1   Superior dispersal ability leads to persistent ecological dominance by *Candida*

2   *pseudoglaebosa* in the *Sarracenia purpurea* metacommunity

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12   **Abstract**

13   A large number of descriptive surveys document changes in microbial communities

14   over time, but direct evidence for the ecological processes mediating succession or

15   causing ecological dominance remains rare. Differential dispersal may be a key

16   mechanism. We surveyed microbial diversity within a metacommunity of pitchers of

17   the model carnivorous plant *Sarracenia purpurea* and discovered the yeast *Candida*

18   *pseudoglaebosa* as ecologically dominant. Its frequency in the metacommunity

19   increased over the growing season, and it was not replaced by other taxa. We next

20   measured its competitive ability in a manipulative laboratory experiment and tracked

21   its dispersal over time in nature. Despite its dominance, *C. pseudoglaebosa* is not a

22   superior competitor. Instead, it is a superior disperser: it arrives in pitchers earlier, and

23   disperses into more pitchers, than other taxa. Differential dispersal across the spatially

24   structured metacommunity of individual pitchers emerges as a key driver of the

25   continuous dominance of *C. pseudoglaebosa* during succession.

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Introduction

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28   Primary microbial succession occurs when a microbial community colonizes

29   and develops on a newly available substrate (Fierer *et al*. 2010). The advent of high-

30   throughput sequencing has revolutionized observational studies of microbial

31   succession, enabling researchers to describe the development of microbial

32   communities in fine detail (Copeland *et al.* 2015, Boynton & Greig 2016, Koenig *et*

33   *al.* 2011, Cutler *et al*. 2014). A variety of successional patterns have been observed.

34   For example, taxon diversity can increase, decrease, or randomly vary with

35   successional time (Cutler *et al.* 2014, Zumsteg *et al.* 2012, Redford & Fierer 2009).

36   However, researchers commonly observe replacement of early-successional taxa by

37   late-successional taxa (*e.g.,* Boynton & Greig 2016, Wolfe *et al*. 2014). An ongoing

38   challenge in microbial ecology is to connect observed ecological patterns to the

39   ecological processes responsible for the patterns.

40   The development of ecological dominance by one or a few microbes over

41   successional time is a particularly intriguing phenomenon (Boynton & Greig, 2016,

42   Findley *et al.* 2013, Jiao *et al.* 2016), in part because of its obvious parallels to plant

43   and animal ecology (Hillebrand *et al.* 2008). Ecological dominance is apparent when

44   one or a few species comprise most of the individuals or biomass in a community

45   (Hillebrand *et al.* 2008). Environmental filtering, superior competitive ability of the

46   dominant species, and ecosystem engineering can all cause dominance (Wolfe *et al.*

47   2014, Albergaria *et al*. 2010, Goddard 2008, Nissen & Arneborg 2003, Williams *et al*.

48   2015). However, the dynamics of microbial dominance have been primarily studied in

49   domesticated systems, and the most frequently reported mechanisms may not be

50   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

76   communities of a metacommunity and the result is a hump-shaped relationship

77   between successional time and species richness (Moquet *et al.* 2003, Sferra *et al.*

78   2017). Species richness is predicted to be low early in a component community's age,

79   to increase as more species disperse into the community, and then to decrease as

80   competitive interactions remove species from the community. Although rarely

81   documented, it is also possible that a particularly good disperser will become

82   dominant and remain dominant (and therefore decreases community diversity) in a

83   metacommunity solely as a result of its dispersal ability.

84   We investigated the contribution of dispersal to ecological dominance over the

85   course of natural microbial succession in pitchers of the carnivorous pitcher plant

86   *Sarracenia purpurea*. *S. purpurea* is a perennial plant native to bogs and savannas in

87   northern and eastern North America (Buckley *et al.* 2003). Each *S. purpurea* plant

88   produces modified vase-shaped leaves, or pitchers (Figure 1a), annually. At first,

89   developing pitchers are entirely closed, sterile chambers (Peterson *et al.* 2008). Once

90   mature, the top portion of each pitcher opens, and open pitchers accumulate rainwater

91   to form small pools of water (phytotelmata). Potential prey (ants and other small

92   insects) are attracted to pitchers (Bennett & Ellison 2009); some prey fall into pitchers

93   and drown, and are then shredded, decomposed, and mineralized by a food web of

94   microorganisms and invertebrates (reviewed in Ellison *et al.* 2003). The pitcher

95   microbial community includes bacteria, algae, and fungi, including culturable yeasts

96   (Cochran-Stafira & von Ende 1998). Pitchers are individual communities within a

97   metacommunity of other pitchers on the same and on different plants.
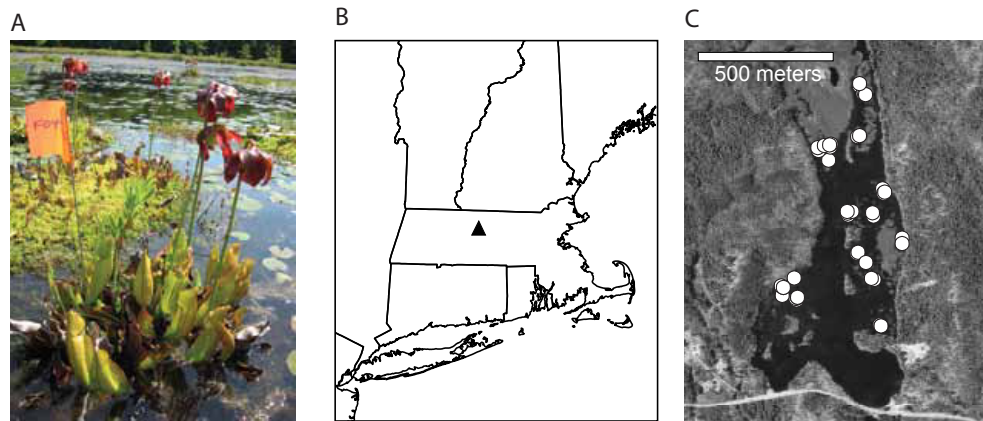
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Figure 1: Example *S. purpurea* plant and study location. (A) One of the study pitcher plants at the edge of a *Sphagnum* island. This photograph was taken early in the growth season and both opened and unopened pitchers are visible. (B) The location 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).

98   We followed fungal succession within individual pitchers in a *Sphagnum* bog

99   in central Massachusetts, where the pitcher growing season lasts for about three

100   months (although pitchers persist for longer and can overwinter, Judd 1959). We first

101   documented that a single yeast species, *Candida pseudoglaebosa*, was numerically

102   dominant in the metacommunity throughout the growing season, and its frequency

103   increased between early and later-successional stages of individual pitchers. We next

104   investigated the ecological processes leading to *C. pseudoglaebosa* dominance.

105   Unlike dominant yeasts in many other systems, *C. pseudoglaebosa* was not an

106   especially good competitor against other tested yeasts. However, it was an especially

107   good disperser. It is one of the first fungi to arrive in pitchers and it maintained high

108   frequency in the pitcher plant metacommunity over the course of the season, even as

109   its frequency within individual pitchers ranged from completely absent to over 90% of

110   sequences. The apparently superior dispersal ability of *C. pseudoglaebosa* leads it to

111   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
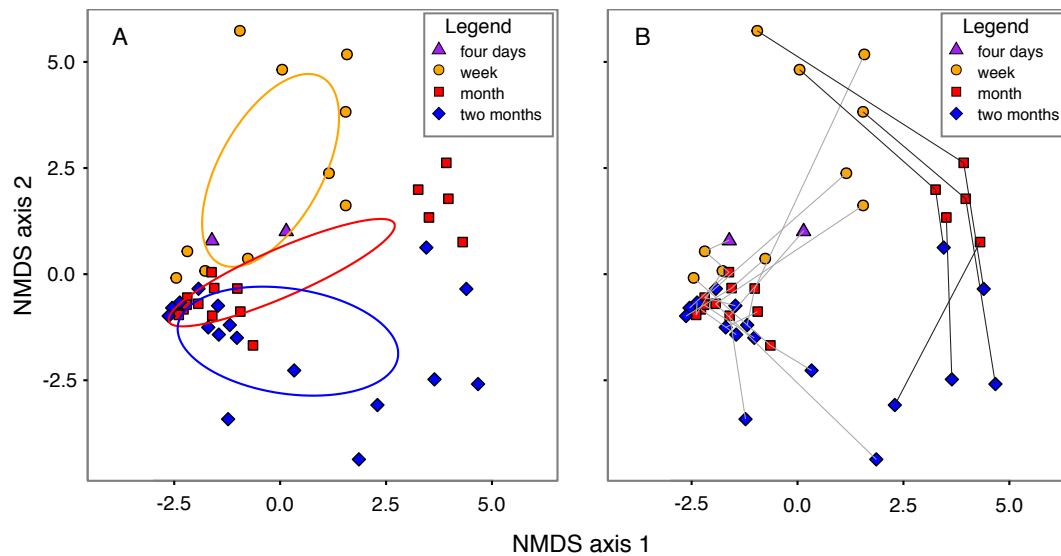
Figure 2: Non-metric Multidimensional Scaling (NMDS) plots of pitcher plant community similarities. Similarities of community OTU compositions were calculated using the Jaccard metric (Jaccard 1912). Purple triangles represent four-day-old communities, orange circles represent week-old communities, red 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).

137   community composition was due to variation in time (distance-based redundancy

138   analysis adjusted $R^2 = 0.03$, F = 1.96, df = 1,27, p = 0.011). This small influence of

139   time on community composition was likely a result of high variation among pitchers.

140   Succession followed two trajectories—five pitchers (hereafter referred to as "pitcher

141   group 1") followed a different successional trajectory from the other twelve ("pitcher

142   group 2")—and there was considerable variation among communities within each

143   trajectory (Figure 2B). Distance among pitchers did not explain significant variation

144   in community composition (partial mantel test of community composition on space

145   controlling for time, mantel statistic r = -0.04, significance = 0.715).

146   Despite the observed variation in fungal community composition, diversity

147   decreased, on average, in pitchers between four days and two months (Figure 3). To

148   determine a sample's diversity, we calculated Hill numbers of orders 0 to 2 (^0D to ^2D)
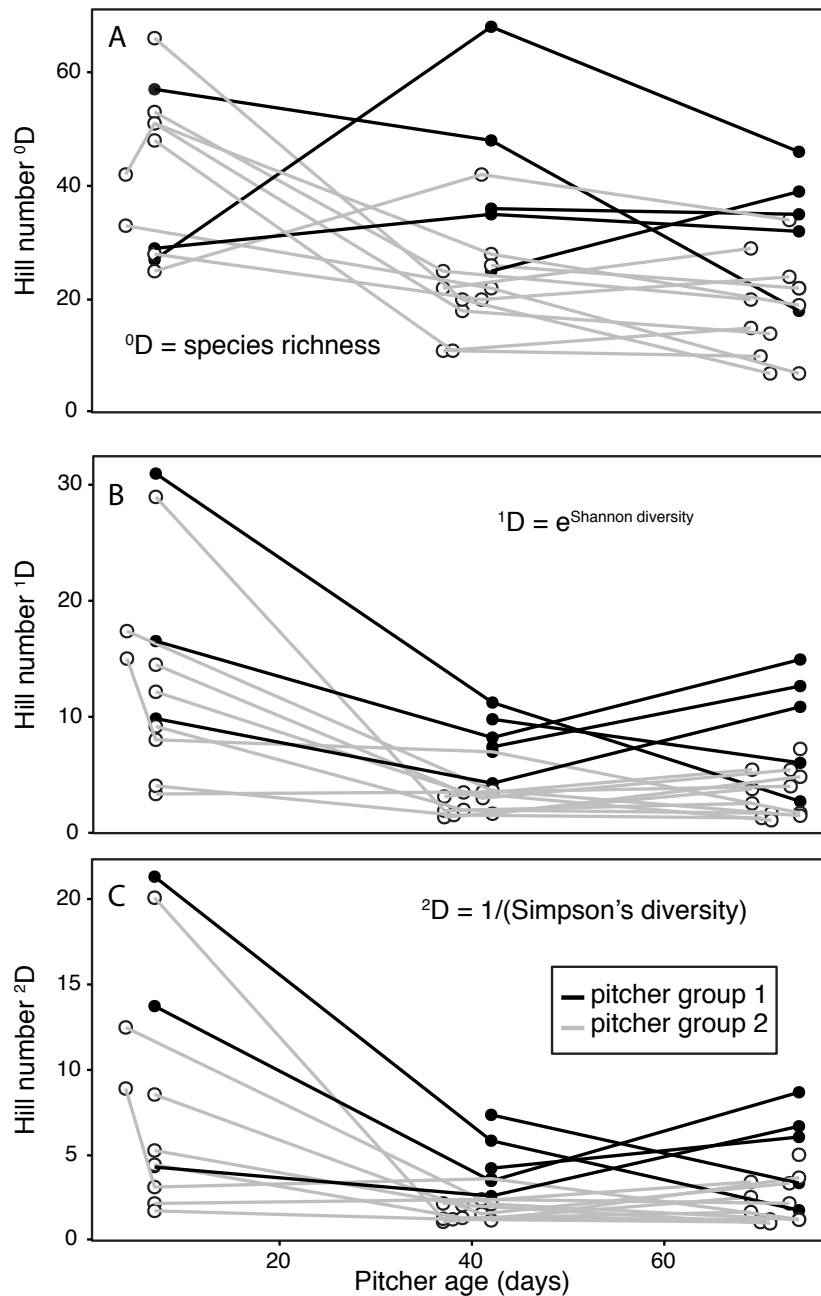
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Figure 3: Hill numbers of orders A) 0, B) 1, and C) 2 in pitchers over time. Data points for communities in the same pitcher are connected with lines. Black lines connect points for pitcher group 1 and gray lines connect points for pitcher group 2.

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   ^0D 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); ^1D declined from

157   14.2 to 5.2 (t=-3.8, df=27, p=0.0008); and ^2D 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*
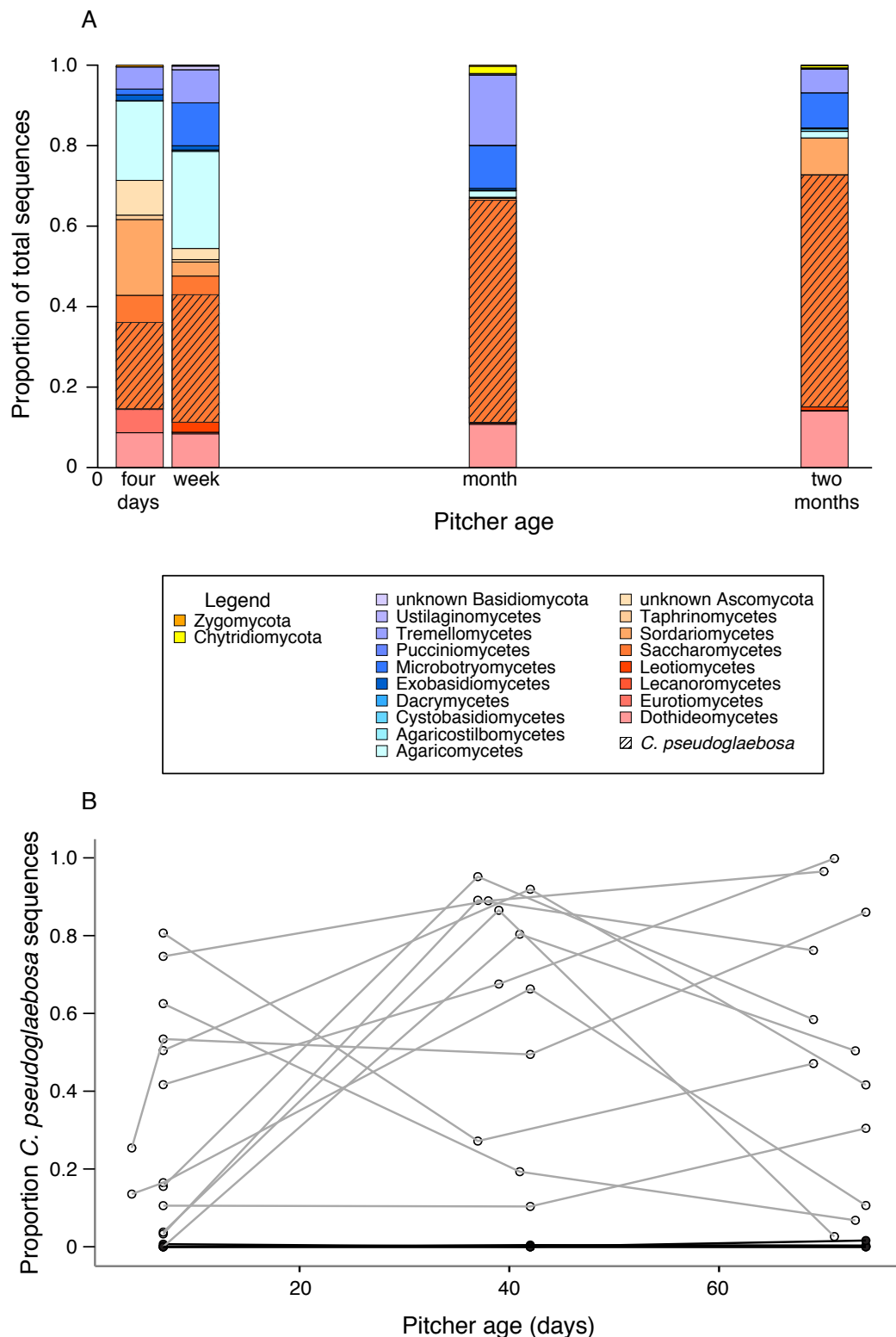
176 *other yeasts*

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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.

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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.*

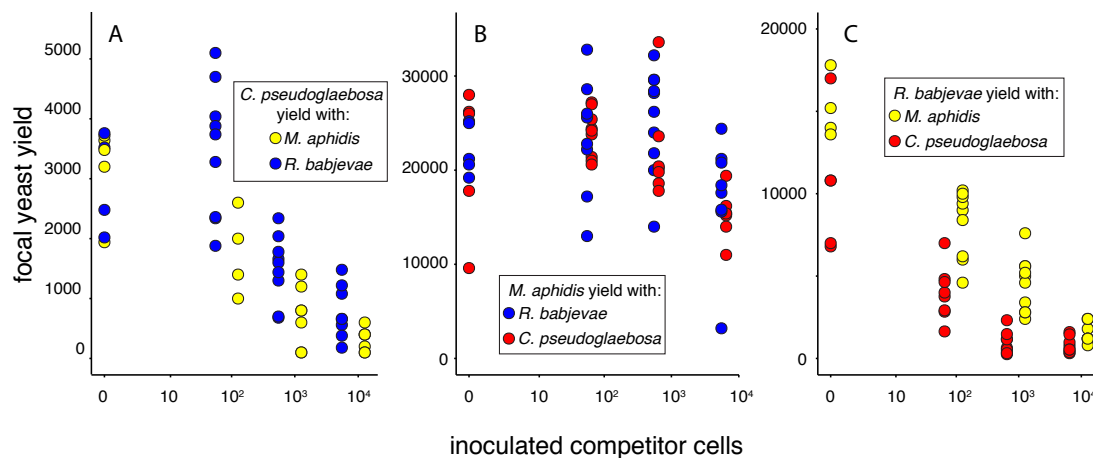202   *pseudoglaebosa* by *R. babjevae* when few *R. babjevae* cells were inoculated, but we

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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*.

203   did not inoculate *M. aphidis* in small enough numbers to confirm *M. aphidis*

204   facilitation of *C. pseudoglaebosa* (Figure 5A). At high numbers of co-inoculated cells,

205   *M. aphidis* had a more detrimental impact on *C. pseudoglaebosa* than *R. babjevae*

206   (F=6.79, df=1,50, p = 0.012, Figure 5, figure supplemental table 2). In contrast, the

207   two interactors of *M. aphidis* had similar effects on its yield: at low and intermediate

208   inoculum sizes, both *R. babjevae* and *C. pseudoglaebosa* promoted *M. aphidis*

209   growth, but at high inoculum sizes, both interactors inhibited *M. aphidis* growth

210   (Figure 5B). Interactions between *R. babjevae* and interactor yeasts were linear

211   (Figure 5C, figure supplemental tables 5-6): *R. babjevae* yield was impeded by

212   interactors regardless of the number of interactor cells present, and *C. pseudoglaebosa*

213   had a more detrimental impact on *R. babjevae* than *M. aphidis* did (F=86.07, df=1,58,

214   p < 0.001, Figure 5, figure supplemental table 6).

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216   C. pseudoglaebosa *is an early disperser in pitchers*

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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.

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229

230   Table 1: Taxon-specific PCR primer sequences used to detect pitcher yeasts

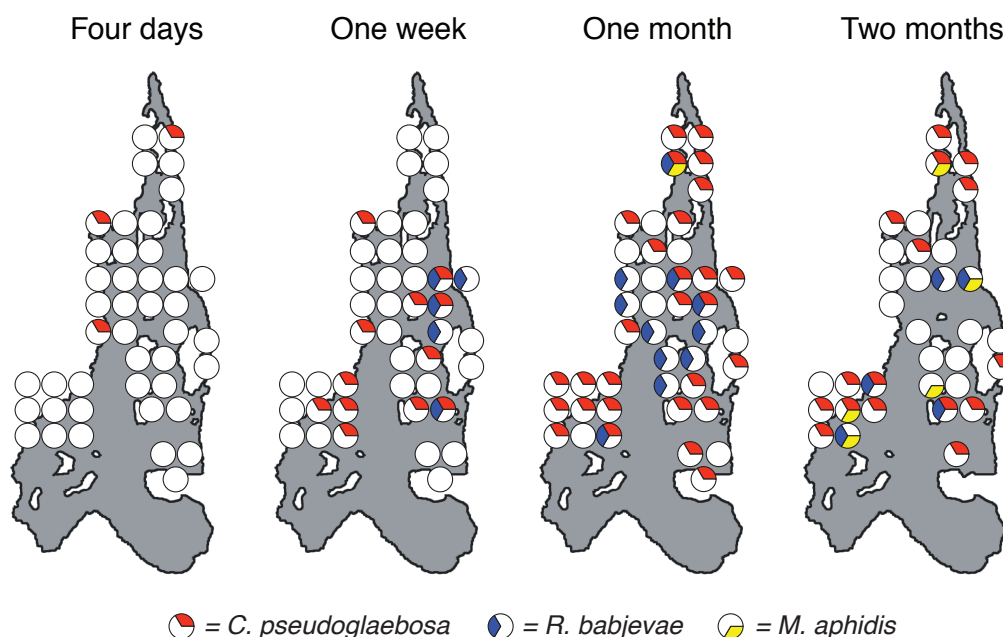| yeast	forward sequence	Tm	reverse sequence	Tm	product length
C. glaebosa	5'-CTGCGGAAGGATCATTACAGT-3'	54.6	5'-TGTTCAGACAACACTGTTCA-3'	51.8	466
R. glutinis	5'-AAGTCGTAACAAGGTTTCCG-3'	52.8	5'-CCCAACTCGGCTCTAGTAAA-3'	53.9	527
P. aphidis	5'-GGTAATGCGGTCGTCTAAAA-3'	52.6	5'-CTCTTCCAAAGAAGCGAGG-3'	53.1	467

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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.

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234

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.*

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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

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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

17

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.

21

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

23

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

24

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 $^{q}D = \left(\sum_{i=1}^{S} p_i^{q}\right)^{1/(1-q)}$, 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

26

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

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.

28

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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