Superior dispersal ability leads to persistent ecological dominance by *Candida pseudoglaebosa* in the *Sarracenia purpurea* metacommunity

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Abstract

A large number of descriptive surveys document changes in microbial communities over time, but direct evidence for the ecological processes mediating succession or causing ecological dominance remains rare. Differential dispersal may be a key mechanism. We surveyed microbial diversity within a metacommunity of pitchers of the model carnivorous plant *Sarracenia purpurea* and discovered the yeast *Candida pseudoglaebosa* as ecologically dominant. Its frequency in the metacommunity increased over 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 taxa. Differential dispersal across the spatially structured metacommunity of individual pitchers emerges as a key driver of the continuous dominance of *C. pseudoglaebosa* during succession.

Introduction

Primary microbial succession occurs when a microbial community colonizes and develops on a newly available substrate (Fierer *et al*. 2010). 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 (Copeland *et al.* 2015, Boynton & Greig 2016, Koenig *et al.* 2011, Cutler *et al*. 2014). A variety of successional patterns have been observed. For example, taxon diversity can increase, decrease, or randomly vary with successional time (Cutler *et al.* 2014, Zumsteg *et al.* 2012, Redford & Fierer 2009). However, researchers commonly observe replacement of early-successional taxa by late-successional taxa (*e.g.,* Boynton & Greig 2016, Wolfe *et al.* 2014). 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 (Boynton & Greig, 2016, Findley *et al.* 2013, Jiao *et al.* 2016), in part because of its obvious parallels to plant and animal ecology (Hillebrand *et al.* 2008). Ecological dominance is apparent when one or a few species comprise most of the individuals or biomass in a community (Hillebrand *et al.* 2008). Environmental filtering, superior competitive ability of the dominant species, and ecosystem engineering can all cause dominance (Wolfe *et al.* 2014, Albergaria *et al*. 2010, Goddard 2008, Nissen & Arneborg 2003, Williams *et al*. 2015). 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

51 example, environmental filtering and competition may be more important in systems
52 where human beings have designed environments to favor domesticated microbes
53 (Wolfe & Dutton 2015) and less important in natural environments with
54 heterogeneous environmental conditions.

55 An overlooked mechanism driving ecological dominance in natural systems
56 may be dispersal. When primary succession occurs on a sterile substrate, all members
57 of the microbial community must first disperse onto the new substrate before
58 establishing in the community. A good disperser may prevent the establishment of
59 other community members through priority effects if it arrives in a habitat first, either
60 by pre-empting or modifying available niches (Fukami 2015). Additionally, good
61 dispersers in nearby communities can impact a microbial community during
62 succession by producing propagules that then disperse into the developing community
63 (Wilson & McTammany 2016).

64 Dispersal may emerge as a key driver of ecological dominance in microbial
65 metacommunities. Metacommunities are physically structured groups of communities
66 in which individual communities are spatially isolated from one another but linked
67 through dispersal (Logue *et al.* 2011, Holyoak *et al.* 2005). Community assembly in
68 metacommunities is a function of dispersal among communities and ecological
69 processes occurring inside each community. Ecological theory explains how dispersal
70 can interact with intracommunity processes to maintain metacommunity diversity
71 (Leibold *et al.* 2004, Holyoak *et al.* 2005). For example, populations occupying low-
72 quality environmental patches can be maintained by dispersal from high-quality
73 patches ("mass effects"), or fitness trade-offs between competitive ability and
74 dispersal ability can mediate species diversity ("patch dynamics"). Theory predicts
75 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 (Moquet *et al.* 2003, Sferra *et al.* 2017). 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 decreases 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 microbial 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 (Buckley *et al.* 2003). Each *S. purpurea* plant produces modified vase-shaped leaves, or pitchers (Figure 1a), annually. At first, developing pitchers are entirely closed, sterile chambers (Peterson *et al.* 2008). 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 (Bennett & Ellison 2009); some prey fall into pitchers and drown, and are then shredded, decomposed, and mineralized by a food web of microorganisms and invertebrates (reviewed in Ellison *et al.* 2003). The pitcher microbial community includes bacteria, algae, and fungi, including culturable yeasts (Cochran-Stafira & von Ende 1998). Pitchers are individual communities within a metacommunity of other pitchers on the same and on different plants.

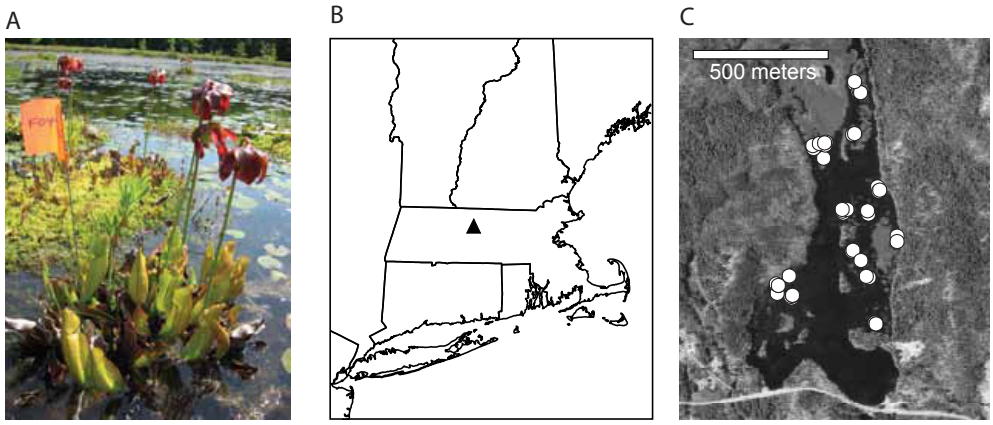

Figure 1: Example *S. purpurea* plant and study location. (A) One of the study pitcher plants at the edge of a *Sphagnum* island. This photograph was taken early in the growth season and both opened and unopened pitchers are visible. (B) The location of Harvard Pond in Massachusetts, USA. (C) Locations of the 43 pitchers sampled for this study. Each white circle represents one pitcher. Note that some pitchers were close enough to one another that the white circles overlap. Maps were created using ArcMap™ Version 9.2 (ESRI 2006); map data are from the Office of Geographic Information, Commonwealth of Massachusetts Information Technology Division (2000) and the National Atlas of the United States (2006).

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, Judd 1959). We first documented that a single yeast species, *Candida pseudoglaebosa*, was numerically dominant in the 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. It 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

112 competitive ability, is an important contributor to ecological dominance in microbial
113 communities.
114
115 **Results**
116 *Fungal communities in pitchers change over successional time*
117 To understand how fungal communities develop in pitchers, we first
118 sequenced entire fungal communities in seventeen pitchers over the course of a
119 growing season. Before selecting pitchers to track for sequencing, we identified 43
120 unopened pitchers on *Sphagnum* islands in Harvard Pond, located in Petersham,
121 Massachusetts (Figure 1). We recorded the opening date of each pitcher and collected
122 water from each pitcher at four days, seven days ("one week"), 34-42 days ("one
123 month"), and 66-74 days ("two months") after opening. At the two month time point,
124 insect herbivores, including moth larvae (Atwater *et al.* 2006), had destroyed ten of
125 the original 43 pitchers, and we could only sample water from 33 pitchers. In the
126 sampled pitchers, the presence of fungal DNA was assayed using the ITS1F/ITS4
127 primer pair (Gardes & Bruns 1993, White *et al.* 1990) Fungi were detectable starting
128 from the first measured timepoint at four days (in 33% of sampled plants), and were
129 widespread after one week, one month, and two months (in 91%, 95%, and 73% of
130 sampled plants, respectively). Seventeen of the pitchers contained detectable fungal
131 DNA at every time point from one week to two months. We sequenced fungal DNA
132 from these seventeen pitchers at all available time points, including four days if
133 available, using 454 sequencing of PCR amplicons of the internal transcribed spacer
134 (ITS) ribosomal region.
135 Fungal succession varied among pitchers. While community composition
136 changed significantly with time (Figure 2A), only a small amount of variation in

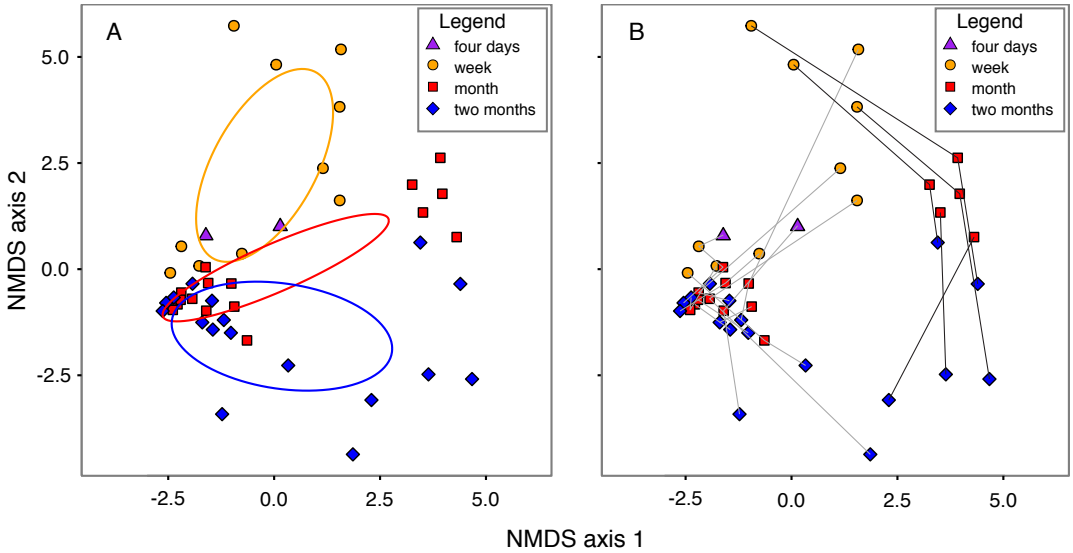

Figure 2: Non-metric Multidimensional Scaling (NMDS) plots of pitcher plant community similarities. Similarities of community OTU compositions were calculated using the Jaccard metric (Jaccard 1912). Purple triangles represent four-day-old communities, orange circles represent week-old communities, red squares represent approximately one-month old communities, and blue diamonds represent approximately two-month-old communities. (A) NMDS plot with similarities among time points highlighted. Ellipses depict 95% confidence intervals of the centroid of each time point. No ellipse is depicted for four-day-old communities because only two were measured. (B) NMDS plot with individual pitchers highlighted. Lines connect measurements for each pitcher. Fungal communities in pitcher group 1 are represented with black lines and fungal communities in pitcher group 2 are represented with gray lines. All points are located at the same coordinates in (A) and (B).

community composition was due to variation in time (distance-based redundancy analysis adjusted $R^2 = 0.03$, $F = 1.96$, $df = 1,27$, $p = 0.011$). 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 $r = -0.04$, significance = 0.715).

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)

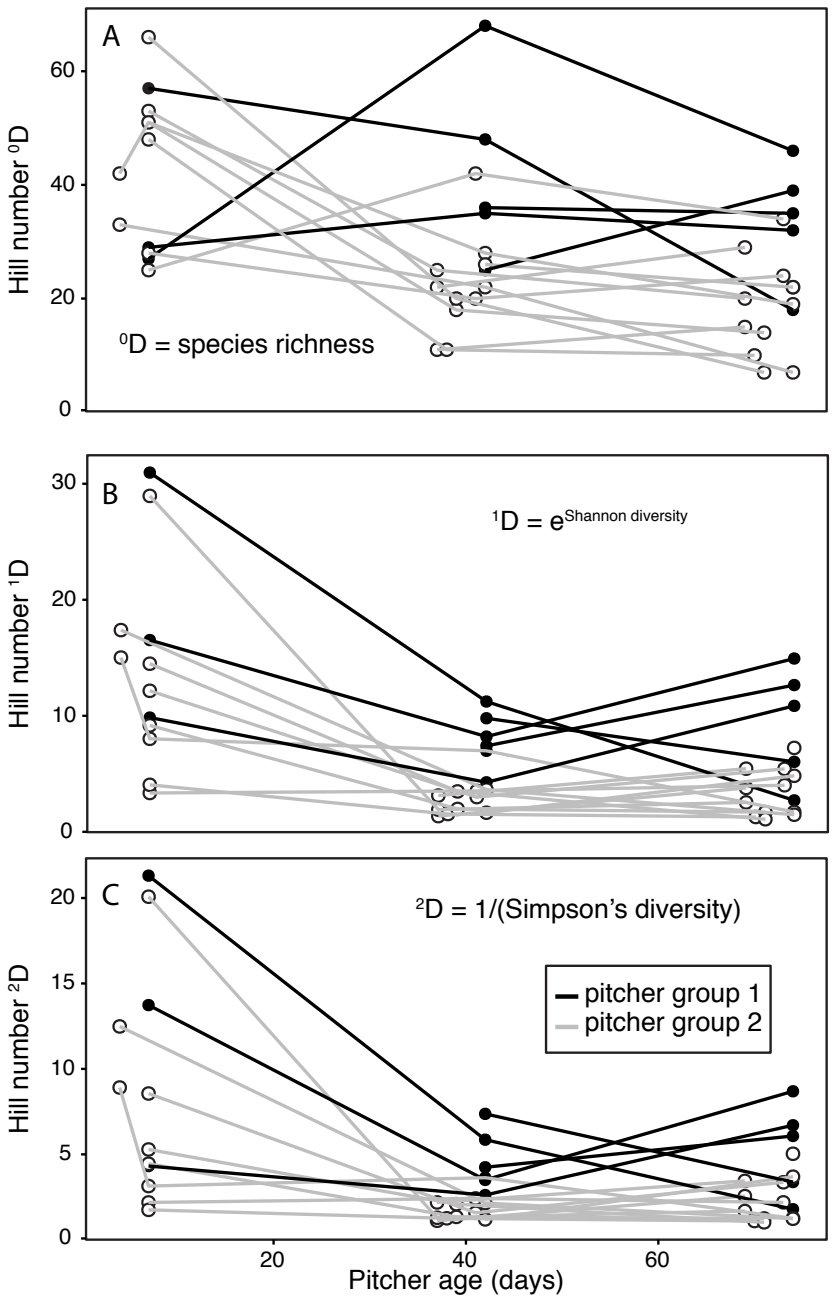

Figure 3: Hill numbers of orders A) 0, B) 1, and C) 2 in pitchers over time. Data points for communities in the same pitcher are connected with lines. Black lines connect points for pitcher group 1 and gray lines connect points for pitcher group 2.

149 for each sample after rarefaction to 1140 sequences per sample. Hill numbers of
150 different orders give community diversity with an emphasis on rare species (low
151 orders) or common species (high orders) (Hill 1973, Chao *et al.* 2014). ^{0}D, ^{1}D, and ^{2}D
152 are equal to operational taxonomic unit (OTU) richness, the exponent of Shannon
153 diversity, and the inverse of Simpson's index, respectively. Diversity as indicated by

154 all calculated Hill numbers decreased between early and late timepoints: on average,
155 ^{0}D declined significantly from 42.5 within the first week (including four day and one
156 week timepoints) to 23.1 after two months (t=-3.6, df=27, p=0.001); ^{1}D declined from
157 14.2 to 5.2 (t=-3.8, df=27, p=0.0008); and ^{2}D declined from 8.9 to 3.2 (t=3.3, df=27,
158 p=0.003).
159
160 C. pseudoglaebosa *is the dominant fungal taxon in pitchers throughout succession*
161 *C. pseudoglaebosa*, in the class Saccharomycetes, was the numerically
162 dominant taxon in the metacommunity, but was not dominant in every pitcher (Figure
163 4). In the metacommunity, *C. pseudoglaebosa* was more frequent than any other
164 taxon at every time point and its frequency increased between early- and late-
165 successional timepoints. Its metacommunity frequency increased from 22% of total
166 sequences at four days to 58% at two months (Figure 4A). However, its frequency did
167 not increase over time in every pitcher. Depending on the pitcher, *C.*
168 *pseudoglaebosa*'s within-pitcher frequency increased over the season, decreased over
169 the season, peaked midway through the season, or dipped midway through the season
170 (Figure 4B). We cannot say whether these increases or decreases in *C.*
171 *pseudoglaebosa* sequence frequency reflect changes in the total cell numbers because
172 we did not measure cell numbers or fungal biomass in pitchers. Pitcher group 1 never
173 contained appreciable *C. pseudoglaebosa*: each pitcher in group 1 contained less than
174 1.7% *C. pseudoglaebosa* sequences regardless of the sampled time point (Figure 4B).
175 C. pseudoglaebosa *is not a superior competitor, but has complex interactions with*
176 *other yeasts*

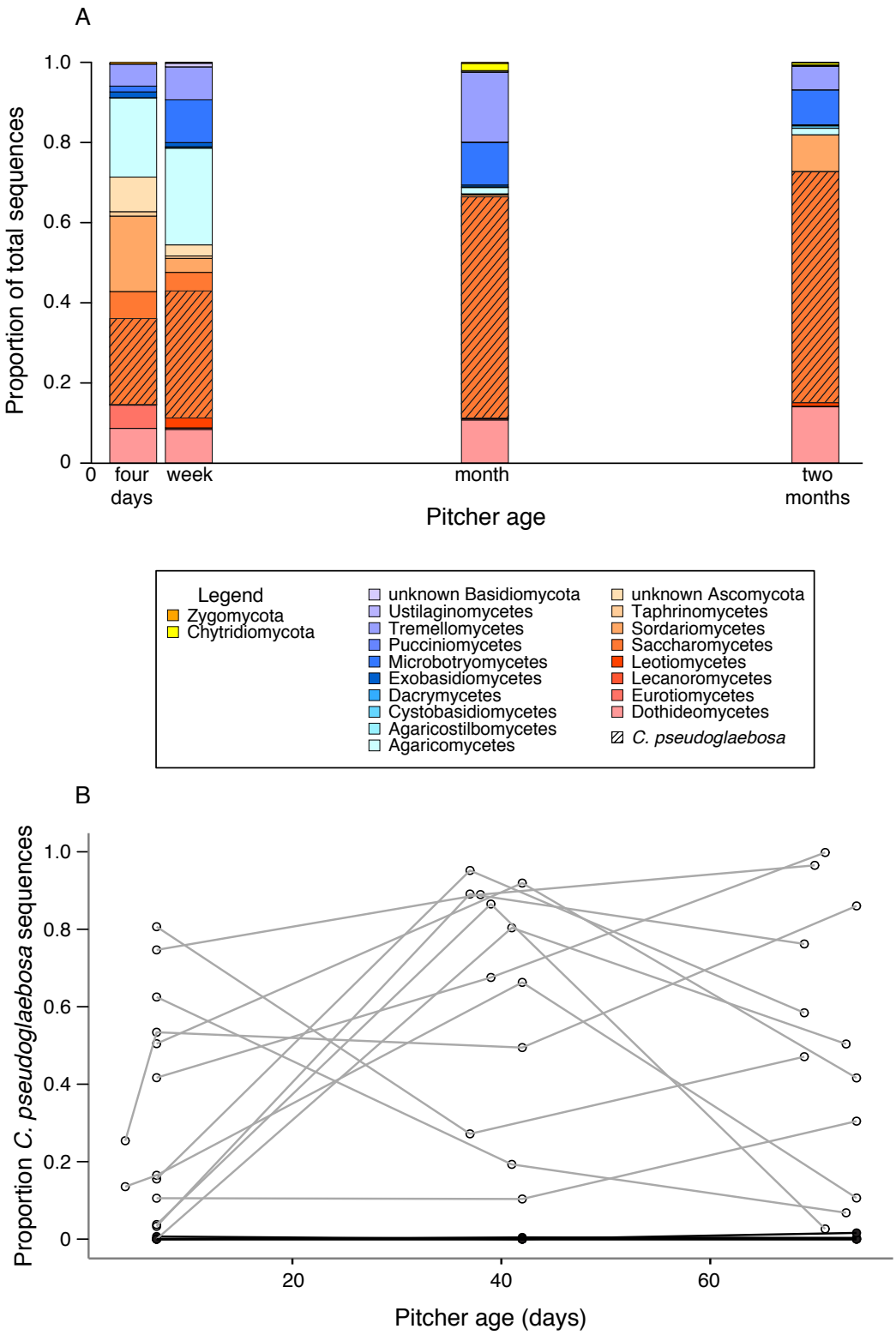

Figure 4: Taxon diversity in pitchers over time. Proportions are reported based on non-rarefied OTU assignments. (A) Taxon diversity in the entire bog metacommunity. Colored bars represent proportions of total sequences for each fungal class (or phylum for basal fungal lineages). The hatched area represents total *C. pseudoglaebosa* frequency for each time point. Note that *C. pseudoglaebosa* is in the class Saccharomycetes and represents over 99% of Saccharomycetes sequences at the one and two month time points. (B) *C. pseudoglaebosa* sequence frequency in individual pitcher communities. Data points for communities in the same pitcher are connected with lines. Black lines connect points for pitcher group 1 and gray lines connect points for pitcher group 2. Data for this figure is non-rarefied OTU data; for rarefied data, see Figure 4 Figure Supplement 1.

178 To better understand how interactions with other yeasts might influence the
179 frequency of *C. pseudoglaebosa*, we grew *C. pseudoglaebosa* and other potentially
180 interacting pitcher yeasts in laboratory microcosms. We followed the strategy
181 advocated by Goldberg and Werner (1983), who suggested determining interacting
182 species' effects on one another by measuring organism performance as the number of
183 interacting individuals increases. We inoculated microcosms with all possible pairs of
184 three culturable pitcher yeasts (*C. pseudoglaebosa*, *Rhodotorula babjevae*, and
185 *Moesziomyces aphidis*). *C. pseudoglaebosa* represented 41%, *R. babjevae* represented
186 2%, and *M. aphidis* represented 0.06% of total sequences in the sequencing dataset.
187 Each low nutrient microcosm contained a focal yeast, which was inoculated as a fixed
188 number of cells, and an interactor, which was inoculated as a varying number of cells.
189 We then let the pairs of yeasts grow in the microcosms and investigated the effects of
190 interactors on each focal yeast using regressions. We evaluated interaction qualities
191 based on the direction (increasing or decreasing focal yeast yield with more
192 interacting cells) and shape (linear or polynomial) of each regression, and we
193 evaluated differences between interactor yeasts based on whether adding interactor
194 yeast identity to each regression improved its fit.
195 Interactions between pitcher plant yeasts ranged from facilitation to
196 competition, depending on the identities of the yeasts and the number of interactor
197 cells present. Under microcosm conditions, interactions between focal yeasts and
198 interactors were polynomial when the focal yeast was *C. pseudoglaebosa* or *M.*
199 *aphidis* (Figures 5A, B, Figure 5 figure supplemental tables 1-4): both yeasts were
200 facilitated by small numbers of co-inoculated cells, but their growth was impeded by
201 larger numbers of co-inoculated cells. Note that we detected facilitation of *C.*
202 *pseudoglaebosa* by *R. babjevae* when few *R. babjevae* cells were inoculated, but we

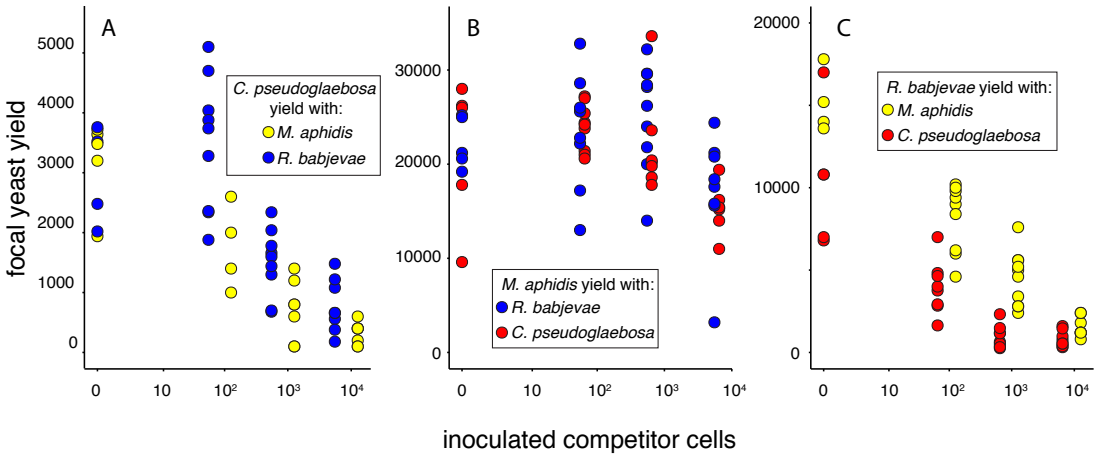

Figure 5: Influence of interacting species on (A) *C. pseudoglaebosa*, (B) *M. aphidis*, and (C) *R. babjevae*. The plots depict the yield of each focal species as a function of the number of cells of an interacting species co-inoculated with the focal species. Interacting species are coded by color: red = *C. pseudoglaebosa*, yellow = *M. aphidis*, and blue = *R. babjevae*.

did not inoculate *M. aphidis* in small enough numbers to confirm *M. aphidis* facilitation of *C. 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, figure supplemental 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, figure supplemental tables 5-6): *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, Figure 5, figure supplemental table 6).

C. pseudoglaebosa *is an early disperser in pitchers*

217 To investigate whether dispersal influences *C. pseudoglaebosa* dominance in
218 pitchers, we observed the arrival times each of the three yeasts mentioned above in
219 pitchers over the *S. purpurea* growth season in Harvard Pond. We surveyed the
220 presence or absence of each yeast in each of the 43 sampled pitchers using taxon-
221 specific PCR primers (Table 1) to determine when each yeast arrived in a pitcher and
222 whether it persisted throughout the season. The three yeasts appeared in pitchers
223 sequentially (Figure 6): *C. pseudoglaebosa* first arrived in pitchers within four days
224 after the pitchers opened; *R. babjevae* arrived between four days and one week after
225 pitchers opened; and *M. aphidis* arrived one week to one month after pitchers opened.
226 Once a yeast colonized a pitcher, it either persisted in or disappeared from that pitcher
227 later in the season, but it did not disappear from the broader metacommunity.
228
229
230 Table 1: Taxon-specific PCR primer sequences used to detect pitcher yeasts

yeast	forward sequence	Tm	reverse sequence	Tm	product length
C. glaebosa	5'-CTGCGGAAGGATCATTACAGT-3'	54.6	5'-TGTTCAGACAACACTGTTCA-3'	51.8	466
R. glutinis	5'-AAGTCGTAACAAGGTTTCCG-3'	52.8	5'-CCCAACTCGGCTCTAGTAAA-3'	53.9	527
P. aphidis	5'-GGTAATGCGGTCGTCTAAAA-3'	52.6	5'-CTCTTCCAAAGAAGCGAGG-3'	53.1	467

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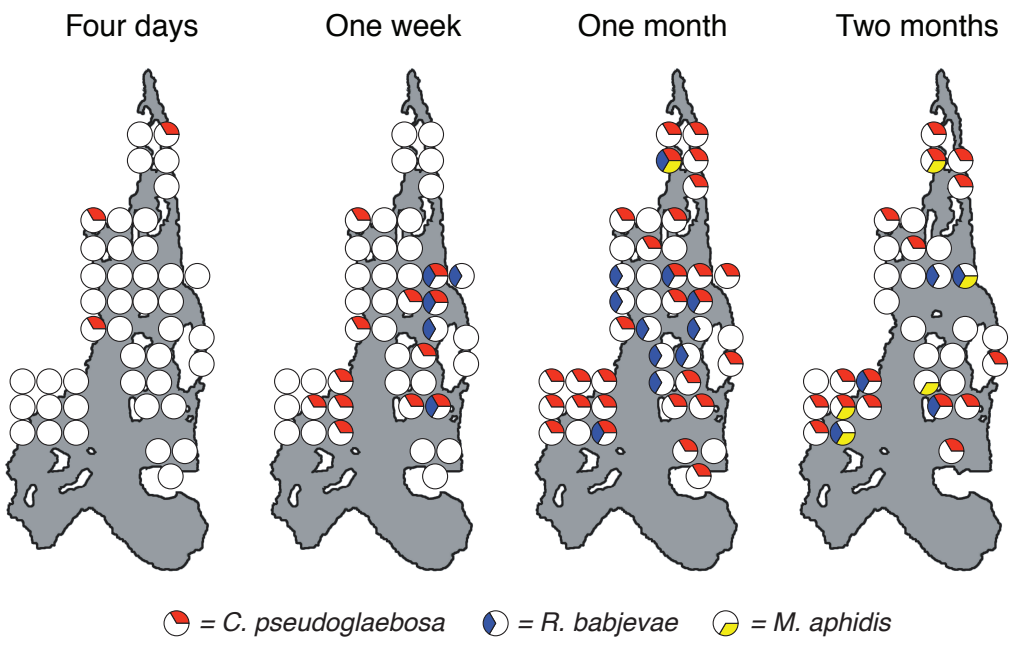

Figure 6: Presences and absences of each of three yeasts in 43 pitchers over time. Each large gray shape represents the Harvard Pond at one of four time points. Circles represent pitchers: completely white circles represent pitchers in which none of the three yeasts were detected, and circles containing colored pie slices represent pitchers in which one or more of the three assayed yeasts were detected. Pie slices are colored by detected yeast: red = *C. pseudoglaebosa*, yellow = *M. aphidis*, and blue = *R. babjevae*. Circles indicate the approximate locations of pitchers, and are offset to make all data visible; see Figure 1C for accurate pitcher locations.

Discussion

C. pseudoglaebosa *is a pitcher metacommunity dominant*

C. pseudoglaebosa was the dominant fungal taxon throughout succession in the *S. purpurea* pitcher metacommunity, although it did not dominate every individual pitcher community. It was the most frequent taxon in the metacommunity at every sampled timepoint, and its frequency in the metacommunity increased after initial colonization (Figure 4). Dominance in the pitcher 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

244 above 90% of all OTUs) to dominate the metacommunity as a whole (Figure 4). The
245 poor competitive performance of *C. pseudoglaebosa* relative to other yeasts in
246 microcosms suggests that overall competitive superiority was not the cause of its
247 dominance (Figure 5). Instead, *C. pseudoglaebosa*'s early dispersal is a more likely
248 cause (Figure 6).

249 Our best explanation for *C. pseudoglaebosa*'s dominance throughout
250 succession in the pitcher metacommunity is that it pre-empted other community
251 members by dispersing and establishing in pitchers before other taxa. Early dispersal
252 likely gave *C. pseudoglaebosa* a numerical advantage by providing the opportunity to
253 begin exponential growth before other members of the fungal community could arrive
254 and begin growing (Scheffer *et al.* 2017). Additionally, once *C. pseudoglaebosa* was
255 established in a pitcher, facilitation by low-frequency interacting taxa may have
256 helped it maintain dominance (Figure 5A). Once established early in the
257 metacommunity, *C. pseudoglaebosa* continued to disperse into new pitchers from one
258 week to one month into the approximately two month long growing season (Figure 6).

259 Although *C. pseudoglaebosa* was the numerically dominant fungal taxon in
260 the metacommunity of pitchers, chance events, dispersal, and interactions among
261 fungi determined whether it was the dominant taxon inside any given pitcher. We
262 expect that interactions with taxa that arrive, by chance, at different times in different
263 pitchers caused the variety of *C. pseudoglaebosa* relative frequency changes observed
264 (*i.e.,* increasing, decreasing, or nonmonotonic, Figure 4B) because interacting yeasts
265 have qualitatively different impacts on *C. pseudoglaebosa* performance depending on
266 the number of interacting cells present (Figure 5A). Individual pitchers experienced
267 priority effects because the timing of taxon arrival in each pitcher (*e.g.,* early arrival
268 of *C. pseudoglaebosa* in a pitcher) determined later community composition (*e.g., C.*

269 *pseudoglaebosa* dominance in the pitcher) (Fukami 2015). Despite the influence of
270 chance events on *C. pseudoglaebosa* dominance in any given pitcher, the early and
271 frequent dispersal of *C. pseudoglaebosa* compared to other yeasts enabled its overall
272 dominance in the metacommunity (Figure 4A, 6).
273
274 *Ecological patterns and processes during pitcher metacommunity succession*
275 In the metacommunity, and in many individual pitchers, *C. pseudoglaebosa*
276 remained dominant through decreases in fungal taxon richness and diversity (Figures
277 3, 4). We did not observe a hump-shaped relationship between pitcher age and species
278 richness, as previously predicted (Figure 3A) (Moquet *et al.* 2003). Species richness
279 instead decreased, even as *R. babjevae* and *M. aphidis* were first dispersing into
280 pitchers late in the season (Figures 3A, 6). It is likely that *C. pseudoglaebosa*
281 repression of taxa through priority effects has a larger influence on species richness
282 than increasing diversity through new dispersal does.
283 Previous studies have also documented biotic and abiotic successional changes
284 in pitchers; while we did not measure the same parameters as these previous studies,
285 we assume that similar changes occurred in our pitcher metacommunity and that *C.*
286 *pseudoglaebosa* maintained its dominance through these changes. For example,
287 previous studies documented decreasing pH with increasing pitcher age, an early peak
288 in prey insect capture during pitchers' life spans (Fish & Hall 1978), and a variety of
289 changes in bacterial, protist, and invertebrate community compositions over time
290 (Gray *et al.* 2012, Miller & terHorst 2012). In bacterial, protist, and invertebrate
291 communities, the identities of dominant taxa changed as succession progressed. In
292 contrast, *C. pseudoglaebosa* remained the dominant fungus throughout succession. *C.*
293 *pseudoglaebosa* appears to be a classical early-successional taxon (Connell & Slatyer

294 1977, Fierer *et al.* 2010) because it disperses early and frequently (Figure 6), but it is
295 not replaced by late-successional taxa.
296 *C. pseudoglaebosa* dominance throughout succession may be enabled by the
297 short lifespans of pitchers in Harvard Pond; *i.e.,* pitchers may not live long enough to
298 enable late-succession fungal taxa to dominate the metacommunity. We sampled
299 pitchers that were up to 66-74 days old, and stopped sampling at this age because 23%
300 of pitchers had been destroyed by moths. However, pitchers in northern *S. purpurea*
301 populations can survive intact through winter conditions (Judd 1959), and pitchers can
302 be active for over a year in the southern United States (Miller & terHorst 2012). We
303 speculate that fungal succession more closely resembles classical successional
304 patterns and the patterns observed for other pitcher guilds (*e.g.,* bacteria,
305 invertebrates) in longer-lived pitchers. For example, it is possible that a strong
306 competitor such as *M. aphidis* could replace *C. pseudoglaebosa* in southern *S.*
307 *purpurea* metacommunities where pitchers are active for many months. However,
308 consistent dominance of a single taxon over succession may be common in microbial
309 habitats that, like northern *S. purpurea* pitchers, have short lifespans but repeatedly
310 become available.
311
312 **Conclusions**
313 In the model pitcher plant metacommunity, taxon dispersal ability has a
314 profound influence on community structure. In particular, *C. pseudoglaebosa*'s ability
315 to disperse into pitchers before other fungal taxa enables it to persist as the dominant
316 taxon in the pitcher metacommunity, even as intertaxon interactions and the
317 stochasticity of individual dispersal events prevent its dominance in every pitcher. It
318 is likely that dispersal ability leads to persistent dominance in a variety of other

319 natural succeeding microbial communities and metacommunities, especially when
320 early dispersal allows a taxon to prevent establishment of other taxa.
321 Future studies of microbial succession should explicitly include
322 metacommunity structure when investigating ecological processes. In the pitcher
323 metacommunity, overall taxon composition changed little over time, with *C.*
324 *pseudoglaebosa* dominant throughout succession (Figure 4A). However, individual
325 pitchers followed a variety of trajectories (Figures 2, 4B, 6). Studies of succession that
326 do not take a metacommunity's structure into account may miss community
327 heterogeneity and the diversity of ecological processes, especially dispersal ability, in
328 play among communities.
329
330 **Materials and Methods**
331 *Study site and field collections*
332 Observations were made on *Sphagnum* islands in Harvard Pond, adjacent to
333 Tom Swamp, a 50 ha *Sphagnum* bog located in Petersham, Massachusetts at 42°30'N,
334 72°12'W (Figure 1; Swan & Gill 2007). The *C. pseudoglaebosa* and *M. aphidis*
335 isolates used in the microcosm study were collected from pitchers in Harvard Pond,
336 and the *R. babjevae* isolate was collected from a pitcher in Swift River Bog, a 2 ha
337 kettlehole bog located 75 km south of Tom Swamp in Belchertown, MA at 42°16'N,
338 72°20'W (Ellison *et al.* 2002). These three yeast isolates were collected in the
339 summer of 2006 and identified by comparing their ribosomal sequences, amplified
340 using the ITS1F/ITS4 and LS1/LR5 primer pairs (Gardes & Bruns 1993, White *et al.*
341 1990, Hausner *et al.* 1993 Vilgalys & Hester 1990), to sequences in the NCBI BLAST
342 database (Zhang *et al.* 2000). We chose *C. pseudoglaebosa*, *R. babjevae*, and *M.*
343 *aphidis* in part because they were all easily cultured from pitchers, and in part because

344 they formed colonies with different morphologies on agar plates: *C. pseudoglaebosa*
345 forms smooth white colonies; *M. aphidis* forms wavy white colonies; and *R. babjevae*
346 forms smooth pink colonies.

347 All *S. purpurea* pitcher water samples for PCR and 454 sequencing were
348 collected in the spring and summer of 2009. In May of 2009, we identified 43
349 unopened *S. purpurea* pitchers on 32 *Sphagnum* islands in Harvard Pond. Pitchers
350 ranged from less than 1 m to 908 m in distance to other pitchers (Figure 1C). We
351 visited each pitcher daily until it opened, and counted pitcher age from the date it
352 opened. For each pitcher water collection, the water inside a pitcher was first mixed
353 by pipetting up and down with a sterile plastic transfer pipette. We then removed
354 about 0.25 ml pitcher water and mixed it with 0.25 ml of CTAB buffer (100 mM Tris
355 pH 8.0, 1.4 M sodium chloride, 20 mM EDTA, 2% CTAB). To the best of our ability,
356 we avoided collecting insect prey or macrofauna in these samples, although any
357 protists and microscopic animals present in our samples were included; collected
358 pitcher water contained no large animal parts and appeared as a cloudy liquid. All
359 samples were flash-frozen in liquid nitrogen within five hours of collection and stored
360 at -20 or -80°C before DNA extraction.

361

362 *PCR assay*

363 We assayed each pitcher water sample for amplifiable DNA from all fungi,
364 using the ITS1F/ITS4 primer pair, and for each of the three yeasts in the microcosm
365 experiment, using the primers in Table 1. Primers to selectively amplify portions of
366 each microcosm yeast's ITS sequence were designed using the NCBI BLAST primer
367 tool (Rozen & Skaletzky 2000). We chose primer sequences to reliably amplify as

368 much of the ITS sequence of each yeast species as possible, while not amplifying
369 other sequences in the BLAST database.
370 To extract DNA from each pitcher water sample before the PCR assay, we
371 first thawed and centrifuged frozen samples at 16.1 g for 10 min and removed the
372 supernatant from each pellet. We then suspended each pellet in 200 μL of breaking
373 buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM sodium chloride, 10
374 mM Tris, and 1 mM EDTA) (Hoffman 1997). We mixed each suspension with about
375 200 μL of 0.5 mm glass beads and 200 μL 25:24:1 chloroform:phenol:isoamyl
376 alcohol. We vortexed each mixture for 2 min, and then centrifuged it for 5 min at 16.1
377 g. After centrifugation, we removed the aqueous layer and mixed it with 2.5 volumes
378 of 95% ethanol and 0.1 volume of 3M sodium acetate (Sambrook & Russell 2001);
379 we incubated each aqueous layer mixture at -20°C for at least three hours. Next, we
380 centrifuged each aqueous layer mixture for 15 min at 16.1 g, and removed the
381 supernatant. Finally, we washed each pellet with 0.5 ml 70% ethanol, centrifuged
382 each mixture for 10 min at 16.1 g, removed the supernatant, and resuspended each
383 pellet in 50 μl water.
384 We then assayed each DNA extract for the presence of each Fungal taxon, or
385 any Fungal DNA in the case of the ITS1F/ITS4 primer pair, using PCR. Each PCR
386 reaction was composed of 7.9 μL water, 0.1 μL GoTaq® Flexi polymerase (Promega),
387 5 μL Flexi buffer with green dye added, 5 μL 5x CES (combinatorial PCR enhancer
388 solution: 2.7 M betaine, 6.7 mM dithiothreitol, 6.7% dimethyl sulfoxide, 55 μg/mL
389 bovine serum albumin) (Ralser *et al.* 2006), 5 μL nucleotide mix, 2 μL magnesium
390 chloride, 1 μL of 10 μM of each primer, and 1 μL undiluted template DNA extract.
391 All reactions were cycled on a Biorad iCycler or myCycler using denaturing,
392 annealing, and extension temperatures of 95, 55, and 72 °C, respectively. We

393 denatured for 85 s, then ran 13 cycles of 35 s denaturing, 55 s annealing, and 45 s
394 extension, followed by 13 cycles that were identical but had a 2 min extension, and
395 finally 9 cycles with a 3 min extension. We ran a subsequent 10 min extension. Two
396 μL of each PCR product were visualized on 1% agarose gels stained with SYBR Safe
397 dye (Invitrogen) and photographed using a U:genius gel documenting system
398 (Syngene) and a Stratagene transilluminator. Photographs of gels were scored for
399 presence or absence of a band. Bands that were too faint to reliably score were run a
400 second time with 6 μL of PCR product per well. Presence of a band on a gel indicated
401 the presence of detectable fungal or yeast species DNA in a water sample.
402 To confirm that primers only amplified sequences from the target yeasts, we
403 randomly selected nine PCR products generated from the *C. pseudoglaebosa* and *R.*
404 *babjevae* primer pairs for sequencing. The primer pair that targets *M. aphidis* only
405 amplified DNA from six pitcher water extracts, and we sequenced all six PCR
406 products for this primer pair. Sequences were identical to or within one base of
407 cultured isolate sequences.
408
409 *Pitcher water fungal DNA amplification and 454 sequencing*
410 We extracted and amplified fungal DNA for fungal community amplicon
411 sequencing using the protocols described above, with the following changes. Gotaq®
412 Hotstart polymerase (Promega) was used instead of Flexi polymerase, and we used 50
413 μM instead of 10 μM of the reverse primer. The forward primer consisted of (in order
414 from 5' to 3') the 454 "A" primer (CCATCTCATCCCTGCGTGTCTCCGACTCAG)
415 concatenated with a 10-bp multiplex tag (454 Life Sciences Corporation 2009), and
416 ITS4; the reverse primer consisted of the 454 "B" primer
417 (CCTATCCCCTGTGTGCCTTGGCAGTCTCAG) concatenated with ITS1F.

418 Multiplex tags were unique to each sample. Reactions were cycled at 95 °C for 15
419 min; 30 cycles of 95 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min; and a final
420 extension of 72 °C for 8 min.
421 Products were purified using Agencourt® *AMPure*® XP (Beckman Coulter)
422 and quantitated using a Qubit® dsDNA HS Assay (Invitrogen) according to the
423 manufacturers' instructions. We combined equimolar concentrations of the products
424 of each of three separate PCR reactions from each DNA extract. The sequencing pool
425 consisted of pooled equimolar concentrations of each pooled PCR product. The pool
426 was sequenced on one-eighth of a 454 Titanium sequencing run by the Duke Genome
427 Sequencing & Analysis Core Resource.
428
429 *454 sequence processing*
430 We processed sequences using QIIME 1.3.0 (Caporaso *et al.* 2010). Low
431 quality sequences were removed and the remaining sequences were assigned to
432 mulitplex barcodes using the default quality filtering settings. Primers and barcodes
433 were trimmed from each sequence and sequences shorter than 200 and longer than
434 1000 bp were removed from each dataset. Sequences were denoised using the QIIME
435 denoiser. We reduced chimeric sequences by trimming the 5.8s and ITS1 portions
436 from all sequences using the Fungal ITS Extractor (Nilsson *et al.* 2010a), and only
437 analyzing the ITS2 portion. The 5.8s ribosomal region lies between the ITS1 and
438 ITS2 spacers, and is conserved among fungi relative to the spacers. We expected most
439 chimeric sequences to form in the 5.8s region and to be composed of ITS1 and ITS2
440 sequences from different templates (Nilsson *et al.* 2010b). We chose operational
441 taxonomic units (OTUs) using the uclust method in QIIME, at 97% similarity (Edgar
442 2010). We discarded all OTUs composed of a single sequence (singleton OTUs)

443 because we assumed that they resulted from sequencing errors. The longest sequence
444 in each remaining cluster was retained as a representative sequence. Of the 141,424
445 total sequences produced, 27,632 were discarded for having lengths less than 200 or
446 more than 1000 bp and 10,938 were discarded because they had low quality or did not
447 have a matching barcode. Sixty-six sequences were discarded because the ITS2
448 subunit could not be extracted and 139 sequences were discarded because they
449 represented singleton OTUs. In total, we retained 102,649 sequences for further
450 analysis. Each pitcher water sample produced between 253 and 4365 sequences. We
451 will deposit the 454 sff files and corresponding FASTA files in the NCBI Small Read
452 Archive database (accession numbers to be added after manuscript acceptance).
453
454 *Sequence taxonomy assignments*
455 To assign genus-level taxonomy to OTUs, we performed a MEGAN analysis
456 of OTU representative sequences on the top ten hits from the NCBI BLAST
457 nucleotide database extracted using BLAST 2.2.25+ and default MEGAN settings
458 (Huson *et al*. 2011, Zhang *et al.* 2000). We discarded OTUs matching organisms not
459 in the kingdom Fungi (plant, animal, and protist sequences), but we assumed that
460 OTUs with no BLAST matches or matching unassigned fungal environmental
461 sequences were fungal sequences not yet identified in the NCBI database. We
462 retained unassigned OTUs for diversity measurements, but did not include them in
463 taxonomy summaries. We reviewed the taxon assignments output by MEGAN
464 manually, and filled in higher-level classifications (*e.g.,* order or class) using Index
465 Fungorum (http://www.indexfungorum.org/) when an OTU was assigned to a genus
466 but not higher-level classifications. We attempted to assign all taxa to genera; if it was
467 not possible to assign a taxon to a genus, we assigned it to the most specific

468 taxonomic group possible. OTUs assigned to the genus *Candida* were further assigned
469 to the species *C. pseudoglaebosa* if they matched either *C. pseudoglaebosa* or its
470 close relative *C. glaebosa* in a BLAST search. When we compared these seven OTUs
471 against sequences representing the type strains of the two *Candida* species (accession
472 numbers KY102342.1 and KY102112.1, respectively), all seven OTUs aligned to *C.*
473 *pseudoglaebosa* better than they did *C. glaebosa*. In total, we detected 553 OTUs, of
474 which 379 were assigned to fungal taxa; fifteen were discarded because they matched
475 non-fungal sequences; and 159 were not assigned. Of the 379 fungal taxa in the
476 temporal data set, 52% were Basidiomycota, 43% Ascomycota, and 5% basal fungal
477 lineages. Sequences, metadata, and OTU tables including taxonomic assignments in
478 QIIME 1.3.0 format are included in the supplementary datasets 2-3.
479
480 *Microcosm interaction assays*
481 Interactions between yeasts were assayed in microcosms designed to mimic
482 pitchers simultaneously colonized by different numbers of two yeast species. We
483 grew microcosms in low-nutrient media designed to mimic natural conditions in
484 pitchers. Microcosms contained sterile yeast extract media (YEM) composed of 1g/L
485 yeast extract in local tap water (Cambridge, MA, USA). Tap water was used instead
486 of deionized water because we wanted the media to include micronutrients present in
487 local rainwater that may be important for pitcher plant yeast growth. The tap water
488 supply in Cambridge, MA, where this experiment was conducted, comes from three
489 Massachusetts reservoirs (Waldron & Bent 2001), and we expected it to have similar
490 inputs as rainwater in Harvard Pond pitchers. Each microcosm was inoculated with a
491 target yeast species and an interactor in 200 μL of liquid yeast media. Each target
492 yeast was inoculated with about 1000 cells per microcosm, and each interactor yeast

493 was inoculated at zero, low, medium, and high cell numbers (0 and approximately
494 100, 1000, and 10,000 cells).
495 Eighteen treatments of yeast mixtures were prepared, with ten replicates each,
496 for a total of 180 microcosms. Before inoculation, yeasts were grown in liquid YEM
497 for 48 hours. Inoculation sizes were measured after inoculation using counts of
498 colony-forming units (CFUs) on solid YEM (YEM plus 1.5% agar). Microcosms
499 were arranged in sterile 96-well polystyrene flat bottom cell culture plates and
500 incubated between 25 and 27 °C, shaking at 700 rpm for 48 hours. After incubation
501 32 microcosms were discarded because of suspected contamination. We diluted each
502 remaining microcosm $1:10^3$ or $1:10^4$ in sterile water, plated it to solid YEM, and
503 counted CFUs on plates containing at least 30 total CFUs. When no CFUs of an
504 inoculated yeast were present on a plate, we conservatively assumed that the yeast
505 was present in the microcosm in numbers just below our detection limit. We
506 calculated total cell numbers assuming one instead of zero CFUs for these yeasts
507 absent from plates. CFU counts before and after incubation are included in
508 supplementary dataset 4.
509
510 *Statistical analyses*
511 OTU datasets rarefied to 1140 sequences were used to produce Non-metric
512 Multidimensional Scaling (NMDS) plots and to compare community similarities, and
513 alpha diversity indices among pitchers. Eight samples contained fewer than 1140
514 sequences and were discarded. Proportions of samples assigned to taxonomic groups
515 were calculated based on the full non-rarefied dataset. Community similarities over
516 time were compared using partial distance-based redundancy analysis (db-RDA) of
517 Jaccard dissimilarity (Jaccard 1912) between each pair of samples with pitcher age as

518 the explanatory variable, conditioned on pitcher identity. A correlation between
519 geographic distance and community similarity was conducted using a partial Mantel
520 test conditioned on pitcher age. Hill numbers of order q=0 or 2 (^{q}D) were calculated
521 as $^{q}\mathrm{D} = \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)}$, where S is the total number of OTUs and p_i is the relative
522 abundance of OTU i; ^{1}D was calculated as the exponent of Shannon diversity (Hill
523 1973, Chao *et al.* 2014). Changes in Hill numbers were modeled over time using
524 repeated-measures linear models controlled for pitcher identity; ^{1}D and ^{2}D were log-
525 transformed before analyses to homogenize variances among timepoints, and ^{0}D was
526 not transformed.

527 We modeled the impact of interactor yeasts on focal yeasts in microcosms
528 using multiple linear and polynomial regressions. Separate regressions were
529 conducted for each focal yeast. For each regression, focal yeast yield was the
530 dependent variable, and both the number of co-inoculated interactor yeast cells and
531 the identity of the interactor yeast were independent variables. We modeled both
532 linear and quadratic relationships between the number of co-inoculated interactor
533 yeast cells and the dependent variable because the relationship did not always appear
534 linear when plotted. Before constructing the regressions, we square-root-transformed
535 focal yeast yield to homogenize variances for the focal yeasts *R. babjevae* and *C.*
536 *pseudoglaebosa*, but left yield untransformed for the focal yeast *M. aphidis*. We also
537 transformed competitor inoculum size by $\log_{10}(x+1)$ because interactor inoculum size
538 was varied on a log scale in the experiment. When comparing the influences of
539 competitor species, we randomly assigned treatments with no interacting yeast
540 inoculum to one of the two interacting yeasts. When selecting the best-fitting
541 regression model, we first established the best-fitting relationship (linear, quadratic, or
542 both) between log-transformed interactor inoculum size and focal yeast yield, and

543 then determined whether adding interactor identity or interactions between interacting
544 yeast identity and inoculum size to the model improved it. The best-fitting model was
545 the one with the lowest Akaike Information Criterion (AIC).
546 All statistical analyses and index calculations were conducted using R version
547 3.3.1 (R Development Core Team, 2016) and the packages vegan, fields, nlme, and
548 GUniFrac (Chen 2018, Pinheiro *et al.* 2016, Oksanen *et al.* 2016, Nychka *et al.* 2016)
549 plots were made using ggplot2 (Wickham 2016)
550

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561

562 **Figure Legends**

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

568 close enough to one another that the white circles overlap. Maps were created using
569 ArcMap™ Version 9.2 (ESRI 2006); map data are from the Office of Geographic
570 Information, Commonwealth of Massachusetts Information Technology Division
571 (2000) and the National Atlas of the United States (2006).
572
573 Figure 2: Non-metric Multidimensional Scaling (NMDS) plots of pitcher plant
574 community similarities. Similarities of community OTU compositions were
575 calculated using the Jaccard metric (Jaccard 1912). Purple triangles represent four-
576 day-old communities, orange circles represent week-old communities, red squares
577 represent approximately one-month old communities, and blue diamonds represent
578 approximately two-month-old communities. (A) NMDS plot with similarities among
579 time points highlighted. Ellipses depict 95% confidence intervals of the centroid of
580 each time point. No ellipse is depicted for four-day-old communities because only
581 two were measured. (B) NMDS plot with individual pitchers highlighted. Lines
582 connect measurements for each pitcher. Fungal communities in pitcher group 1 are
583 represented with black lines and fungal communities in pitcher group 2 are
584 represented with gray lines. All points are located at the same coordinates in (A) and
585 (B).
586
587 Figure 3: Hill numbers of orders A) 0, B) 1, and C) 2 in pitchers over time. Data
588 points for communities in the same pitcher are connected with lines. Black lines
589 connect points for pitcher group 1 and gray lines connect points for pitcher group 2.
590
591 Figure 4: Taxon diversity in pitchers over time. Proportions are reported based on
592 non-rarefied OTU assignments. (A) Taxon diversity in the entire bog metacommunity.

593 Colored bars represent proportions of total sequences for each fungal class (or phylum
594 for basal fungal lineages). The hatched area represents total *C. pseudoglaebosa*
595 frequency for each time point. Note that *C. pseudoglaebosa* is in the class
596 Saccharomycetes and represents over 99% of Saccharomycetes sequences at the one
597 and two month time points. (B) *C. pseudoglaebosa* sequence frequency in individual
598 pitcher communities. Data points for communities in the same pitcher are connected
599 with lines. Black lines connect points for pitcher group 1 and gray lines connect
600 points for pitcher group 2. Data for this figure is non-rarefied OTU data; for rarefied
601 data, see Figure 4 Figure Supplement 1.
602
603 Figure 4 Figure Supplement 1: Taxon diversity in pitchers over time; rarefied
604 sequence data. The OTU table was rarefied to 1140 sequences per sample. (A) Taxon
605 diversity in the entire bog metacommunity. Colored bars represent proportions of total
606 sequences for each fungal class (or phylum for basal fungal lineages). The hatched
607 area represents total *C. pseudoglaebosa* frequency for each time point. Note that *C.*
608 *pseudoglaebosa* is in the class Saccharomycetes and represents over 99% of
609 Saccharomycetes sequences at the one and two month time points. (B) *C.*
610 *pseudoglaebosa* sequence frequency in individual pitcher communities. Data points
611 for communities in the same pitcher are connected with lines. Black lines connect
612 points for pitcher group 1 and gray lines connect points for pitcher group 2. Note that
613 data for some pitchers are missing at some time points because these samples were
614 discarded during sample rarefaction.
615
616 Figure 5: Influence of interacting species on (A) *C. pseudoglaebosa*, (B) *M. aphidis*,
617 and (C) *R. babjevae*. The plots depict the yield of each focal species as a function of

618 the number of cells of an interacting species co-inoculated with the focal species.

619 Interacting species are coded by color: red = *C. pseudoglaebosa*, yellow = *M. aphidis*,

620 and blue = *R. babjevae*.

621

622 Figure 6: Presences and absences of each of three yeasts in 43 pitchers over time.

623 Each large gray shape represents the Harvard Pond at one of four time points. Circles

624 represent pitchers: completely white circles represent pitchers in which none of the

625 three yeasts were detected, and circles containing colored pie slices represent pitchers

626 in which one or more of the three assayed yeasts were detected. Pie slices are colored

627 by detected yeast: red = *C. pseudoglaebosa*, yellow = *M. aphidis*, and blue = *R.*

628 *babjevae*. Circles indicate the approximate locations of pitchers, and are offset to

629 make all data visible; see Figure 1C for accurate pitcher locations.

630

631

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