseudoglaebosa* first arrived in pitchers within four days after
249 the pitchers opened; *R. babjevae* arrived between four days and one week after pitchers
250 opened; and *M. aphidis* arrived one week to one month after pitchers opened. Once a
251 yeast colonized a pitcher, it either persisted in or disappeared from that pitcher later in the
252 season, but it did not disappear from the broader metacommunity.
253
254 **Discussion**
255 C. pseudoglaebosa *is a pitcher metacommunity dominant*
256 *C. pseudoglaebosa* was the dominant fungal taxon throughout succession in the *S.*
257 *purpurea* pitcher metacommunity, although it did not dominate every individual pitcher
258 community. It was the most frequent fungal taxon in the metacommunity at every
259 sampled timepoint, and its frequency in the metacommunity increased after initial
260 colonization (Figure 4). The observation that *C. pseudoglaebosa* sequences were the most
261 frequently found in the metacommunity is consistent with observations that *C.*
262 *pseudoglaebosa* is the most readily cultured yeast taxon in pitchers, and that it is as
263 geographically widespread as the pitcher plant *S. purpurea* itself, found at sites spanning
264 Florida, Newfoundland, and British Columbia (40). Dominance in the pitcher
265 metacommunity was in part a result of the metacommunity structure itself: while *C.*

pseudoglaebosa was not dominant in every pitcher, it was dominant in enough pitchers (in some cases with a frequency above 90% of all OTUs) to dominate the metacommunity as a whole (Figure 4). The poor competitive performance of *C. pseudoglaebosa* relative to other yeasts in microcosms suggests that overall competitive superiority was not the cause of its dominance (Figure 5). Instead, *C. pseudoglaebosa*'s early dispersal is a more likely cause (Figure 6). Dispersal ability appears to enable *C. pseudoglaebosa* to maintain dominance in the metacommunity even when it is not dominant inside every pitcher it colonizes.

Our best explanation for *C. pseudoglaebosa*'s dominance is that it pre-empts other community members by reaching and establishing in pitchers before other taxa arrive. Early dispersal likely gives *C. pseudoglaebosa* a numerical advantage by providing the opportunity to begin exponential growth before other members of the fungal community can arrive and begin growing (41). Additionally, once *C. pseudoglaebosa* is established in a pitcher, facilitation by low-frequency interacting taxa may help it to maintain dominance (Figure 5A). Once established in the metacommunity, *C. pseudoglaebosa* will continue to disperse into new pitchers throughout the growing season (Figure 6).

However, we do not understand how yeasts, including *C. pseudoglaebosa*, disperse into pitchers. Pitchers are sterile before they open, and all pitcher-inhabiting organisms must reach the pitcher habitat from the external environment (27). Because yeasts are ubiquitous in nature (42), potential sources of yeast inocula are numerous and include rainfall, older pitchers, the surfaces of other bog plants, surface or pore water of the bog itself, and surrounding forests and upland soils. It is also likely that pitcher invertebrates bring yeasts into pitchers, perhaps from older pitchers. *C. pseudoglaebosa*,

289 or a close relative, was previously found associating with the pitcher-endemic mosquito
290 *W. smithii*, and could persist in the hindguts of the mosquito *Aedes agypti* in a laboratory
291 experiment (43). However, we have no direct observations of insects introducing yeasts
292 into sterile pitchers.
293
294 *Ecological patterns within pitchers*
295 Although *C. pseudoglaebosa* was the numerically dominant fungal taxon in the
296 metacommunity of pitchers, chance events, dispersal, and interactions among fungi
297 appear to determine whether it is the dominant taxon inside any given pitcher. We
298 observed both facilitation and inhibition in microcosms (Figure 5). While we did not
299 investigate the mechanisms driving these interactions, inhibition may result from
300 differences in abilities to exploit resources or from direct antagonism (44). We further
301 hypothesize that interactions with the taxa that arrive, by chance, at different times in
302 different pitchers caused the variety of *C. pseudoglaebosa* relative frequency changes
303 observed (*i.e.*, increasing, decreasing, or nonmonotonic, Figure 4B) because different
304 interacting yeasts have qualitatively different impacts on *C. pseudoglaebosa* depending
305 on the number of interacting cells present (Figure 5A). In general, individual pitchers
306 experienced priority effects: the timing of taxon arrival in each pitcher (*e.g.*, early arrival
307 of *C. pseudoglaebosa* in a pitcher) determined later community composition (*e.g.*, *C.*
308 *pseudoglaebosa* dominance in the pitcher) (19). Despite the influence of chance events on
309 *C. pseudoglaebosa* dominance in any given pitcher, the early and frequent dispersal of *C.*
310 *pseudoglaebosa* compared to other yeasts enabled its overall dominance in the
311 metacommunity (Figure 4A, 6).

A variety of other ecological processes may influence *C. pseudoglaebosa* frequency changes in individual pitchers (Figure 4B). In addition to interacting with other yeast species, *C. pseudoglaebosa* most likely interacts with bacteria. Bacteria might influence *C. pseudoglaebosa* dominance by altering pitcher environment pH or making nutrients available to *C. pseudoglaebosa*, or *C. pseduoglaebosa* might compete with bacteria for nutrients. Similarly, invertebrates in the pitcher food web community (29) likely influence *C. pseudoglaebosa* frequency, perhaps through predation.

Interactions between fungi and insect prey may also influence fungal communities and *C. pseudoglaebosa* frequencies in pitchers. *S. purpurea* pitchers generally trap most of their prey early in development, and this prey can be the only input into *S. purpurea* food webs (49). We observed an overall decline in fungal colonization late in succession (73% of pitchers with detectable fungi after two months, down from a high of 95%; note that only 33% of pitchers contained detectable fungi at the four day timepoint, probably because fungi had not yet had enough time to colonize sterile pitchers (27)). We attribute this decline to old pitchers experiencing die-offs of their fungal communities after all trapped prey were digested and nutrients were exhausted. In this way, pitchers resemble laboratory batch cultures, each of which has a limited amount of exhaustible nutrients. Variation in the quality and quantity of these exhaustible nutrients may also influence variation in *C. pseudoglaebosa* frequency among pitchers. Overall, while the superior dispersal ability of *C. pseudoglaebosa* allowed it to maintain its dominance across pitchers in the metacommunity, interactions with the pitcher host, other microbes, and insect prey were likely responsible for the fate of *C. pseudoglaebosa* in any given pitcher.

Ecological patterns and processes during pitcher metacommunity succession

In the metacommunity, and in many individual pitchers, *C. pseudoglaebosa* remained dominant through decreases in fungal taxon richness and diversity (Figures 3, 4). We did not observe a hump-shaped relationship between pitcher age and species richness, as previously predicted (Figure 3A) (24). Species richness instead decreased, even as *R. babjevae* and *M. aphidis* were first dispersing into pitchers late in the season (Figures 3A, 6). It is likely that *C. pseudoglaebosa* repression of taxa through priority effects has a larger influence on species richness than does new dispersal by other species, which would increase diversity.

Previous studies have also documented biotic and abiotic successional changes in pitchers; while we did not measure the same parameters as these previous studies, we assume that similar changes occurred in our pitcher metacommunity and that *C. pseudoglaebosa* maintained its dominance through these changes. For example, previous studies have documented decreasing pH with increasing pitcher age, an early peak in prey insect capture during pitchers' life spans (45), and a variety of changes in bacterial, protist, and invertebrate community compositions over time (46, 47). In bacterial, protist, and invertebrate communities, the identities of dominant taxa changed as succession progressed. In contrast, *C. pseudoglaebosa* remained the dominant fungus throughout succession. *C. pseudoglaebosa* appears to be a classical early-successional taxon (1, 48) because it disperses early and frequently (Figure 6), but it is not replaced by late-successional taxa.

Unlike classical early-successional taxa, *C. pseudoglaebosa* maintains dominance in the metacommunity over time and is not consistently replaced. In contrast, dominant

early successional taxa are replaced over time by dominant late successional taxa in most studied successional systems (8, 45, 48, 50). Changes in the abiotic and biotic environment generally cause this turnover of dominant taxa (1, 48). In classical models of succession, early-successional taxa are either superior dispersers, or are good at establishing in uncolonized habitats, or both. Late-successional taxa either require facilitation by early-successional taxa, or are tolerant of late-successional environmental conditions, or both (1, 48). Superior competitive ability and/or ecosystem engineering can also be responsible for late-successional dominance. For example, directly antagonistic interactions are often responsible for replacement of one species by another during fungal succession on decaying wood (51, 52). In contrast, *C. pseudoglaebosa* does not require environmental changes to achieve late-successional dominance, as it is already present and dominant early in succession, nor is it replaced by superior competitors late in succession.

C. pseudoglaebosa dominance throughout succession may be enabled by the short lifespans of pitchers in Harvard Pond; *i.e.*, pitchers may not live long enough to enable late-succession fungal taxa to dominate the metacommunity. We sampled pitchers that were up to 66-74 days old, and stopped sampling at this age because 23% of pitchers had been destroyed by moths. However, pitchers in northern *S. purpurea* populations can survive intact through winter conditions (32), and pitchers can be active for over a year in the southern United States (47). We speculate that fungal succession more closely resembles classical successional patterns and the patterns observed for other pitcher guilds (*e.g.*, bacteria, invertebrates) in longer-lived pitchers. For example, it is possible that a strong competitor such as *M. aphidis* could replace *C. pseudoglaebosa* in southern

S. purpurea metacommunities where pitchers are active for many months. However, consistent dominance of a single taxon over succession may be common in microbial habitats that, like northern *S. purpurea* pitchers, have short lifespans but repeatedly become available.

Conclusions

In the model pitcher plant metacommunity, taxon dispersal ability has a profound influence on community structure. In particular, *C. pseudoglaebosa*'s ability to disperse into pitchers before other fungal taxa enables it to persist as the dominant taxon in the pitcher metacommunity, even as intertaxon interactions and the stochasticity of individual dispersal events prevent its dominance in every pitcher. It is likely that dispersal ability leads to persistent dominance in a variety of other natural succeeding microbial communities and metacommunities, especially when early dispersal allows a taxon to prevent establishment of other taxa. But to establish dispersal as a general mechanism causing dominance in microbial ecosystems, it would be useful to track its dynamics in other metacommunities.

Future studies of microbial succession should explicitly include metacommunity structure when investigating ecological processes. In the pitcher metacommunity, overall taxon composition changed little over time, with *C. pseudoglaebosa* dominant throughout succession (Figure 4A). However, individual pitchers followed a variety of trajectories (Figures 2, 4B, 6). Studies of succession that do not take a metacommunity's structure into account may miss community heterogeneity and the diversity of ecological processes, especially dispersal ability, in play among communities.

404

405 **Materials and Methods**

406 *Study site and field collections*

407 Observations were made on *Sphagnum* islands in Harvard Pond, adjacent to Tom
408 Swamp, a 50 ha *Sphagnum* bog located in Petersham, Massachusetts at 42°30'N,
409 72°12'W (Figure 1) (53). The *C. pseudoglaebosa* and *M. aphidis* isolates used in the
410 microcosm study were collected from pitchers in Harvard Pond, and the *R. babjevae*
411 isolate was collected from a pitcher in Swift River Bog, a 2 ha kettlehole bog located 75
412 km south of Tom Swamp in Belchertown, MA at 42°16'N, 72°20'W (54). These three
413 yeast isolates were collected in the summer of 2006 and identified by comparing their
414 ribosomal sequences, amplified using the ITS1F/ITS4 and LS1/LR5 primer pairs (34, 35,
415 55, 56), to sequences in the NCBI BLAST database (57). We chose *C. pseudoglaebosa*,
416 *R. babjevae*, and *M. aphidis* in part because they were all easily cultured from pitchers,
417 and in part because they formed colonies with different morphologies on agar plates: *C.*
418 *pseudoglaebosa* forms smooth white colonies; *M. aphidis* forms wavy white colonies;
419 and *R. babjevae* forms smooth pink colonies.

420 All *S. purpurea* pitcher water samples for PCR and 454 sequencing were collected
421 in the spring and summer of 2009. In May of 2009, we identified 43 unopened *S.*
422 *purpurea* pitchers on 32 *Sphagnum* islands in Harvard Pond. Pitchers ranged from less
423 than 1 m to 908 m in distance to other pitchers (Figure 1C). To the best of our
424 knowledge, all pitchers were taken from different rosettes. However, rosettes can be
425 joined via rhizomes hidden underwater or underground, and we did not confirm that all
426 sampled pitchers were from genetically distinct plants. We visited each pitcher daily until

it opened, and counted pitcher age from the date it opened. For each pitcher water collection, the water inside a pitcher was first mixed by pipetting up and down with a sterile plastic transfer pipette. We then removed about 0.25 ml pitcher water and mixed it with 0.25 ml of CTAB buffer (100 mM Tris pH 8.0, 1.4 M sodium chloride, 20 mM EDTA, 2% CTAB). To the best of our ability, we avoided collecting insect prey or macrofauna in these samples, although any protists and microscopic animals present in our samples were included; collected pitcher water contained no large animal parts and appeared as a cloudy liquid. All samples were flash-frozen in liquid nitrogen within five hours of collection and stored at -20 or -80°C before DNA extraction.

PCR assay

We assayed each pitcher water sample for amplifiable DNA from all fungi, using the ITS1F/ITS4 primer pair, and for each of the three yeasts in the microcosm experiment, using the primers in Table 3. Primers to selectively amplify portions of each microcosm yeast's ITS sequence were designed using the NCBI BLAST primer tool (58). We chose primer sequences to reliably amplify as much of the ITS sequence of each yeast species as possible, while not amplifying other sequences in the BLAST database.

To extract DNA from each pitcher water sample before the PCR assay, we first thawed and centrifuged frozen samples at 16.1 g for 10 min and removed the supernatant from each pellet. We then suspended each pellet in 200 µL of breaking buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM sodium chloride, 10 mM Tris, and 1 mM EDTA) (59). We mixed each suspension with about 200 µL of 0.5 mm glass beads and 200 µL 25:24:1 chloroform:phenol:isoamyl alcohol. We vortexed each mixture for 2 min,

and then centrifuged it for 5 min at 16.1 g. After centrifugation, we removed the aqueous layer and mixed it with 2.5 volumes of 95% ethanol and 0.1 volume of 3M sodium acetate (60); we incubated each aqueous layer mixture at -20°C for at least three hours. Next, we centrifuged each aqueous layer mixture for 15 min at 16.1 g, and removed the supernatant. Finally, we washed each pellet with 0.5 ml 70% ethanol, centrifuged each mixture for 10 min at 16.1 g, removed the supernatant, and resuspended each pellet in 50 µl water.

We then assayed each DNA extract for the presence of each Fungal taxon, or any Fungal DNA in the case of the ITS1F/ITS4 primer pair, using PCR. Each PCR reaction was composed of 7.9 µL water, 0.1 µL GoTaq® Flexi polymerase (Promega), 5 µL Flexi buffer with green dye added, 5 µL 5x CES (combinatorial PCR enhancer solution: 2.7 M betaine, 6.7 mM dithiothreitol, 6.7% dimethyl sulfoxide, 55 µg/mL bovine serum albumin) (61), 5 µL nucleotide mix, 2 µL magnesium chloride, 1 µL of 10 µM of each primer, and 1 µL undiluted template DNA extract. All reactions were cycled on a Biorad iCycler or myCycler using denaturing, annealing, and extension temperatures of 95, 55, and 72 °C, respectively. We denatured for 85 s, then ran 13 cycles of 35 s denaturing, 55 s annealing, and 45 s extension, followed by 13 cycles that were identical but had a 2 min extension, and finally 9 cycles with a 3 min extension. We ran a subsequent 10 min extension. Two µL of each PCR product were visualized on 1% agarose gels stained with SYBR Safe dye (Invitrogen) and photographed using a U:genius gel documenting system (Syngene) and a Stratagene transilluminator. Photographs of gels were scored for presence or absence of a band. Bands that were too faint to reliably score were run a

second time with 6 µL of PCR product per well. Presence of a band on a gel indicated the presence of detectable fungal or yeast species DNA in a water sample.

To confirm that primers only amplified sequences from the target yeasts, we randomly selected nine PCR products generated from the *C. pseudoglaebosa* and *R. babjevae* primer pairs for sequencing. The primer pair that targets *M. aphidis* only amplified DNA from six pitcher water extracts, and we sequenced all six PCR products for this primer pair. Sequences were identical to or within one base of cultured isolate sequences.

Pitcher water fungal DNA amplification and 454 sequencing

We extracted and amplified fungal DNA for fungal community amplicon sequencing using the protocols described above, with the following changes. Gotaq® Hotstart polymerase (Promega) was used instead of Flexi polymerase, and we used 50 µM instead of 10 µM of the reverse primer. The forward primer consisted of (in order from 5' to 3') the 454 "A" primer (CCATCTCATCCCTGCGTGTCTCCGACTCAG) concatenated with a 10-bp multiplex tag (62), and ITS4; the reverse primer consisted of the 454 "B" primer (CCTATCCCCTGTGTGCCTTGGCAGTCTCAG) concatenated with ITS1F. Multiplex tags were unique to each sample. Reactions were cycled at 95 °C for 15 min; 30 cycles of 95 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min; and a final extension of 72 °C for 8 min.

Products were purified using Agencourt® *AMPure*® XP (Beckman Coulter) and quantitated using a Qubit® dsDNA HS Assay (Invitrogen) according to the manufacturers' instructions. We combined equimolar concentrations of the products of

each of three separate PCR reactions from each DNA extract. The sequencing pool consisted of pooled equimolar concentrations of each pooled PCR product. The pool was sequenced on one-eighth of a 454 Titanium sequencing run by the Duke Genome Sequencing & Analysis Core Resource.

454 sequence processing

We processed sequences using QIIME 1.3.0 (63). Low quality sequences were removed and the remaining sequences were assigned to multiplex barcodes using the default quality filtering settings. Primers and barcodes were trimmed from each sequence and sequences shorter than 200 and longer than 1000 bp were removed from each dataset. Sequences were denoised using the QIIME denoiser. We reduced chimeric sequences by trimming the 5.8s and ITS1 portions from all sequences using the Fungal ITS Extractor (64), and only analyzing the ITS2 portion. The 5.8s ribosomal region lies between the ITS1 and ITS2 spacers, and is conserved among fungi relative to the spacers. We expected most chimeric sequences to form in the 5.8s region and to be composed of ITS1 and ITS2 sequences from different templates (65). We chose operational taxonomic units (OTUs) using the uclust method in QIIME, at 97% similarity (66). We discarded all OTUs composed of a single sequence (singleton OTUs) because we assumed that they resulted from sequencing errors. The longest sequence in each remaining cluster was retained as a representative sequence. Of the 141,424 total sequences produced, 27,632 were discarded for having lengths less than 200 or more than 1000 bp and 10,938 were discarded because they had low quality or did not have a matching barcode. Sixty-six sequences were discarded because the ITS2 subunit could not be extracted and 139

sequences were discarded because they represented singleton OTUs. In total, we retained 102,649 sequences for further analysis. Each pitcher water sample produced between 253 and 4365 sequences. We will deposit the 454 sff files and corresponding FASTA files in the NCBI Small Read Archive database (accession numbers to be added after manuscript acceptance).

Sequence taxonomy assignments

We used the uclust method in QIIME and the UNITE database (dynamic release 01-12-2017) to assign taxonomy to a representative sequence for each OTU with a minimum percent similarity of 80% (66, 67). We assumed that unassigned OTUs were fungal sequences not yet in the UNITE database, and discarded one OTU assigned to the kingdom Rhizaria. We retained unassigned OTUs for diversity measurements, but did not include them in taxonomy summaries. OTUs assigned to the genera *Candida*, *Rhodotorula*, and *Moesziomyces* were manually curated and compared with sequences for type strains in the NCBI database (68). In total, we detected 553 OTUs, of which 348 were assigned to fungal taxa, one was assigned to a kingdom other than Fungi, and 204 were not assigned. Of the 348 fungal taxa, 50% (174) were Basidiomycota, 47% (162) were Ascomycota, and 0.3% (12) were basal fungal lineages. Sequences, metadata, and OTU tables including taxonomic assignments in QIIME 1.3.0 format will be submitted to the Edmond Open Access Data Repository after manuscript acceptance.

Microcosm interaction assays

Interactions between yeasts were assayed in microcosms designed to mimic pitchers simultaneously colonized by different numbers of two or more yeast species. Interacting yeasts grew in low-nutrient media designed to mimic natural conditions in pitchers. While sterilized pitcher liquid would be the most realistic media for microcosms, the quantities needed were unavailable. Instead, microcosms contained sterile yeast extract media (YEM) composed of 1g/L yeast extract in local tap water (Cambridge, MA, USA). Tap water was used instead of deionized water because we wanted the media to include micronutrients present in local rainwater that may be important for pitcher plant yeast growth. The tap water supply in Cambridge, MA, where this experiment was conducted, comes from three Massachusetts reservoirs (69), and we expected it to have similar inputs as rainwater in Harvard Pond pitchers. Each microcosm was inoculated with a target yeast species and an interactor in 200 µL of liquid yeast media. Each target yeast was inoculated with about 1000 cells per microcosm, and each interactor yeast was inoculated at zero, low, medium, and high cell numbers (0 and approximately 100, 1000, and 10,000 cells).

Eighteen treatments of yeast mixtures were prepared, with ten replicates each, for a total of 180 microcosms. Before inoculation, yeasts were grown in liquid YEM for 48 hours. Inoculation sizes were measured after inoculation using counts of colony-forming units (CFUs) on solid YEM (YEM plus 1.5% agar). Microcosms were arranged in sterile 96-well polystyrene flat bottom cell culture plates and incubated between 25 and 27 °C, shaking at 700 rpm for 48 hours. After incubation 32 microcosms were discarded because of suspected contamination. We diluted each remaining microcosm $1{:}10^3$ or $1{:}10^4$ in sterile water, plated it to solid YEM, and counted CFUs on plates containing at least 30

563 total CFUs. The three species were distinguished by colony morphology (see above).
564 When no CFUs of an inoculated yeast were present on a plate, we conservatively
565 assumed that the yeast was present in the microcosm in numbers just below our detection
566 limit. We calculated total cell numbers assuming one instead of zero CFUs for these
567 yeasts absent from plates. CFU counts before and after incubation will be submitted to
568 the Edmond Open Access Data Repository after manuscript acceptance.
569
570 *Statistical analyses*
571 OTU datasets rarefied to 1143 sequences were used to produce Non-metric
572 Multidimensional Scaling (NMDS) plots and to compare community similarities, and
573 alpha diversity indices among pitchers. Eight samples contained fewer than 1143
574 sequences and were discarded. Proportions of samples assigned to taxonomic groups
575 were calculated based on the full non-rarefied dataset. Community similarities over time
576 were compared using partial distance-based redundancy analysis (db-RDA) of Jaccard
577 dissimilarity (70) between each pair of samples with pitcher age as the explanatory
578 variable, conditioned on pitcher identity. A correlation between geographic distance and
579 community similarity was conducted using a partial Mantel test conditioned on pitcher
580 age. Hill numbers of order q=0 or 2 (^{q}D) were calculated as $^{q}D = \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)}$,
581 where S is the total number of OTUs and p_i is the relative abundance of OTU i; ^{1}D was
582 calculated as the exponent of Shannon diversity (37, 38). Changes in Hill numbers were
583 modeled over time using repeated-measures linear models controlled for pitcher identity;
584 ^{1}D and ^{2}D were log-transformed before analyses to homogenize variances among
585 timepoints, and ^{0}D was not transformed.

We modeled the impact of interactor yeasts on focal yeasts in microcosms using multiple linear and polynomial regressions. Separate regressions were conducted for each focal yeast. For each regression, focal yeast yield was the dependent variable, and both the number of co-inoculated interactor yeast cells and the identity of the interactor yeast were independent variables. We modeled both linear and quadratic relationships between the number of co-inoculated interactor yeast cells and the dependent variable because the relationship did not always appear linear when plotted. Before constructing the regressions, we square-root-transformed focal yeast yield to homogenize variances for the focal yeasts *R. babjevae* and *C. pseudoglaebosa*, but left yield untransformed for the focal yeast *M. aphidis*. We also transformed competitor inoculum size by $\log_{10}(x+1)$ because interactor inoculum size was varied on a log scale in the experiment. When comparing the influences of competitor species, we randomly assigned treatments with no interacting yeast inoculum to one of the two interacting yeasts. When selecting the best-fitting regression model, we first established the best-fitting relationship (linear, quadratic, or both) between log-transformed interactor inoculum size and focal yeast yield, and then determined whether adding interactor identity or interactions between interacting yeast identity and inoculum size to the model improved it. The best-fitting model was the one with the lowest Akaike Information Criterion (AIC).

All statistical analyses and index calculations were conducted using R version 3.3.1 (71) and the packages vegan, fields, nlme, and GUniFrac (72-75). Plots were made using ggplot2 (76).

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617

618 **Data Accessibility**

619 We will deposit the 454 sff files and corresponding FASTA files in the NCBI Small Read
620 Archive database after manuscript acceptance. We will deposit representative sequences
621 for each OTU, metadata, OTU tables including taxonomic assignments in QIIME 1.3.0
622 format, and raw data from the competition experiment to the Edmond Open Access Data
623 Repository after manuscript acceptance.

624

625 **Figure Legends**

626 Figure 1: Example *S. purpurea* plant and study location. (A) One of the study pitcher
627 plants at the edge of a *Sphagnum* island. This photograph was taken early in the growth
628 season and both opened and unopened pitchers are visible. (B) The location of Harvard
629 Pond in Massachusetts, USA. (C) Locations of the 43 pitchers sampled for this study.
630 Each white circle represents one pitcher. Note that some pitchers were close enough to

631 one another that the white circles overlap. Maps were created using ArcMap™ Version
632 9.2 (77); map data for panel B are from (79) and map data from panel C are from (78).
633
634 Figure 2: Non-metric Multidimensional Scaling (NMDS) plots of pitcher plant
635 community similarities. Similarities of community OTU compositions were calculated
636 using the Jaccard metric (70). Purple triangles represent four-day-old communities,
637 orange circles represent week-old communities, red squares represent approximately one-
638 month old communities, and blue diamonds represent approximately two-month-old
639 communities. (A) NMDS plot with similarities among time points highlighted. Ellipses
640 depict 95% confidence intervals of the centroid of each time point. No ellipse is depicted
641 for four-day-old communities because only two were measured. (B) NMDS plot with
642 individual pitchers highlighted. Lines connect measurements for each pitcher. Fungal
643 communities in pitcher group 1 are represented with black lines and fungal communities
644 in pitcher group 2 are represented with gray lines. All points are located at the same
645 coordinates in (A) and (B).
646
647 Figure 3: Hill numbers of orders A) 0, B) 1, and C) 2 in pitchers over time. Data points
648 for communities in the same pitcher are connected with lines. Black lines connect points
649 for pitcher group 1 and gray lines connect points for pitcher group 2.
650
651 Figure 4: Taxon diversity in pitchers over time. Proportions are reported based on non-
652 rarefied OTU assignments. (A) Taxon diversity in the entire bog metacommunity.
653 Colored bars represent proportions of total sequences for each fungal class (or phylum for

654 basal fungal lineages). Unclassified sequences were not included in this figure. The
655 hatched area represents total *C. pseudoglaebosa* frequency for each time point. Note that
656 *C. pseudoglaebosa* is in the class Saccharomycetes and represents over 99% of
657 Saccharomycetes sequences at the one and two month time points. (B) *C.*
658 *pseudoglaebosa* sequence frequency in individual pitcher communities. Data points for
659 communities in the same pitcher are connected with lines. Black lines connect points for
660 pitcher group 1 and gray lines connect points for pitcher group 2.
661
662 Figure 5: Influence of interacting species on (A) *C. pseudoglaebosa*, (B) *M. aphidis*, and
663 (C) *R. babjevae*. The plots depict the yield of each focal species as a function of the
664 number of cells of an interacting species co-inoculated with the focal species. Interacting
665 species are coded by color: red = *C. pseudoglaebosa*, yellow = *M. aphidis*, and blue = *R.*
666 *babjevae*.
667
668 Figure 6: Presences and absences of each of three yeasts in 43 pitchers over time. Each
669 large gray shape represents the Harvard Pond at one of four time points. Circles represent
670 pitchers: completely white circles represent pitchers in which none of the three yeasts
671 were detected, and circles containing colored pie slices represent pitchers in which one or
672 more of the three assayed yeasts were detected. Pie slices are colored by detected yeast:
673 red = *C. pseudoglaebosa*, yellow = *M. aphidis*, and blue = *R. babjevae*. Circles indicate
674 the approximate locations of pitchers, and are offset to make all data visible; see Figure
675 1C for accurate pitcher locations. Maps were adapted from (78).
676

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Table 1: Model selection for each focal yeast

Response variable	Explanatory variables[§]	df	F	p	adj-R^2	AIC
number of *C. pseudoglaebosa* cells[*]	inoculum size	1, 51	79.37	<.001	0.60	405.64
	(inoculum size)^2	1, 51	111.1	<.001	0.68	394.09
	inoculum size + (inoculum size)^2	2, 50	54.65	<.001	0.67	395.97
	(inoculum size)^2 + competitor identity	**2, 50**	**65.26**	**<.001**	**0.71**	**389.34**
	(inoculum size)^2 + competitor identity x (inoculum size)^2	2, 50	60.02	<.001	0.69	392.51
	(inoculum size)^2 + competitor identity + competitor identity x (inoculum size)^2	3, 49	42.74	<.001	0.71	391.25
number of *M. aphidis* cells	inoculum size	1, 58	5.37	.02	0.07	1211.77
	(inoculum size)^2	1, 58	11.97	.001	0.16	1205.83
	inoculum size + (inoculum size)^2	**2, 57**	**10.86**	**<.001**	**0.25**	**1199.71**
	inoculum size + (inoculum size)^2 + competitor identity	3, 56	7.64	<.001	0.25	1200.51
	inoculum size + (inoculum size)^2 + competitor identity x inoculum size	3, 56	7.73	<.001	0.26	1200.28
	inoculum size + (inoculum size)^2 + competitor identity x (inoculum size)^2	3, 56	7.68	<.001	0.25	1200.28
number of *R. babjevae* cells[*]	inoculum size	1, 59	92.25	<.001	0.60	542.62
	(inoculum size)^2	1, 59	67.64	<.001	0.53	553.45
	inoculum size + (inoculum size)^2	2, 58	45.56	<.001	0.60	544.44
	inoculum size + competitor identity	**2, 58**	**155.7**	**<.001**	**0.84**	**489.12**
	inoculum size + competitor identity x inoculum size	2, 58	109.0	<.001	0.78	506.87
	inoculum size + competitor identity + competitor identity x inoculum size	3, 57	102.1	<.001	0.83	491.06

* number of *C. pseudoglaebosa* and *R. babjevae* cells were square-root-transformed

§ inoculum size was $\log_{10}(x+1)$-transformed

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900 Table 2: ANOVA tables for each best-fitting models
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Response variable	Explanatory variable§	df	SS	MS	F	p
number of *C. pseudoglaebosa* cells*	(inoculum size)^2	1	10235.2	10235.2	123.73	<.001
	competitor identity	1	561.4	561.4	6.79	.012
	residuals	50	4136.3	82.7		
number of *M. aphidis* cells	inoculum size	1	173667504	173667504	6.6684	.012
	(inoculum size)^2	1	391953317	391953317	15.0501	<.001
	residuals	57	1484466512	26043272		
number of *R. babjevae* cells*	inoculum size	1	36941	36941	225.28	<.001
	competitor identity	1	14114	14114	86.07	<.001
	residuals	58	9511	164		

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903 * number of *C. pseudoglaebosa* and *R. babjevae* cells were square-root-transformed
904 § inoculum size was $\log_{10}(x+1)$-transformed
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908 Table 3: Taxon-specific PCR primer sequences used to detect pitcher yeasts
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yeast	forward sequence	Tm	reverse sequence	Tm	product length
C. pseudoglaebosa	CTGCGGAAGGATCATTACAGT	54.6	TGTTCAGACAACACTGTTCA	51.8	466
R. babjevae	AAGTCGTAACAAGGTTTCCG	52.8	CCCAACTCGGCTCTAGTAA	53.9	527
M. aphidis	GGTAATGCGGTCGTCTAAAA	52.6	CTCTTCCAAAGAAGCGAGG	53.1	467

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