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

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Abstract

Dominant taxa often emerge during microbial succession. It is not always clear what makes a taxon dominant, and dispersal ability may be an overlooked mechanism promoting dominance late in succession. We investigated mechanisms leading to dominance of the yeast *Candida pseudoglaebosa* in the model carnivorous pitcher plant (*Sarracenia purpurea*) microbial metacommunity. *C. pseudoglaebosa* was the dominant taxon in the metacommunity, but not every individual pitcher community, throughout succession. Its frequency in the metacommunity increased between early and late-successional stages, and it was not replaced by other taxa. Despite its dominance, *C. pseudoglaebosa* was not a superior competitor. Instead, it was a superior disperser: it arrived in pitchers earlier, and dispersed into more pitchers, than other taxa. We attribute the continuous dominance of *C. pseudoglaebosa* in *S. purpurea* pitchers to its dispersal ability and the spatial structure of the

metacommunity. Dispersal ability influences dominance throughout microbial 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 changes in microbial communities in fine detail (Copeland *et al.* 2015, Boynton & Greig 2016, Koenig *et al.* 2011, Cutler *et al*. 2014). These observational studies have described a variety of successional patterns. For example, taxon diversity can increase, decrease, or randomly vary with successional time (Cutler *et al.* 2014, Zumsteg *et al.* 2012, Redford & Fierer 2009). Researchers also often observe replacement of early-successional taxa by late-successional taxa with time (*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.

One frequently observed pattern in microbial systems is the development of ecological dominance over successional time (Boynton & Greig, 2016, Findley *et al.* 2013, Jiao *et al*. 2016). Ecological dominance is a pattern where 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 taxon, and ecosystem engineering can all lead to dominance (Wolfe *et al.* 2014, Albergaria *et al.* 2010, Goddard 2008, Nissen & Arneborg 2003, Williams *et al.* 2015). However, dominance in microbial succession has been primarily studied in domesticated systems, and the most frequently reported mechanisms may not be responsible for

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

56 Dispersal may contribute to microbial dominance over succession. When
57 primary succession happens on a sterile substrate, all members of the microbial
58 community must first disperse onto the new substrate before establishing in the
59 community. A good disperser may prevent the establishment of other community
60 members through priority effects if it arrives in a habitat first, either by pre-empting
61 or modifying available niches (Fukami 2015). Additionally, similar nearby
62 communities can impact a microbial community during succession by producing
63 propagules that then disperse into the developing community.

64 Naturally succeeding microbial communities are often physically structured in
65 groups, or metacommunities, where individual communities are spatially isolated
66 from one another but linked through dispersal (Logue *et al.* 2011, Holyoak *et al.*
67 2005). Community assembly in metacommunities is a function of ecological
68 processes occurring inside each community and dispersal among communities.
69 Ecological theory explains how dispersal can interact with intracommunity processes
70 to maintain metacommunity diversity (Leibold *et al.* 2004, Holyoak *et al.* 2005). For
71 example, populations occupying low-quality environmental patches can be
72 maintained by dispersal from high-quality patches ("mass effects"), or fitness trade-
73 offs between competitive ability and dispersal ability can maintain species diversity
74 ("patch dynamics"). Theory predicts that dispersal and competition interact during
75 succession in the individual component communities of a metacommunity to result in

76 a hump-shaped relationship between successional time and species richness (Moquet
77 *et al.* 2003, Sferra *et al.* 2017). Species richness is predicted to be low early in a
78 component community's age, to increase as more species disperse into the
79 community, and then to decrease as competitive interactions remove species from the
80 community. It is also possible that a particularly highly dispersing species becomes
81 dominant (and therefore decreases community diversity) in a metacommunity solely
82 as a result of its dispersal ability. Dispersal contrasts with other mechanisms leading
83 to dominance because it may allow an apparently inferior competitor to become
84 dominant through high dispersal ability.

85 We investigated the contribution of dispersal ability to ecological dominance
86 over the course of a natural microbial succession in the carnivorous pitcher plant
87 *Sarracenia purpurea*. *S. purpurea* is a perennial plant native to bogs and savannas in
88 northern and eastern North America (Buckley *et al.* 2003). It produces modified vase-
89 shaped leaves, or pitchers (Figure 1a), annually. Developing pitchers are first entirely
90 closed, sterile chambers (Peterson *et al.* 2008). Once mature, the top portion of each
91 pitcher opens, and open pitchers accumulate rainwater to form small pools of water
92 (phytotelmata). Potential prey (ants and other small insects) are attracted to pitchers
93 (Bennett & Ellison 2009); some prey fall into pitchers and drown, and are then
94 shredded, decomposed, and mineralized by a food web of microorganisms and
95 invertebrates (reviewed in Ellison *et al.* 2003). The pitcher microbial community
96 includes bacteria, algae, and fungi, including culturable yeasts (Cochran-Stafira &
97 von Ende 1998).

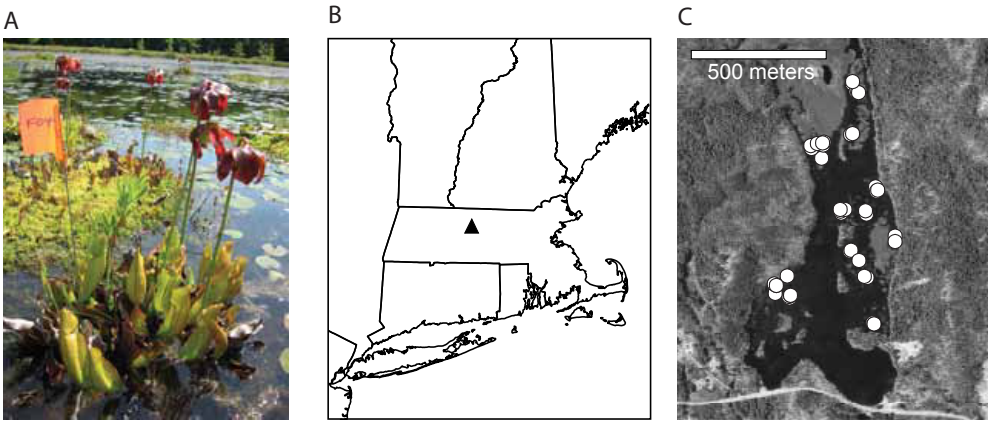

Figure 1: Example *S. purpurea* plant and study location. (A) One of the study pitcher plants at the edge of a *Sphagnum* island. This photograph was taken early in the growth season and both opened and unopened pitchers are visible. (B) The location of Harvard Pond in Massachusetts, USA. (C) Locations 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 pitcher fungal succession in a *Sphagnum* bog in central Massachusetts where the pitcher growing season lasts for about three months (although pitchers persist longer and can overwinter, Judd 1959). A single yeast taxon, *Candida pseudoglaebosa*, was numerically dominant in the metacommunity throughout pitcher growth, and its frequency increased between early and later-successional stages. We used further observations and experiments to investigate the ecological processes leading to *C. pseudoglaebosa* dominance. Unlike dominant yeasts in many other systems, *C. pseudoglaebosa* is not an especially good competitor against other tested yeasts. However, it is an especially good disperser. It is one of the first fungi to arrive in pitchers and maintains high frequency in the pitcher plant metacommunity over the course of the season, even as its frequency varies among pitchers from completely absent to over 90% of sequences. The 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.

113

114 **Results**

115 *Fungal communities in pitchers change over successional time*

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

134 Fungal succession varied among pitchers. While community composition
135 changed significantly with time (Figure 2A), only a small amount of variation in
136 community composition was due to variation in time (distance-based redundancy
137 analysis adjusted $R^2 = 0.03$, $F = 1.96$, $df = 1,27$, $p = 0.011$). This small influence of

138 time on community composition was likely a result of high variation among pitchers.
139 Succession followed two trajectories—five pitchers (hereafter referred to as "pitcher
140 group 1") followed a different successional trajectory from the other twelve ("pitcher
141 group 2")—and there was considerable variation among communities within each
142 trajectory (Figure 2B). Distance among pitchers did not explain significant variation
143 in community composition (partial mantel test of community composition on space
144 controlling for time, mantel statistic r = -0.04, significance = 0.715).

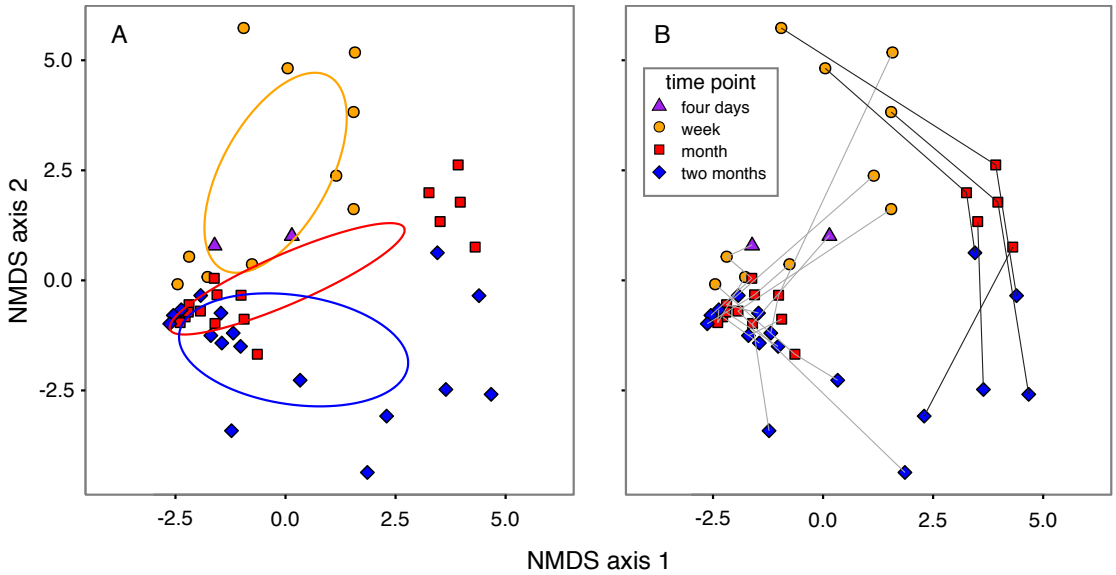

Figure 2: Non-metric Multidimensional Scaling (NMDS) plots of pitcher plant community similarities. Similarities of community OTU compositions were calculated using the Jaccard metric (Jaccard, 1902). (A) NMDS plot with similarities among time points highlighted. Ellipses depict 95% confidence intervals 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 pitcher group 2 are represented with gray lines. All points are located at the same coordinates in (A) and (B).

145 Despite the observed variation in fungal community composition, diversity
146 decreased, on average, in pitchers between four days and two months (Figure 3). To
147 determine a sample's diversity, we calculated Hill numbers of orders 0 to 2 (^{0}D to ^{2}D)
148 for each sample after rarefaction to 1040 sequences per sample. Hill numbers of
149 different orders give community diversity with an emphasis on rare species (low
150 orders) or common species (high orders) (Hill 1973, Chao *et al.* 2014). ^{0}D, ^{1}D, and ^{2}D

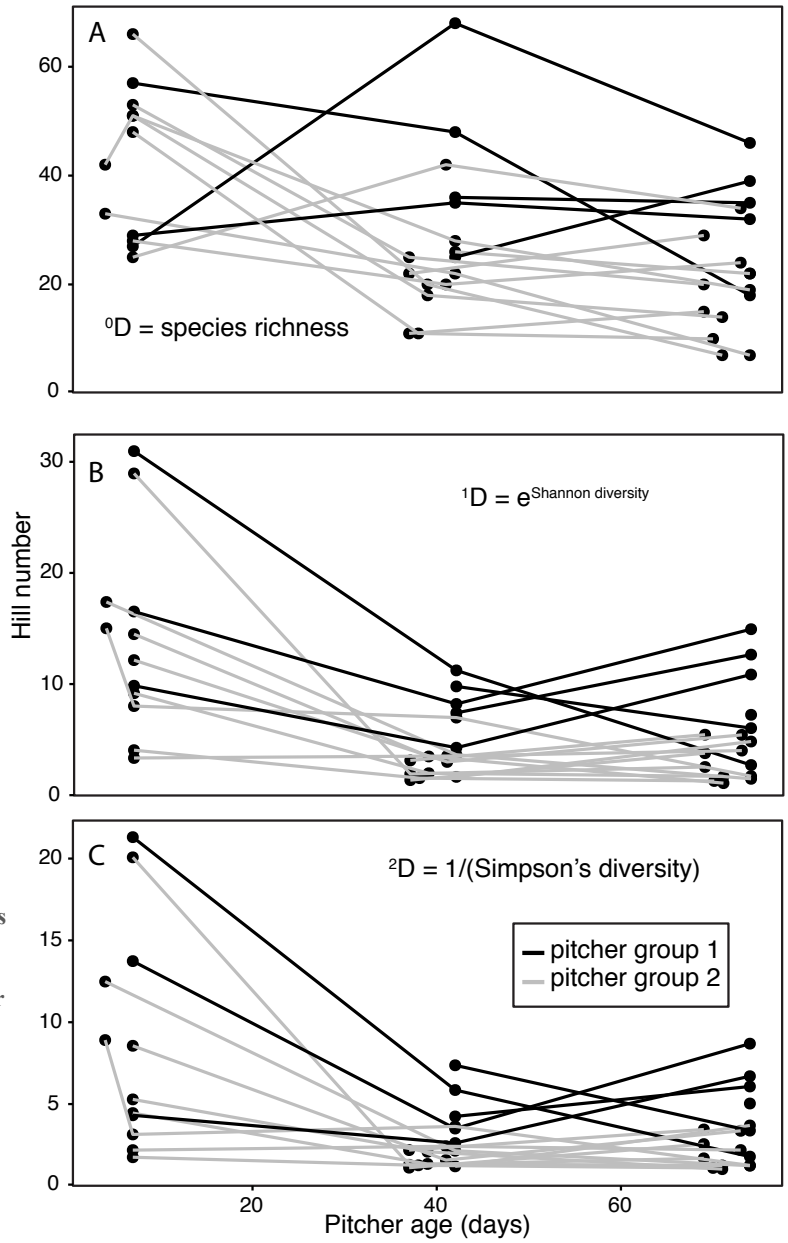

Figure 3: Hill numbers of orders A) 0, B) 1, and C) 2 in pitchers over time. Data points for communities in the same pitcher are connected with lines. Black lines connect points for pitcher group 1 and gray lines connect points for pitcher group 2.

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, ^{0}D declined significantly from 42.5 within the first week (including four day and one week timepoints) to 23.1 after two months (t=-3.6, df=27, p=0.001); ^{1}D declined from 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, p=0.003).

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

160 *C. pseudoglaebosa*, in the class Saccharomycetes, was the numerically
161 dominant taxon in the metacommunity, but was not dominant in every pitcher (Figure
162 4). In the metacommunity, *C. pseudoglaebosa* was more frequent than any other
163 taxon at every time point and its frequency increased between early- and late-
164 successional timepoints. Its metacommunity frequency increased from 22% of total
165 sequences at four days to 58% at two months (Figure 4A). However, its frequency did
166 not increase over time in every pitcher. Instead, its within-pitcher frequency increased
167 over the season, decreased over the season, peaked midway through the season, or
168 dipped midway through the season, depending on the pitcher (Figure 4B). We cannot
169 say whether these increases or decreases in *C. pseudoglaebosa* sequence frequency
170 reflect changes in the total cell numbers because we did not measure cell numbers or
171 fungal biomass in pitchers. Pitcher group 1 never contained appreciable *C.*
172 *pseudoglaebosa*: each pitcher in group 1 contained less than 1.7% *C. pseudoglaebosa*
173 sequences regardless of the sampled time point (Figure 4B).

174

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

177 To better understand how interactions with other yeasts might influence the
178 frequency of *C. pseudoglaebosa*, we grew *C. pseudoglaebosa* and other potentially
179 interacting pitcher yeasts in laboratory microcosms. We followed the strategy
180 advocated by Goldberg and Werner (1983), who suggested determining interacting
181 species' effects on one another by measuring organism performance as the number of
182 interacting individuals increases. We inoculated microcosms with all possible pairs of
183 three culturable pitcher yeasts (*C. pseudoglaebosa*, *Rhodotorula babjevae*, and
184

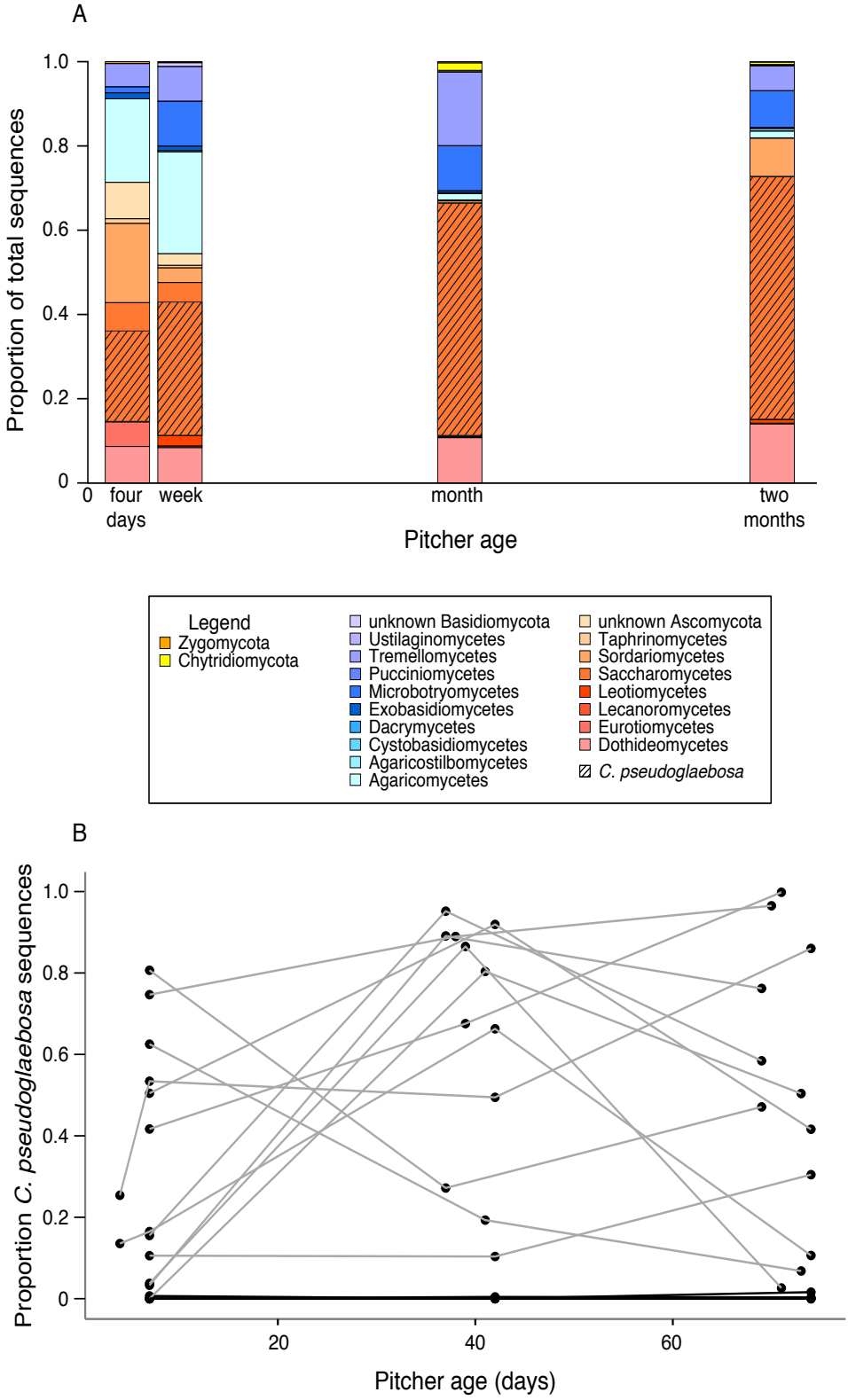

Figure 4: Taxon diversity in pitchers over time. Proportions are reported based on non-rarefied OTU assignments. (A) Taxon diversity in the entire bog metacommunity. Colored bars represent proportions of total sequences for each fungal class (or phylum for basal fungal lineages). The hatched area represents 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.

186 *Moesziomyces aphidis*). *C. pseudoglaebosa* represented 41%, *R. babjevae* represented
187 2%, and *M. aphidis* represented 0.06% of total sequences in the sequencing dataset.
188 Each low nutrient microcosm contained a focal yeast, which was inoculated as a fixed
189 number of cells, and an interactor, which was inoculated as a varying number of cells.
190 We then let the pairs of yeasts grow in the microcosms and investigated the effects of
191 interactors on each focal yeast using regressions. We evaluated interaction qualities
192 based on the direction (increasing or decreasing focal yeast yield with more
193 interacting cells) and shape of each regression (linear or polynomial), and we
194 evaluated differences between interactor yeasts based on whether adding interactor
195 yeast identity to each regression improved its fit.

196 Interactions between pitcher plant yeasts ranged from facilitation to
197 competition, depending on the identities of the yeasts and the number of interactor
198 cells present. Under microcosm conditions, interactions between focal yeasts and
199 interactors were polynomial when the focal yeast was *C. pseudoglaebosa* or *M.*
200 *aphidis* (Figures 5A, B, Supplemental tables 1-4): both yeasts were facilitated by

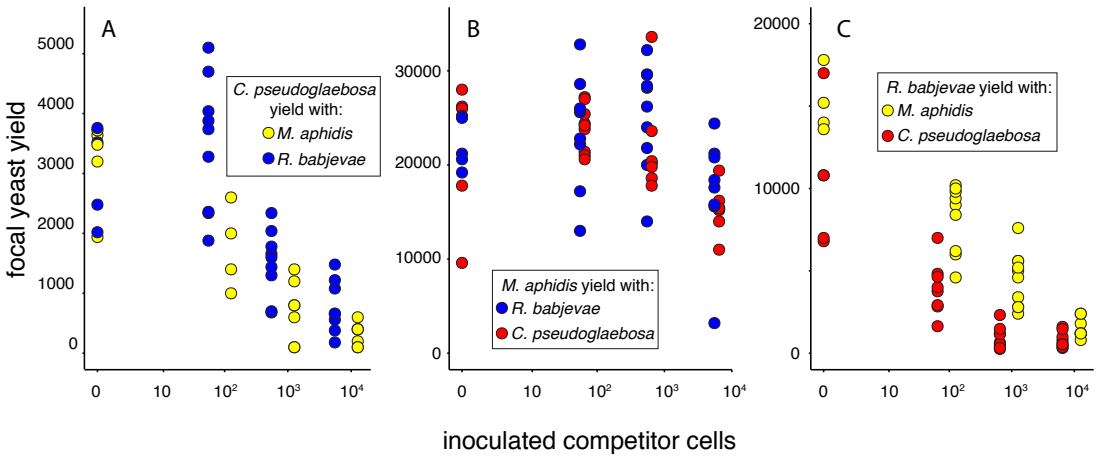

Figure 5: Influence of interacting species on (A) *C. pseudoglaebosa*, (B) *M. aphidis*, and (C) *R. babjevae*. The plots depict the yield of each focal species as a function of the number of cells of an interacting species co-inoculated with the focal species. Interacting species are coded by color: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

201 small numbers of co-inoculated cells, but their growth was impeded by larger
202 numbers of co-inoculated cells. Note that we detected facilitation of *C.*
203 *pseudoglaebosa* by *R. babjevae* when few *R. babjevae* cells were inoculated, but we
204 did not inoculate *M. aphidis* in small enough numbers to confirm *M. aphidis*
205 facilitation of *C. pseudoglaebosa* (Figure 5A). At high numbers of co-inoculated cells,
206 *M. aphidis* had a more detrimental impact on *C. pseudoglaebosa* than *R. babjevae*
207 ($F=6.79$, $df=1,50$, $p = 0.012$, Supplemental table 2). In contrast, the two interactors of
208 *M. aphidis* had similar effects on its yield: at low and intermediate inoculum sizes,
209 both *R. babjevae* and *C. pseudoglaebosa* promoted *M. aphidis* growth, but at high
210 inoculum sizes, both interactors inhibited *M. aphidis* growth (Figure 5B). Interactions
211 between *R. babjevae* and interactor yeasts were linear (Figure 5C, Supplemental
212 tables 5-6): *R. babjevae* yield was impeded by interactors regardless of the number of
213 interactor cells present, and *C. pseudoglaebosa* had a more detrimental impact on *R.*
214 *babjevae* than *M. aphidis* did ($F=86.07$, $df=1,58$, $p < 0.001$, Supplemental table 6).
215

216 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 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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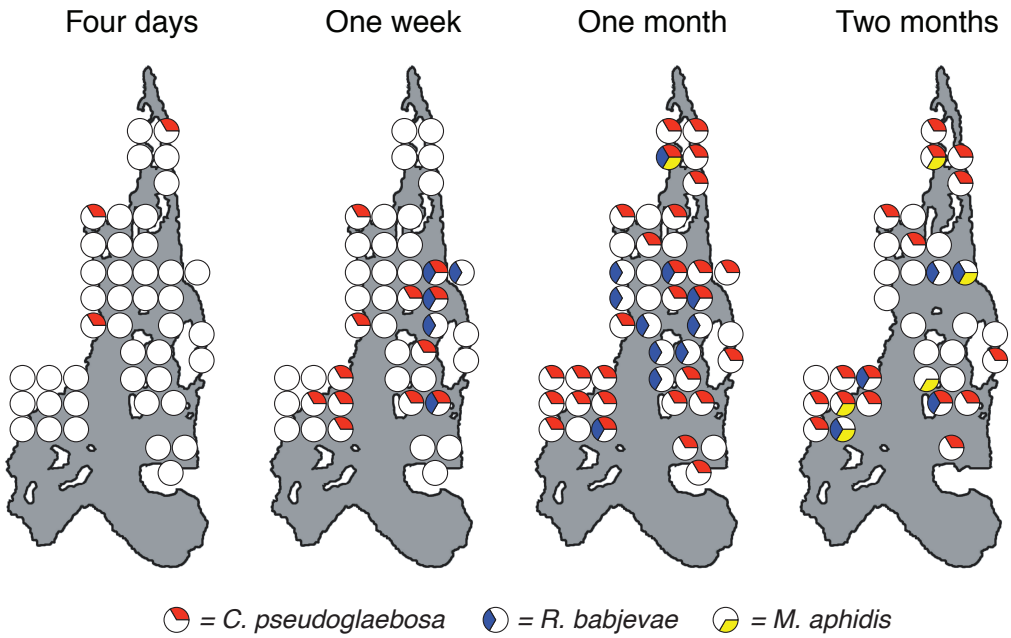

Figure 6: Presences and absences of each of three yeasts in 43 pitchers over time. Each large gray shape represents the Harvard Pond at one of four time points. Circles represent pitchers: completely white circles represent pitchers in which none of the three yeasts were detected, and circles containing 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.

231 **Discussion**

232 C. pseudoglaebosa *is a pitcher metacommunity dominant*

233 *C. pseudoglaebosa* was the dominant fungal taxon throughout succession in
234 the *S. purpurea* pitcher metacommunity, although it did not dominate every individual
235 pitcher community. It was the most frequent taxon in the metacommunity at every
236 sampled timepoint, and its frequency in the metacommunity increased after initial
237 colonization (Figure 4). Dominance in the pitcher metacommunity was in part a result
238 of the metacommunity structure itself: while *C. pseudoglaebosa* was not dominant in
239 every pitcher, it was dominant in enough pitchers (in some cases with a frequency
240 above 90% of all OTUs) to dominate the metacommunity as a whole (Figure 4). The
241 poor competitive performance of *C. pseudoglaebosa* relative to other yeasts in
242 microcosms suggests that overall competitive superiority was not the cause of its
243 dominance (Figure 5). Instead, *C. pseudoglaebosa*'s early dispersal is a more likely
244 cause (Figure 6).

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

256 Although *C. pseudoglaebosa* was the numerically dominant fungal taxon in
257 the metacommunity of pitchers, chance events, dispersal, and interactions among
258 fungi determined whether it is the dominant taxon inside any given pitcher. We expect
259 that interactions with taxa that arrive, by chance, at different times in different
260 pitchers caused the variety of *C. pseudoglaebosa* relative frequency changes observed
261 in pitchers (*i.e.,* increasing, decreasing, or nonmonotonic, Figure 4B) because
262 interacting yeasts have qualitatively different impacts on *C. pseudoglaebosa*
263 performance depending on the number of interacting cells present (Figure 5).
264 Individual pitchers experienced priority effects because the timing of taxon arrival in
265 each pitcher (*e.g.,* early arrival of *C. pseudoglaebosa* in a pitcher) determined later
266 community composition (*e.g., C. pseudoglaebosa* dominance in the pitcher) (Fukami
267 2015). Despite the influence of chance events on *C. pseudoglaebosa* dominance in
268 any given pitcher, the early and frequent dispersal of *C. pseudoglaebosa* compared to
269 other yeasts enabled its overall dominance in the metacommunity (Figure 4A, 6).
270
271 *Ecological patterns and processes during pitcher metacommunity succession*
272 In the metacommunity, and in many individual pitchers, *C. pseudoglaebosa*
273 remained dominant through decreases in fungal taxon richness and diversity (Figures
274 3, 4). We did not observe a hump-shaped relationship between pitcher age and species
275 richness, as previously predicted (Figure 3A) (Moquet *et al.* 2003). Species richness
276 instead decreased, even as *R. babjevae* and *M. aphidis* were first dispersing into
277 pitchers late in the season (Figures 3A, 6). It is likely that *C. pseudoglaebosa*
278 repression of taxa through priority effects has a larger influence on species richness
279 than increasing diversity through new dispersal does.

280 Previous studies have also documented biotic and abiotic successional changes
281 in pitchers; while we did not measure the same parameters as these previous studies,
282 we assume that similar changes occurred in our pitcher metacommunity and that *C.*
283 *pseudoglaebosa* maintained its dominance through these changes. For example,
284 previous studies documented decreasing pH with increasing pitcher age, an early peak
285 in prey insect capture during pitchers' life spans, and a variety of changes in bacterial,
286 protist, and invertebrate community compositions over time (Fish & Hall 1978, Gray
287 *et al.* 2012, Miller & terHorst 2012). In bacterial, protist, and invertebrate
288 communities, the identities of dominant taxa changed as succession progressed. In
289 contrast, *C. pseudoglaebosa* remained the dominant fungus throughout succession. *C.*
290 *pseudoglaebosa* appears to be a classical early-successional taxon (Connell & Slatyer
291 1977, Fierer *et al.* 2010) because it disperses early and frequently (Figure 6), but it is
292 not replaced by late-successional taxa.
293 *C. pseudoglaebosa* dominance throughout succession may be enabled by the
294 short lifespans of pitchers in Harvard Pond; *i.e.,* pitchers may not live long enough to
295 enable late-succession fungal taxa to dominate the metacommunity. We sampled
296 pitchers that were up to 66-74 days old, and stopped sampling at this age because 23%
297 of pitchers had been destroyed by moths. However, pitchers in northern *S. purpurea*
298 populations can survive intact through winter conditions (Judd 1959), and pitchers can
299 be active for over a year in the southern United States (Miller & terHorst 2012). We
300 speculate that fungal succession more closely resembles classical successional
301 patterns and the patterns observed for other pitcher guilds (*e.g.,* bacteria,
302 invertebrates) in longer-lived pitchers. For example, it is possible that a strong
303 competitor such as *M. aphidis* could replace *C. pseudoglaebosa* in southern *S.*
304 *purpurea* metacommunities where pitchers are active for many months. However,

305 consistent dominance of a single taxon over succession may be common in microbial
306 habitats that, like northern *S. purpurea* pitchers, have short lifespans but repeatedly
307 become available.
308
309 **Conclusions**
310 In the model pitcher plant metacommunity, taxon dispersal ability has a
311 profound influence on community structure. In particular, *C. pseudoglaebosa*'s ability
312 to disperse into pitchers before other fungal taxa enables it to persist as the dominant
313 taxon in the pitcher metacommunity, even as intertaxon interactions and the
314 stochasticity of individual dispersal events prevent its dominance in many pitchers. It
315 is likely that dispersal ability leads to persistent dominance in a variety of other
316 natural succeeding microbial communities and metacommunities, especially when
317 early dispersal allows a taxon to prevent establishment of other taxa.
318 Future studies of microbial succession should explicitly include
319 metacommunity structure when investigating ecological processes. In the pitcher
320 metacommunity, overall taxon composition changed little over time, with *C.*
321 *pseudoglaebosa* dominant throughout succession (Figure 4A). However, individual
322 pitchers followed a variety of trajectories (Figures 2, 4B, 6). Studies of succession that
323 do not take a metacommunity's structure into account may miss community
324 heterogeneity and the diversity of ecological processes, especially dispersal ability, in
325 play among communities.
326
327 **Materials and Methods**
328 *Study site and field collections*

329 Observations were made on *Sphagnum* islands in Harvard Pond, adjacent to
330 Tom Swamp, a 50 ha *Sphagnum* bog located in Petersham, Massachusetts at 42°30'N,
331 72°12'W (Figure 1; Swan & Gill 2007). The *C. pseudoglaebosa* and *M. aphidis*
332 isolates used in the microcosm study were collected from pitchers in Harvard Pond,
333 and the *R. babjevae* isolate was collected from a pitcher in Swift River Bog, a 2 ha
334 kettlehole bog located 75 km south of Tom Swamp in Belchertown, MA at 42°16'N,
335 72°20'W (Ellison *et al.* 2002). These three yeast isolates were collected in the
336 summer of 2006 and identified by comparing their ribosomal sequences, amplified
337 using the ITS1F/ITS4 and LS1/LR5 primer pairs (Gardes & Bruns 1993, White *et al.*
338 1990, Hausner *et al.* 1993 Vilgalys & Hester 1990), to sequences in the NCBI BLAST
339 database (Zhang *et al.* 2000). We chose *C. pseudoglaebosa*, *R. babjevae*, and *M.*
340 *aphidis* in part because they were all easily cultured from pitchers, and in part because
341 they formed colonies with different morphologies on agar plates: *C. pseudoglaebosa*
342 forms smooth white colonies; *M. aphidis* forms wavy white colonies; and *R. babjevae*
343 forms smooth pink colonies.
344 All *S. purpurea* pitcher water samples for PCR and 454 sequencing were
345 collected in the spring and summer of 2009. In May of 2009, we identified 43
346 unopened *S. purpurea* pitchers on 32 *Sphagnum* islands in Harvard Pond. Pitchers
347 ranged from less than 1 m to 908 m in distance to other pitchers (Figure 1C). We
348 visited each pitcher daily until it opened, and counted pitcher age from the date it
349 opened. For each pitcher water collection, the water inside a pitcher was first mixed
350 by pipetting up and down with a sterile plastic transfer pipette. We then removed
351 about 0.25 ml pitcher water and mixed it with 0.25 ml of CTAB buffer (100 mM Tris
352 pH 8.0, 1.4 M sodium chloride, 20 mM EDTA, 2% CTAB). To the best of our ability,
353 we avoided collecting insect prey or macrofauna in these samples, although any

354 protists and microscopic animals present in our samples were included; collected
355 pitcher water contained no large animal parts and appeared as a cloudy liquid. All
356 samples were flash-frozen in liquid nitrogen within five hours of collection and stored
357 at -20 or -80°C before DNA extraction.
358
359 *PCR assay*
360 We assayed each pitcher water sample for amplifiable DNA from all fungi,
361 using the ITS1F/ITS4 primer pair, and for each of the three yeasts in the microcosm
362 experiment, using the primers in Table 1. Primers to selectively amplify portions of
363 each microcosm yeast's ITS sequence were designed using the NCBI BLAST primer
364 tool (Rozen & Skaletzky 2000). We chose primer sequences to reliably amplify as
365 much of the ITS sequence of each yeast species as possible, while not amplifying
366 other sequences in the BLAST database.
367 To extract DNA from each pitcher water sample before the PCR assay, we
368 first thawed and centrifuged frozen samples at 16.1 g for 10 min and removed the
369 supernatant from each pellet. We then suspended each pellet in 200 μL of breaking
370 buffer (2% Triton X-100, 1% sodium dodecyl sulfate, 100 mM sodium chloride, 10
371 mM Tris, and 1 mM EDTA) (Hoffman 1997). We mixed each suspension with about
372 200 μL of 0.5 mm glass beads and 200 μL 25:24:1 chloroform:phenol:isoamyl
373 alcohol. We vortexed each mixture for 2 min, and then centrifuged it for 5 min at 16.1
374 g. After centrifugation, we removed the aqueous layer and mixed it with 2.5 volumes
375 of 95% ethanol and 0.1 volume of 3M sodium acetate (Sambrook & Russell 2001);
376 we incubated each aqueous layer mixture at -20°C for at least three hours. Next, we
377 centrifuged each aqueous layer mixture for 15 min at 16.1 g, and removed the
378 supernatant. Finally, we washed each pellet with 0.5 ml 70% ethanol, centrifuged

379 each mixture for 10 min at 16.1 g, removed the supernatant, and resuspended each
380 pellet in 50 µl water.
381 We then assayed each DNA extract for the presence of each Fungal taxon, or
382 any Fungal DNA in the case of the ITS1F/ITS4 primer pair, using PCR. Each PCR
383 reaction was composed of 7.9 µL water, 0.1 µL GoTaq® Flexi polymerase (Promega),
384 5 µL Flexi buffer with green dye added, 5 µL 5x CES (combinatorial PCR enhancer
385 solution: 2.7 M betaine, 6.7 mM dithiothreitol, 6.7% dimethyl sulfoxide, 55 µg/mL
386 bovine serum albumin) (Ralser *et al.* 2006), 5 µL nucleotide mix, 2 µL magnesium
387 chloride, 1 µL of 10 µM of each primer, and 1 µL undiluted template DNA extract.
388 All reactions were cycled on a Biorad iCycler or myCycler using denaturing,
389 annealing, and extension temperatures of 95, 55, and 72 °C, respectively. We
390 denatured for 85 s, then ran 13 cycles of 35 s denaturing, 55 s annealing, and 45 s
391 extension, followed by 13 cycles that were identical but had a 2 min extension, and
392 finally 9 cycles with a 3 min extension. We ran a subsequent 10 min extension. Two
393 µL of each PCR product were visualized on 1% agarose gels stained with SYBR Safe
394 dye (Invitrogen) and photographed using a U:genius gel documenting system
395 (Syngene) and a Stratagene transilluminator. Photographs of gels were scored for
396 presence or absence of a band. Bands that were too faint to reliably score were run a
397 second time with 6 µL of PCR product per well. Presence of a band on a gel indicated
398 the presence of detectable fungal or yeast species DNA in a water sample.
399 To confirm that primers only amplified sequences from the target yeasts, we
400 randomly selected nine PCR products generated from the *C. pseudoglaebosa* and *R.*
401 *babjevae* primer pairs for sequencing. The primer pair that targets *M. aphidis* only
402 amplified DNA from six pitcher water extracts, and we sequenced all six PCR

403 products for this primer pair. Sequences were identical to or within one base of
404 cultured isolate sequences.
405
406 *Pitcher water fungal DNA amplification and 454 sequencing*
407 We extracted and amplified fungal DNA for fungal community amplicon
408 sequencing using the protocols described above, with the following changes. Gotaq®
409 Hotstart polymerase (Promega) was used instead of Flexi polymerase, and we used 50
410 μM instead of 10 μM of the reverse primer. The forward primer consisted of (in order
411 from 5' to 3') the 454 "A" primer (CCATCTCATCCCTGCGTGTCTCCGACTCAG)
412 concatenated with a 10-bp multiplex tag (454 Life Sciences Corporation 2009), and
413 ITS4; the reverse primer consisted of the 454 "B" primer
414 (CCTATCCCCTGTGTGCCTTGGCAGTCTCAG) concatenated with ITS1F.
415 Multiplex tags were unique to each sample. Reactions were cycled at 95 °C for 15
416 min; 30 cycles of 95 °C for 1 min, 51 °C for 1 min, 72 °C for 1 min; and a final
417 extension of 72 °C for 8 min.
418 Products were purified using Agencourt® *AMPure*® XP (Beckman Coulter)
419 and quantitated using a Qubit® dsDNA HS Assay (Invitrogen) according to the
420 manufacturers' instructions. We combined equimolar concentrations of the products
421 of each of three separate PCR reactions from each DNA extract. The sequencing pool
422 consisted of pooled equimolar concentrations of each pooled PCR product. The pool
423 was sequenced on one-eighth of a 454 Titanium sequencing run by the Duke Genome
424 Sequencing & Analysis Core Resource.
425
426 *454 sequence processing*

427 We processed sequences using QIIME 1.3.0 (Caporaso *et al.* 2010). Low
428 quality sequences were removed and the remaining sequences were assigned to
429 mulitplex barcodes using the default quality filtering settings. Primers and barcodes
430 were trimmed from each sequence and sequences shorter than 200 and longer than
431 1000 bp were removed from each dataset. Sequences were denoised using the QIIME
432 denoiser. We reduced chimeric sequences by trimming the 5.8s and ITS1 portions
433 from all sequences using the Fungal ITS Extractor (Nilsson *et al.* 2010a), and only
434 analyzing the ITS2 portion. The 5.8s ribosomal region lies between the ITS1 and
435 ITS2 spacers, and is conserved among fungi relative to the spacers. We expected most
436 chimeric sequences to form in the 5.8s region and to be composed of ITS1 and ITS2
437 sequences from different templates (Nilsson *et al.* 2010b). We chose operational
438 taxonomic units (OTUs) using the uclust method in QIIME, at 97% similarity (Edgar
439 2010). We discarded all OTUs composed of a single sequence (singleton OTUs)
440 because we assumed that they resulted from sequencing errors. The longest sequence
441 in each remaining cluster was retained as a representative sequence. Of the 141,424
442 total sequences produced, 27,632 were discarded for having lengths less than 200 or
443 more than 1000 bp and 10,938 were discarded because they had low quality or did not
444 have a matching barcode. Sixty-six sequences were discarded because the ITS2
445 subunit could not be extracted and 139 sequences were discarded because they
446 represented singleton OTUs. In total, we retained 102,649 sequences for further
447 analysis. Each pitcher water sample produced between 253 and 4365 sequences.
448
449 *Sequence taxonomy assignments*
450 To assign genus-level taxonomy to OTUs, we performed a MEGAN analysis
451 of OTU representative sequences on the top ten hits from the NCBI BLAST

452 nucleotide database extracted using BLAST 2.2.25+ and default MEGAN settings
453 (Huson *et al*. 2011, Zhang *et al.* 2000). We discarded OTUs matching organisms not
454 in the kingdom Fungi (plant, animal, and protist sequences), but we assumed that
455 OTUs with no BLAST matches or matching unassigned fungal environmental
456 sequences were fungal sequences not yet identified in the NCBI database. We
457 retained unassigned OTUs for diversity measurements, but did not include them in
458 taxonomy summaries. We reviewed the taxon assignments output by MEGAN
459 manually, and filled in higher-level classifications (*e.g.,* order or class) using Index
460 Fungorum (http://www.indexfungorum.org/) when an OTU was assigned to a genus
461 but not higher-level classifications. We attempted to assign all taxa to genera; if it was
462 not possible to assign a taxon to a genus, we assigned it to the most specific
463 taxonomic group possible. OTUs assigned to the genus *Candida* were further assigned
464 to the species *C. pseudoglaebosa* if they matched either *C. pseudoglaebosa* or its
465 close relative *C. glaebosa* in a BLAST search. When we compared these seven OTUs
466 against sequences representing the type strains of the two *Candida* species (accession
467 numbers KY102342.1 and KY102112.1, respectively), all seven OTUs aligned to *C.*
468 *pseudoglaebosa* better than they did *C. glaebosa*. In total, we detected 553 OTUs, of
469 which 379 were assigned to fungal taxa; fifteen were discarded because they matched
470 non-fungal sequences; and 159 were not assigned. Of the 379 fungal taxa in the
471 temporal data set, 52% were Basidiomycota, 43% Ascomycota, and 5% basal fungal
472 lineages.
473
474 *Microcosm interaction assays*
475 Interactions between yeasts were assayed in microcosms designed to mimic
476 pitchers simultaneously colonized by different numbers of two yeast species. We

477 grew microcosms in low-nutrient media designed to mimic natural conditions in
478 pitchers. Microcosms contained sterile yeast extract media (YEM) composed of 1g/L
479 yeast extract in local tap water (Cambridge, MA, USA). Tap water was used instead
480 of deionized water because we wanted the media to include micronutrients present in
481 local rainwater that may be important for pitcher plant yeast growth. The tap water
482 supply in Cambridge, MA, where this experiment was conducted, comes from three
483 Massachusetts reservoirs (Waldron & Bent 2001), and we expected it to have similar
484 inputs as rainwater in Harvard Pond pitchers. Each microcosm was inoculated with a
485 target yeast species and an interactor in 200 μL of liquid yeast media. Each target
486 yeast was inoculated with about 1000 cells per microcosm, and each interactor yeast
487 was inoculated at zero, low, medium, and high cell numbers (0 and approximately
488 100, 1000, and 10,000 cells).
489 Eighteen treatments of yeast mixtures were prepared, with ten replicates each,
490 for a total of 180 microcosms. Before inoculation, yeasts were grown in liquid YEM
491 for 48 hours. Inoculation sizes were measured after inoculation using counts of
492 colony-forming units (CFUs) on solid YEM (YEM plus 1.5% agar). Microcosms
493 were arranged in sterile 96-well polystyrene flat bottom cell culture plates and
494 incubated between 25 and 27 °C, shaking at 700 rpm for 48 hours. After incubation
495 32 microcosms were discarded because of suspected contamination. We diluted each
496 remaining microcosm, plated it to solid YEM, and counted CFUs on plates containing
497 at least 30 total CFUs. When no CFUs of an inoculated yeast were present on a plate,
498 we conservatively assumed that the yeast was present in the microcosm in numbers
499 just below our detection limit. We calculated total cell numbers assuming one instead
500 of zero CFUs for these yeasts absent from plates.
501

502 *Statistical analyses*

503 OTU datasets rarefied to 1040 sequences were used to produce Non-metric
504 Multidimensional Scaling (NMDS) plots and to compare community similarities, and
505 alpha diversity indices among pitchers. Eight samples contained fewer than 1040
506 sequences and were discarded. Proportions of samples assigned to taxonomic groups
507 were calculated based on the full non-rarefied dataset. Community similarities over
508 time were compared using partial distance-based redundancy analysis (db-RDA) of
509 Jaccard dissimilarity (Jaccard 1912) between each pair of samples with pitcher age as
510 the explanatory variable, conditioned on pitcher identity. A correlation between
511 geographic distance and community similarity was conducted using a partial Mantel
512 test conditioned on pitcher age. Hill numbers of order q=0 or 2 (^{q}D) were calculated
513 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
514 abundance of OTU i; ^{1}D was calculated as the exponent of Shannon diversity (Hill
515 1973, Chao *et al.* 2014). Changes in Hill numbers were modeled over time using
516 repeated-measures linear models controlled for pitcher identity; ^{1}D and ^{2}D were log-
517 transformed before analyses to homogenize variances among timepoints, and ^{0}D was
518 not transformed.

519 We modeled the impact of interactor yeasts on focal yeasts in microcosms
520 using multiple linear and polynomial regressions. Separate regressions were
521 conducted for each focal yeast. For each regression, focal yeast yield was the
522 dependent variable, and both the number of co-inoculated interactor yeast cells and
523 the identity of the interactor yeast were independent variables. We modeled both
524 linear and quadratic relationships between the number of co-inoculated interactor
525 yeast cells and the dependent variable because the relationship did not always appear
526 linear when plotted. Before constructing the regressions, we square-root-transformed

527 focal yeast yield to homogenize variances for the focal yeasts *R. babjevae* and *C.*
528 *pseudoglaebosa*, but left yield untransformed for the focal yeast *M. aphidis*. We also
529 transformed competitor inoculum size by $\log_{10}(x+1)$ because interactor inoculum size
530 was varied on a log scale in the experiment. When comparing the influences of
531 competitor species, we randomly assigned treatments with no interacting yeast
532 inoculum to one of the two interacting yeasts. When selecting the best-fitting
533 regression model, we first established the best-fitting relationship (linear, quadratic, or
534 both) between log-transformed interactor inoculum size and focal yeast yield, and
535 then determined whether adding interactor identity or interactions between interacting
536 yeast identity and inoculum size to the model improved it. The best-fitting model was
537 the one with the lowest Akaike Information Criterion (AIC).
538 All statistical analyses and index calculations were conducted using R version
539 3.3.1 (R Development Core Team, 2016) and the packages vegan, fields, nlme, and
540 GUniFrac (Chen 2018, Pinheiro *et al.* 2016, Oksanen *et al.* 2016, Nychka *et al.* 2016)
541 plots were made using ggplot2 (Wickham 2016)
542

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Supplemental Table 1: Model selection for the focal yeast *Candida glaebosa* (*C. glaebosa* yield square-root-transformed, and inoculum size $\log_{10}(x+1)$-transformed):

explanatory variables	df	F	p	adj-R^2	AIC
inoculum size	1, 51	79.37	<0.001	0.60	405.64
(inoculum size)^2	1, 51	111.1	<0.001	0.68	394.09
inoculum size + (inoculum size)^2	2, 50	54.65	<0.001	0.67	395.97
(inoculum size)^2 + competitor identity	**2, 50**	**65.26**	**<0.001**	**0.71**	**389.34**
(inoculum size)^2 + competitor identity x (inoculum size)^2	2, 50	60.02	<0.001	0.69	392.51
(inoculum size)^2 + competitor identity + competitor identity x (inoculum size)^2	3, 49	42.74	<0.001	0.71	391.25

Supplemental Table 2: ANOVA table for the best-fitting model for *C. glaebosa*:

	df	Sum of Squares	Mean Squares	F	p
(inoculum size)^2	1	10235.2	10235.2	123.73	<0.001
competitor identity	1	561.4	561.4	6.79	0.012
residuals	50	4136.3	82.7		

Supplemental Table 3: Model selection for the focal yeast *Pseudozyma aphidis* (*P. aphidis* yield untransformed, and inoculum size $\log_{10}(x+1)$-transformed):

explanatory variables	df	F	p	adj-R^2	AIC
inoculum size	1, 58	5.37	0.02	0.07	1211.77
(inoculum size)^2	1, 58	11.97	0.001	0.16	1205.83
inoculum size + (inoculum size)^2	**2, 57**	**10.86**	**<0.001**	**0.25**	**1199.71**
inoculum size + (inoculum size)^2 + competitor identity	3, 56	7.64	<0.001	0.25	1200.51
inoculum size + (inoculum size)^2 + competitor identity x inoculum size	3, 56	7.73	<0.001	0.26	1200.28
inoculum size + (inoculum size)^2 + competitor identity x (inoculum size)^2	3, 56	7.68	<0.001	0.25	1200.28

Supplemental Table 4: ANOVA table for the best-fitting model for *P. aphidis*:

	df	Sum of Squares	Mean Squares	F	p
inoculum size	1	173667504	173667504	6.6684	0.012
(inoculum size)^2	1	391953317	391953317	15.0501	<0.001
residuals	57	1484466512	26043272		

Supplemental Table 5: Model selection for the focal yeast *Rhodotorula glutinis* (*R. glutinis* yield square-root-transformed, and inoculum size $\log_{10}(x+1)$-transformed):

explanatory variables	df	F	p	adj-R^2	AIC
inoculum size	1, 59	92.25	<0.001	0.60	542.62
(inoculum size)^2	1, 59	67.64	<0.001	0.53	553.45
inoculum size + (inoculum size)^2	2, 58	45.56	<0.001	0.60	544.44
inoculum size + competitor identity	**2, 58**	**155.7**	**<0.001**	**0.84**	**489.12**
inoculum size + competitor identity x inoculum size	2, 58	109.0	<0.001	0.78	506.87
inoculum size + competitor identity + competitor identity x inoculum size	3, 57	102.1	<0.001	0.83	491.06

Supplemental Table 6: ANOVA table for the best-fitting model for *R. glutinis*:

	df	Sum of Squares	Mean Squares	F	p
inoculum size	1	36941	36941	225.28	<0.001
competitor identity	1	14114	14114	86.07	<0.001
residuals	58	9511	164		