

Top-down versus bottom-up cohesiveness in microbial community coalescence

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Abstract

The abstract goes here.

Introduction

Microbial communities often invade one another. This has been observed, for instance, in river courses where terrestrial microbes mix with aquatic microorganisms [1–3] or in soil communities being invaded as a result of tillage and outplanting [4] or by aerially dispersed bacteria and fungi [5]. Gut microbiomes can invade external communities through the host animal secretions [6], and the skin microbiota is also subject to invasions when making contact with environmental sources of microbes [7].

The phenomenon by which entire microbiomes invade one another has been termed *community coalescence* [8]. Ecologists have long contemplated the idea that interactions between multiple co-invading species can produce correlated invasional outcomes [8–18]. However, and in spite of its clear potential importance, the role of coalescence in microbiome assembly is only beginning to be addressed and little is known about the mechanisms that govern it and its potential implications. Early mathematical models of community-community invasions [9, 19] as well as more recent work [20–23] suggest that high-order invasion effects are common during community coalescence. Communities that have a previous history of coexistence may exhibit an emergent “cohesiveness” which produces correlated invasional outcomes among species from the same community [15, 24]. The situation where ecological partners in the invading community recruit each other into the final coalesced community has been called *ecological co-selection* [24, 25].

The mechanisms of ecological co-selection during community coalescence are still poorly understood. Do a few key species recruit everyone else, or are collective interactions among all species (including the rarer members of the community) relevant for coalescence outcomes? While it is reasonable to expect species with larger population sizes to have a proportionally oversized effect, natural communities tend to be highly diverse [26] and the role played by the less abundant species has long been subject to debate [27]. Laboratory cultures have also been found to contain uneven distributions of multiple strains that feed off the metabolic secretions of the dominant species [28, 29]. The fate of these sub-dominant taxa may be dependent on the invasional success of their dominant species, or, alternatively, the dominant itself may owe its dominance (at least in part) to cross-feeding or other forms of facilitation from the rarer members of the population. These scenarios would give rise to “top-down” or “bottom-up” community cohesiveness, respectively. Either of these forms of co-selection could, in principle, be positive (recruitment) or negative (antagonism), as illustrated in Figure 1e. Which of these situations are typically found in nature? Previous theoretical and computational studies suggest that the answer is determined by the type and strength of the interactions of the community members with one another and with the environment [20, 22, 23], but addressing this question has been experimentally challenging in the past [24, 25].

In previous work, we have shown that a large amount of soil and plant microbiomes can be cultured *ex situ* in synthetic minimal environments with a single supplied limiting resource under serial growth-dilution cycles [29] (Figure 1a-b). Under these conditions, environmental microbiomes spontaneously re-assemble into complex multi-species communities sustained by dense cross-feeding facilitation networks [29]. In addition, and just like

48 in natural consortia, species abundance distributions in these communities are generally long-tailed and uneven
49 (Figure 1d and Figure S1), with the dominant (most abundant) species typically comprising most of the biomass
50 (median = 46%, Figure S1). Because these communities are easy to manipulate and grow in high throughput, and
51 are largely made up by culturable members, they represent good test cases to investigate ecological co-selection
52 during community coalescence. Here we focus on the dominants and ask whether they can co-select or be co-
53 selected by the sub-dominant species in their communities (henceforth referred to as their *cohorts*, Figure 1c).

54 Our results indicate that co-selection varies in direction and strength depending on the supplied limiting re-
55 source. This primary resource, in turn, has been shown to shape the structure and interactions of the communities
56 [30]. We observe that, when top-down co-selection is weak, bottom-up co-selection can be very strong, with
57 positive co-selection being far more common than negative co-selection. We then turn to a Microbial Consumer-
58 Resource Model (MicroCRM) [29, 31, 32] that is able to capture the dynamics of microbial communities domi-
59 nated by metabolic interactions, as is the case for the ones assembled in our experimental conditions [29, 30]. We
60 show that the empirically observed trends in ecological co-selection are reproduced with minimal model assump-
61 tions, and that the recurrence of top-down and bottom-up co-selection is determined by the configuration of the
62 cross-feeding networks in the MicroCRM. Our findings indicate that collective interactions play an important role
63 at dictating community structure during coalescence.

64 Results & Discussion

65 We collected eight natural microbiomes from different soil and plant environmental samples (Figure 1a) and used
66 them to inoculate our synthetic communities, which were stabilized in serial batch-culture biorreactors for 84
67 generations in synthetic minimal media containing either glutamine or citrate as the only supplied carbon source
68 (Figure 1b, Methods: Stabilization of environmental communities in simple synthetic environments). We chose
69 these two carbon sources because they are metabolized through different pathways in bacteria [33, 34], and we
70 hypothesize that communities assembled in either resource will be supported by cross-feeding networks of distinct
71 sets of metabolites [29, 30] thus leading to potentially variable degrees of community cohesiveness and coales-
72 cence outcomes [18, 20, 21, 23]. We isolated the dominant species of every community (Methods: Isolation
73 of dominant species) and identified them by Sanger-sequencing their 16S rRNA gene (Methods: Determination
74 of community composition by 16S sequencing), which correctly matched the dominant Exact Sequence Variant
75 (ESV) [35, 36] found through community-level 16S Illumina sequencing (Figure S1). These dominants remained
76 at high frequency after seven additional transfers with the exception of two of the citrate communities and one
77 of the glutamine communities (where the dominants were presumably a transiently dominating species) that were
78 excluded from further analysis (Figure S1). Similarly, pairs of communities where the dominants shared a same
79 16S sequence and had similar colony morphology were excluded (Figure S1).

80 Top-down ecological co-selection

81 If communities being coalesced were highly cohesive from the top-down, the dominant species would co-select the
82 rarer members of its community during coalescence (Figure 1e, left panels). In this scenario, we would expect the
83 outcome of community coalescence to be predicted by which of the two dominants is most competitive in pairwise
84 competition. Analogously, competition between dominants should be affected only weakly by the presence or ab-
85 sence of the cohorts, that would play a passive role under these conditions. To test this hypothesis, we performed
86 all pairwise competitions between dominant species in glutamine and citrate environments by mixing them 1:1 on
87 their native media and propagating the cultures for seven serial transfers, roughly 42 generations (Methods: Coa-
88 lescence, competition and invasion experiments). We then carried out all possible pairwise community coalescence
89 experiments by mixing equal volumes of the communities and propagating the resulting cultures for seven extra
90 transfers (Figure 1f). The frequencies of all species in both community-community and dominant-dominant com-
91 petitions were determined by 16S Illumina sequencing (Methods: Determination of community composition by
92 16S sequencing).

93 We found that, for communities assembled in the glutamine environment, the relative frequency of a dominant
94 against another in head-to-head pairwise competition is barely predictive of its relative frequency against that
95 same other dominant when the cohorts are present too, i.e. during community coalescence (Figure 2a red dots,
96 $R^2 = 0.04, p > 0.05$). This correlation is significantly higher for the citrate communities (Figure 2a blue dots,
97 $R^2 = 0.83, p < 10^{-8}$). This suggests that, in the glutamine environments, head-to-head competition of dominants
98 is heavily influenced by higher order effects introduced by the rare taxa of the communities. On the other hand, the
99 cohorts seem to play a more passive role in the citrate environments. To test the effects of top-down co-selection
100 at the community level, we quantified the distances between the invasive and coalesced communities using the
101 relative Bray-Curtis similarity (Methods: Metrics of community distance) and compared them to the outcomes of

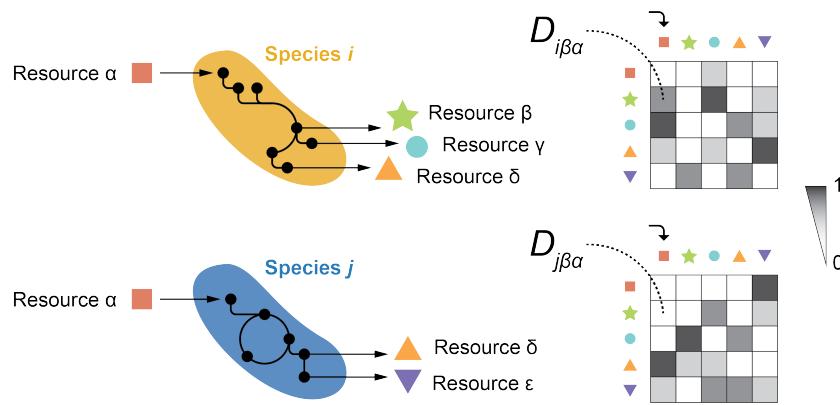
102 the pairwise competitions between dominants alone. We again noticed differences between glutamine and citrate
103 communities: for the former, the pairwise competitive ability of an invasive dominant is only weakly predictive of
104 the performance of the invasive community in coalescence (Figure 2b left panel, $R^2 = 0.15$, $p < 0.05$). For the
105 latter, the structure of the coalesced communities tends to be more strongly dictated by the result of the dominant-
106 dominant competition (Figure 2b middle panel, $R^2 = 0.57$, $p < 10^{-4}$). Alternative quantifications of community
107 distance yield similar results, with weaker effects when the metric used accounts only for the presence/absence
108 of specific species and not for their relative abundance in the communities (Figure S2). All these metrics include
109 the presence of the dominant species themselves. To better disentangle the effect that these dominants have on the
110 other members of their communities, we repeated the analysis this time excluding the dominant species from the
111 compositional data, finding that our results still hold (Figure S3).

112 Together, these observations suggest that the strength of top-down co-selection depends on the environment
113 where communities are assembled and coalescence takes place. Communities stabilized with citrate as the primary
114 supplied resource display a strong degree of top-down cohesiveness, with the fates of the sub-dominant species
115 determined to a large extent by dominant-dominant pairwise competition. This competition is, in turn, only weakly
116 affected by the presence of the cohorts. For glutamine communities, although some level of top-down co-selection
117 is consistent with our data, the cohorts do not appear to just be passively responding to their dominants but rather
118 playing an active role in community coalescence.

119 To investigate the determinants of top-down co-selection and the factors modulating its strength, we ran a
120 set of simulations of community coalescence. We used a Microbial Consumer-Resource Model (MicroCRM)
121 [29, 31] as implemented in the Community Simulator package for Python [32] (Box 1). We chose this modeling
122 framework because communities assembled under our experimental conditions (natural microbiomes re-assembled
123 into multispecies communities through serial growth-dilution cycles in synthetic minimal media with a single
124 carbon source) have been shown to be sustained by dense metabolic cross-feeding networks [29, 30] for which the
125 MicroCRM provides a good description. Based on previous experimental work [30, 38, 39], we allowed different
126 species in our model to secrete different sets of byproducts when metabolizing a same resource (Box 1). To
127 reproduce our experimental protocol *in silico*, we first generated a library of resources and two non-overlapping
128 pools of species. Each pool was used to seed a collection of 100 invasive and 100 resident communities respectively
129 by randomly choosing 50 species and allowing them to stabilize through 20 growth-dilution cycles. We then mixed
130 these stable communities in pairs to simulate our coalescence and dominant-dominant competition experiments
131 (Methods: Simulations). We found that the MicroCRM was able to capture a correlation between the head-to-head
132 pairwise competition of dominants and the outcome of community coalescence (Figure 2b, right panel), further
133 supporting the idea that top-down ecological co-selection can consistently emerge from metabolic interactions
134 across species.

135 **Box 1: A Microbial Consumer-Resource Model for community coalescence**

136 The Microbial Consumer-Resource Model (MicroCRM) [29, 31, 32] is a modeling framework based on the
 137 classic MacArthur's consumer resource model [37]. It encodes the dynamics of a system with S species and
 138 M resources in terms of a consumer preference matrix \mathbf{c} and a metabolic matrix \mathbf{D} , with an additional set of
 139 parameters controlling the species maintenance costs (m_i for species i), the resource energy densities (w_α for
 140 resource α), the energy to growth rate conversion factor (g_i for species i) and the leakage fraction, i.e. the
 141 amount of energy lost as byproducts when a resource is consumed (l_α for resource α). The element c_{ia} of the
 142 consumer preference matrix represents the uptake rate of resource α by species i (although the relationship
 143 between c_{ia} and the uptake rate can be more complex in modeling scenarios that are not considered here, see
 144 [29, 31, 32]). Experimental evidence suggests that individual species can secrete different sets of metabolites
 145 to the environment when growing on a same primary resource [30, 38, 39]. Thus, we define \mathbf{D} as a three-
 146 dimensional matrix where the element $D_{i\beta\alpha}$ represents the energy flux in the form of resource β that is secreted
 147 by species i when it metabolizes resource α . Note that $D_{i\beta\alpha}$ need not be equal to $D_{j\beta\alpha}$ if $i \neq j$ (see illustration
 148 below).



150 The following equations describe the kinetics of the abundances of the i -th species (denoted as N_i) and
 151 the α -th resource (denoted as R_α):

$$\frac{dN_i}{dt} = g_i N_i \left[\sum_\alpha (1 - l_\alpha) w_\alpha c_{i\alpha} R_\alpha - m_i \right] \quad (1)$$

$$\frac{dR_\alpha}{dt} = - \sum_j N_j c_{j\alpha} R_\alpha + \sum_j \sum_\beta N_j c_{j\beta} R_\beta \left[l_\beta D_{j\beta\alpha} \frac{w_\beta}{w_\alpha} \right] \quad (2)$$

152 These equations can take slightly different forms in certain cases, e.g. if the primary resource is supplied con-
 153 tinuously instead of at the beginning of each growth cycle [31, 32], but they are a good approximation for the
 154 community dynamics between consecutive serial dilutions in our setup. Here, we assembled *in silico* com-
 155 munities by randomly sampling a set of species from a pool, then integrating equations 1 and 2, diluting the
 156 final abundances, replenishing the primary resource, and repeating the process until generational equilib-
 157 rium was achieved (Methods: Simulations). Coalescence simulations were carried out following the same logic,
 158 this time seeding the coalesced communities by mixing the invasive and resident ones instead of sampling
 159 from a species pool.

168 **Bottom-up co-selection during community coalescence**

169 Our data indicates that the primary resource supplied to the communities can modulate the effect that the cohorts
170 have in the dominants pairwise competition ([Figure 2a](#)) and the strength of top-down co-selection ([Figure 2b](#),
171 left and middle panels). The fact that our model captures these trends suggests that this might be a result of the
172 metabolic interactions between community members, including the rarer taxa. To investigate the potential role
173 of the cohorts in coalescence, i.e. whether the dominants may be co-selected for or against by them ([Figure 1e](#),
174 right panels), we ran a new set of simulations this time invading resident communities with the dominants alone
175 ([Methods: Simulations](#)). We compared the invasion success of the dominants in isolation with respect to our
176 previous simulations where they invaded accompanied by their cohorts. The invasion success of the dominants
177 was quantified by their relative abundance in the final stabilized communities. Whenever positive bottom-up
178 ecological co-selection is strong, we expect to see dominants reaching higher invasion success with their cohorts
179 than by themselves ([Figure 3b](#), green shaded region). On the other hand, a high degree of bottom-up antagonism
180 would result in dominants invading more effectively alone than in the presence of their cohorts ([Figure 3b](#), red
181 shaded region). Alternatively, if both forms of bottom-up co-selection are weak, we would see a similar invasion
182 success regardless of the presence or absence of the cohort ([Figure 3b](#), gray shaded region).

183 [Figure 3b](#) shows that, in the simulations, many dominants could not invade on their own (or could only do
184 so at very low final relative abundances, below 0.1) but were able to reach high frequencies when they were
185 accompanied by their cohorts. This indicates that positive bottom-up co-selection is frequent and potentially very
186 strong, while negative bottom-up co-selection is far more uncommon. We then asked whether the ability of the
187 pairwise competition of dominants to predict coalescence outcomes was dependent on the strength of bottom-up
188 co-selection. We divided our simulations into two subsets: the first one was comprised of the instances where
189 positive bottom-up co-selection was strong (i.e. dots in the green shaded region of [Figure 3b](#)), the second set
190 included all other cases (dots near the diagonal of [Figure 3b](#)). We reexamined our original simulations and plotted
191 the frequency of the invasive dominant in pairwise competition versus the relative similarity between the invasive
192 and coalesced communities, i.e. the same plot as in [Figure 2b](#), for each subset. We found that when bottom-up
193 positive co-selection is strong, the pairwise competition of dominants is not predictive of coalescence outcomes
194 ([Figure 3c](#), left panel) and vice-versa ([Figure 3c](#), right panel).

195 We then asked whether this trend was also observed *in vitro*. We went back to our laboratory cultures and car-
196 ried out a new round of experiments where we invaded the resident communities with the invasive dominants alone
197 ([Methods: Coalescence, competition and invasion experiments](#)). After stabilization, we quantified species abun-
198 dance through 16S Illumina sequencing ([Methods: Determination of community composition by 16S sequencing](#)).
199 Again, we observed that bottom-up co-selection is far more common in its positive than in its negative form ([Fig-
200 ure 3d](#)). Interestingly, bottom-up recruitment appears to be more frequent in the glutamine environments than in
201 the citrate ones, consistent with our hypothesis that metabolic interactions among species are key in determining
202 the strength and direction of ecological co-selection. We then repeated our analysis in [Figure 3c](#), this time splitting
203 our data according to the observed strength of bottom-up co-selection instead of the primary carbon source as we
204 had done in [Figure 2b](#). Our findings were in line with the model prediction: pairwise competition between domi-
205 nants is only predictive of coalescence outcomes if bottom-up co-selection is weak ([Figure 3e](#), $R^2 = 0.07$, $p > 0.05$
206 when bottom-up co-selection is strong; $R^2 = 0.37$, $p < 10^{-4}$ when bottom-up co-selection is weak).

207 **Understanding the mechanisms of ecological co-selection: a minimal model of community coalescence**

208 To better understand the underlying mechanisms that govern the emergence of ecological co-selection, we devel-
209 oped a minimal model of community coalescence. This model is comprised of two communities with two species
210 each as illustrated in [Figure 4a](#). Within each community, the dominant species (s_1 and s_3 in the resident and inva-
211 sive communities respectively) is able to utilize the primary resource that is supplied externally (R_1). As a result
212 of R_1 metabolism, the dominants secrete a single byproduct off which the sub-dominant species (s_2 and s_4 respec-
213 tively) can feed. Finally, these sub-dominants secrete an additional resource that can again be metabolized by the
214 respective dominants. This is the simplest structure where both top-dwn and bottom-up cohesiveness can emerge,
215 with their relative strengths being determined by how effectively the species can utilize each other's metabolic
216 byproducts. Several parameters can modulate the direction of community cohesiveness, and even in this simple
217 model there can be complex interactions between them. For instance, a sub-dominant that is very efficient at me-
218 tabolizing the dominant secretions will therefore be a strong producer of secondary secretions, in turn increasing
219 the metabolic flow towards the dominant itself.

220 **Community hierarchy regulates the strength of bottom-up co-selection**

221 How do the ideas above scale to more complex and diverse communities?

222 We reason that the choice of species-specific metabolic architectures is necessary to potentially generate cohe-
223 siveness at the community level during coalescence. If the secretions of all species were identical (or only slightly
224 different), higher order cross-feeding effects would be very unspecific: the establishment of new invasive species
225 –given that they could outcompete resident taxa within their metabolic niches, i.e. more effectively feed off the
226 same resource or set of resources as them– would not alter (or only do so moderately) the metabolic flows through
227 the rest of the community’s cross-feeding network. On the other hand, said network could undergo a profound
228 and further-reaching restructuring if the invasive species secreted very different sets of metabolites with respect
229 to the resident ones, potentially disabling existing niches and/or enabling new ones where more invaders could
230 be co-selected. For a similar reason, we argue that the sparsity of the metabolic matrix could also modulate the
231 emergence of cohesiveness in the face of coalescence. A dense metabolic matrix corresponds to a situation where
232 all species secrete a wide variety of byproducts. New-coming invasive species that secrete similar byproducts as
233 resident ones –even if they do so in different relative amounts– might only induce moderate quantitative changes in
234 the metabolic fluxes. But if the sets of secretions are qualitatively different, co-selection of species adapted to each
235 of those sets becomes possible. These ideas are supported by experimental observations suggesting that species
236 with a history of coexistence make up cohesive communities with highly specific cross-feeding configurations
237 [28–30].

238 Conclusions

239 Understanding the mechanisms underlying the responses of microbial communities to invasions is an essential
240 but poorly understood question in microbial ecology [8]. Theory has suggested that communities may exhibit an
241 emergent cohesiveness [9, 15, 20, 21], leading to members of the same community recruiting one another during
242 community-community invasions. Our results provide direct experimental evidence of ecological co-selection in
243 a large number of community coalescence experiments, and highlight the critical role played by the rarer, sub-
244 dominant species in the generation of community cohesiveness.

245 Our data suggests that the strength and direction of ecological co-selection is modulated by the underlying
246 metabolic networks that shape the structure of communities assembled in synthetic minimal conditions [29, 30].
247 This network is in turn regulated by the supplied primary carbon source in our minimal laboratory conditions.
248 This idea is supported by the observation that a Microbial Consumer-Resource Model captures the trends observed
249 experimentally when we enable a large variation in the metabolic fluxes across species. The model also predicts a
250 trade-off between the strength of bottom-up co-selection and the ability of dominant-dominant pairwise competi-
251 tion to dictate coalescence outcomes, which we have confirmed experimentally. These observations, together with
252 previous results in different systems [24] as well as theoretical predictions [9, 19–23], suggest that collective inter-
253 actions between microbes and the environment should be generically expected to produce ecological co-selection
254 during community coalescence.

255 Additional work will be necessary to further clarify the relationship between metabolic feedbacks, community
256 cohesiveness and ecological co-selection. The experimental system that we introduced in this work can be eas-
257 ily expanded so that large numbers of community coalescence experiments can be carried out in parallel. It thus
258 represents a promising tool to explore the properties of microbial community coalescence in high throughput and
259 test quantitative theories about its role in microbiome assembly. On the other hand, coalescence of communities
260 under different settings (e.g. in spatially structured environments) might be governed by additional factors. Un-
261 derstanding them and quantifying their relative contributions in natural communities remains an open question in
262 ecology.

263 **Methods**

264 **Stabilization of environmental communities in simple synthetic environments**

265 Communities were stabilized *ex situ* as described in [29]. In short, environmental samples (soil, leaves...) within
266 one meter radius in eight different geographical locations were collected with sterile tweezers or spatulas into 50mL
267 sterile tubes ([Figure 1a](#)). One gram of each sample was allowed to sit at room temperature in 10mL of phosphate
268 buffered saline (1×PBS) containing 200µg/mL cycloheximide to suppress eukaryotic growth. After 48h, samples
269 were mixed 1:1 with 80% glycerol and kept frozen at -80°C. Starting microbial communities were prepared by
270 scrapping the frozen stocks into 200µL of 1×PBS and adding a volume of 4µL to 500µL of synthetic minimal
271 media (1×M9) supplemented with 200µg/mL cycloheximide and 0.07 C-mol/L glutamine or sodium citrate as
272 the carbon source in 96 deep-well plates (1.2mL; VWR). Cultures were then incubated still at 30°C to allow
273 for re-growth. After 48h, samples were fully homogenized and biomass increase was followed by measuring the
274 optical density (620nm) of 100µL of the cultures in a Multiskan FC plate reader (Thermo Scientific). Communities
275 were stabilized [29] by passaging 4µL of the cultures into 500µL of fresh media (1×M9 with the carbon source)
276 every 48h for a total of 12 transfers at a dilution factor of 1:100, roughly equivalent to 80 generations per culture
277 ([Figure 1b](#)). Cycloheximide was not added to the media after the first two transfers.

278 **Isolation of dominant species**

279 For each community, the most abundant colony morphotype at the end of the ninth transfer was selected ([Figure 1c](#)),
280 resuspended in 100µL 1×PBS and serially diluted (1:10). Next, 20µL of the cells diluted to 10⁻⁶ were plated in the
281 corresponding synthetic minimal media and allowed to regrow at 30°C for 48h. Dominants were then identified,
282 inoculated into 500µL of fresh media and incubated still at 30°C for 48h. After this period, the communities
283 stabilized for eleven transfers and the isolated dominants were ready for the competition experiments at the onset
284 of the twelfth transfer.

285 **Coalescence, competition and invasion experiments**

286 All possible pairwise dominant-dominant and community-community competition experiments were performed
287 by mixing equal volumes (4µL) of each of the eight communities or eight dominants at the onset of the twelfth
288 transfer. Competitions were set up in their native media, i.e. in 500µL of 1×M9 supplemented with 0.07 C-mol/L
289 of either glutamine or citrate in 96 deep-well plates. Plates were incubated at 30°C for 48h. Pairwise competitions
290 were further propagated for seven serial transfers (roughly 42 generations, [Figure 1f](#)) by transferring 8µL of each
291 culture to fresh media (500µL).

292 **Determination of community composition by 16S sequencing**

293 The sequencing protocol was identical to that described in [29]. Community samples were collected by spinning
294 down at 3500rpm for 25min in a bench-top centrifuge at room temperature; cell pellets were stored at -80°C
295 before processing. To maximize Gram-positive bacteria cell wall lysis, the cell pellets were re-suspended and
296 incubated at 37°C for 30min in enzymatic lysis buffer (20mM Tris-HCl, 2mM sodium EDTA, 1.2% Triton X-100)
297 and 20mg/mL of lysozyme from chicken egg white (Sigma-Aldrich). After cell lysis, the DNA extraction and
298 purification was performed using the DNeasy 96 protocol for animal tissues (Qiagen). The clean DNA in 100µL
299 elution buffer of 10mM Tris-HCl, 0.5mM EDTA at pH 9.0 was quantified using Quan-iT PicoGreen dsDNA Assay
300 Kit (Molecular Probes, Inc.) and normalized to 5ng/µL in nuclease-free water (Qiagen) for subsequent 16S rRNA
301 Illumina sequencing. 16S rRNA amplicon library preparation was performed following a dual-index paired-end
302 approach [40]. Briefly, PCR amplicon libraries of V4 regions of the 16S rRNA were prepared sing dual-index
303 primers (F515/R805), then pooled and sequenced using the Illumina MiSeq chemistry and platform. Each sample
304 went through a 30-cycle PCR in duplicate of 20µL reaction volumes using 5ng of DNA each, dual index primers,
305 and AccuPrime Pfx SuperMix (Invitrogen). The thermocycling procedure includes a 2min initial denaturation step
306 at 95°C, and 30 cycles of the following PCR scheme: (a) 20-second denaturation at 95°C, (b) 15-second annealing
307 at 55°C, and (c) 5-minute extension at 72°C. The duplicate PCR products of each sample were pooled, purified,
308 and normalized using SequalPrep PCR cleanup and normalization kit (Invitrogen). Barcoded amplicon libraries
309 were then pooled and sequenced using Illumina Miseq v2 reagent kit, which generated 2×250bp paired-end reads
310 at the Yale Center for Genome Analysis (YCGA). The sequencing reads were demultiplexed on QIIME 1.9.0 [41].
311 The barcodes, indexes, and primers were removed from raw reads, producing FASTQ files with both the forward
312 and reverse reads for each sample, ready for DADA2 analysis [36]. DADA2 version 1.1.6 was used to infer unique
313 biological exact sequence variants (ESVs) for each sample and naïve Bayes was used to assign taxonomy using
314 the SILVA version 123 database [42, 43].

315 **Metrics of community distance**

316 Beta-diversity indexes between the invasive and coalesced communities or the resident and coalesced communities
 317 were computed using various similarity metrics. For two arbitrary communities with ESV abundances represented
 318 by the vectors $\mathbf{x} = (x_1, x_2, \dots, x_S)$ and $\mathbf{y} = (y_1, y_2, \dots, y_S)$ (where x_i and y_i represent the relative abundance of the
 319 i th ESV in each community respectively and S is the total number of ESVs), the Bray-Curtis similarity $BC(\mathbf{x}, \mathbf{y})$
 320 is calculated as [44]

$$BC(\mathbf{x}, \mathbf{y}) = \sum_i \min(x_i, y_i) \quad (3)$$

321 The Jensen-Shannon similarity $JS(\mathbf{x}, \mathbf{y})$ is defined as one minus the Jensen-Shannon distance (which is, in turn,
 322 the square root of the Jensen-Shannon divergence [45])

$$JS(\mathbf{x}, \mathbf{y}) = 1 - \sqrt{\frac{1}{2}KL(\mathbf{x}, \mathbf{m}) + \frac{1}{2}KL(\mathbf{y}, \mathbf{m})} \quad (4)$$

323 where $\mathbf{m} = (\mathbf{x} + \mathbf{y}) / 2$ and KL denotes the Kullback-Leibler divergence [46]

$$KL(\mathbf{x}, \mathbf{y}) = \sum_i x_i \log_2 \left(\frac{x_i}{y_i} \right) \quad (5)$$

324 Using base-two logarithms ensures that the metric is bounded between 0 and 1. The Jaccard similarity is given by
 325 $J(\mathbf{x}, \mathbf{y})$ [47]

$$J(\mathbf{x}, \mathbf{y}) = \frac{|\mathbf{x} \cap \mathbf{y}|}{|\mathbf{x} \cup \mathbf{y}|} \quad (6)$$

326 Additionally, we quantified coalescence outcomes by examining the fraction of the endemic cohort of the original
 327 communities that persists in the coalesced one. We call $E(\mathbf{x}, \mathbf{y})$ to the fraction of endemic species of \mathbf{x} that are also
 328 found in \mathbf{y} .

329 For all the metrics above, we quantified the relative similarity between the invasive and the coalesced communi-
 330 ties using relative metrics (denoted as Q):

$$Q(\mathbf{x}_I, \mathbf{x}_R, \mathbf{x}_C) = \frac{F(\mathbf{x}_I, \mathbf{x}_C)}{F(\mathbf{x}_I, \mathbf{x}_C) + F(\mathbf{x}_R, \mathbf{x}_C)} \quad (7)$$

331 where the subindices I, R and C correspond to the invasive, resident and coalesced communities respectively,
 332 and F represents one of BC (Bray-Curtis similarity), JS (Jensen-Shannon similarity), J (Jaccard similarity) or E
 333 (endemic survival) defined above.

334 **Simulations**

335 We used the Community Simulator package [32] and included new features for our simulations. In the package,
 336 species are characterized by their resource uptake rates (c_{ia} for species i and resource α), and they all share a
 337 common metabolic matrix \mathbf{D} . The element $D_{\alpha\beta}$ of this matrix represents the fraction of energy in the form of
 338 resource α secreted when resource β is consumed. Here we implemented a new operation mode in which species
 339 can secrete different metabolites (and/or in different abundances) when consuming a same resource. We call $D_{i\alpha\beta}$ to
 340 the fraction of energy in the form of resource α secreted by species i when consuming resource β . In the Community
 341 Simulator underlying Microbial Consumer-Resource Model, this means that the energy flux $J_{i\beta}^{\text{out}}$ [29, 31] now takes
 342 the form

$$J_{i\beta}^{\text{out}} = \sum_{\alpha} D_{i\beta\alpha} l_{\alpha} J_{i\alpha}^{\text{in}} \quad (8)$$

343 The documentation for the Community Simulator contains detailed descriptions of the model formulation, param-
 344 eters and package use. For the updated package with the new functionality, see [Data & code availability](#).

345 For our simulations, we first generated a library of 2400 species divided into three specialist families of 800
 346 members each and a generalist family of 240 members. We split this library into two non-overlapping pools of
 347 1320 species each. We randomly sampled 50 species from each pool in equal ratios to seed 100 resident and 100
 348 invasive communities respectively. We then let grow and diluted the communities serially, replenishing the primary
 349 resource after each dilution. We repeated the process 20 times to ensure generational equilibrium was achieved
 350 [29]. We then performed the *in silico* experiments by using the generationally stable communities to seed 100
 351 coalesced communities that were again stabilized as described previously. Similarly, we identified the dominant

352 (most abundant) species of every resident and invasive community to carry out pairwise competition and single
353 invasion simulations.

354 Most other parameters were set to the defaults of the original Community Simulator package, with the only
355 exception of the maintenance costs (m) which are set to zero for all species (equivalent to assuming cell death is
356 negligible through the duration of our growth cycles) and the sparsity of the metabolic matrices (s) which is set to
357 0.9 to generate significant variability in the secretion fluxes across different species (see main text).

358 **Data & code availability**

359 Experimental data and code for the analysis, as well as code for the simulations and the updated Community
360 Simulator package with instructions for enabling the new features are in github.com/jdiazc9/coalescence.

361 **Acknowledgements**

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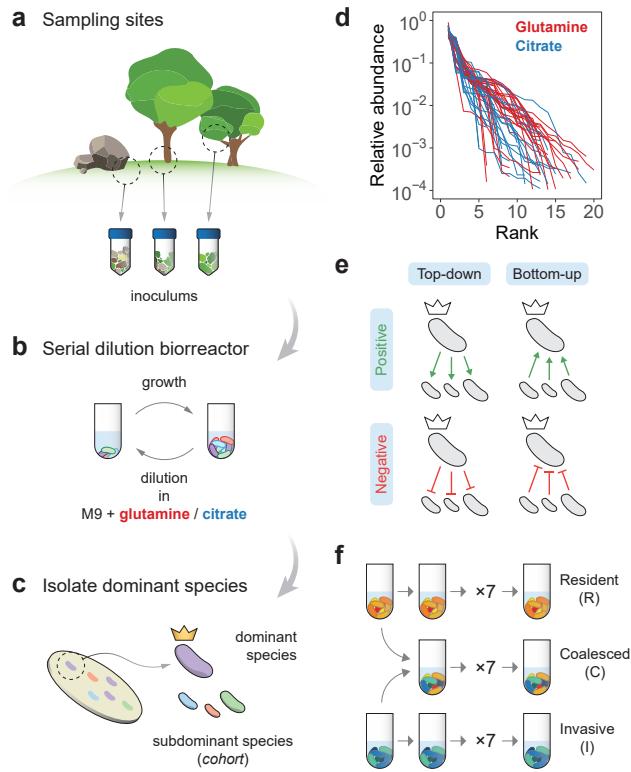
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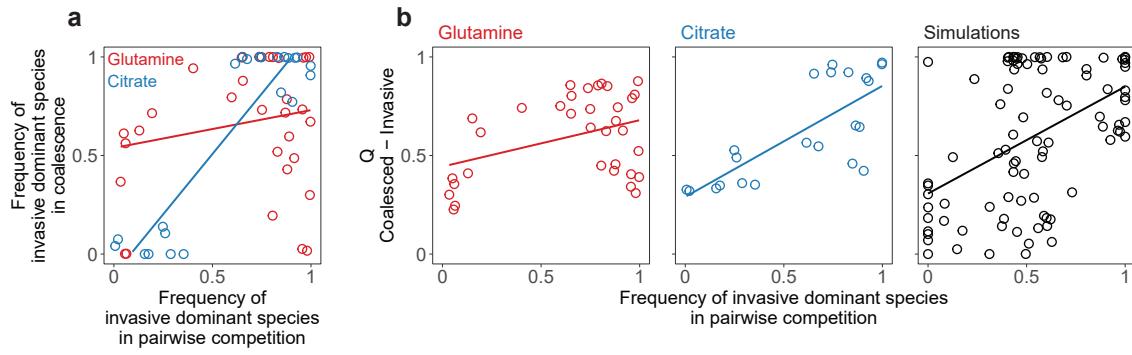
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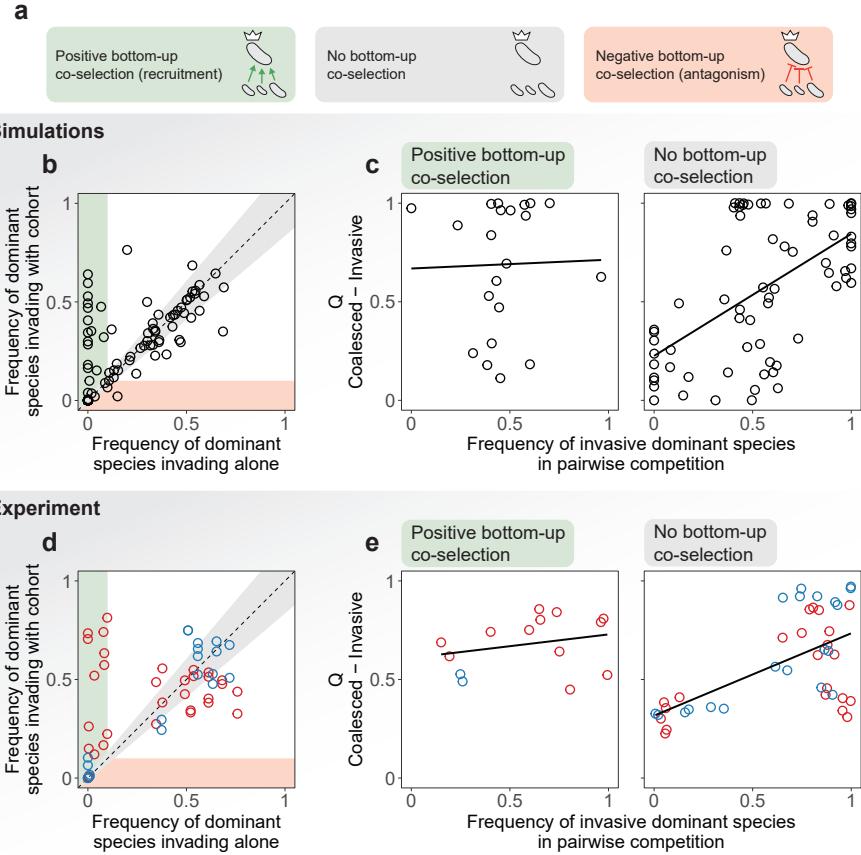


471 **Figure 1. Overview of the experimental protocol.** **a.** Environmental samples collected from eight different locations were
 472 used to inoculate our communities. **b.** Communities were stabilized in serial batch culture bioreactors [29] in minimal synthetic
 473 media with glutamine or citrate as the only supplied carbon source. **c.** Communities were plated in minimal media agar plates
 474 and the most abundant species (the “dominants”) from each community were isolated. We refer to the set of sub-dominant
 475 species as the “cohorts”. **d.** Rank-frequency distributions of all eight communities stabilized in either glutamine (red) or citrate
 476 (blue), sequenced at a depth of 10^{-4} reads. Three biological replicates per community are shown. Community compositions are
 477 skewed and long-tailed. **e.** Our hypothesis is that ecological co-selection can take place from the top-down, i.e. the dominant
 478 co-selecting the cohort, or from the bottom-up, i.e. the cohort co-selecting the dominant. Both forms of co-selection can be
 479 positive (recruitment) or negative (antagonism). **f.** Illustration of the protocol of our coalescence experiments. All pairs of
 480 communities were inoculated into fresh minimal media supplemented with the same carbon source where communities had
 481 been previously stabilized. The coalesced (C) and original resident (R) and invasive (I) communities were then serially diluted
 482 and allowed to grow for seven additional transfers.

484

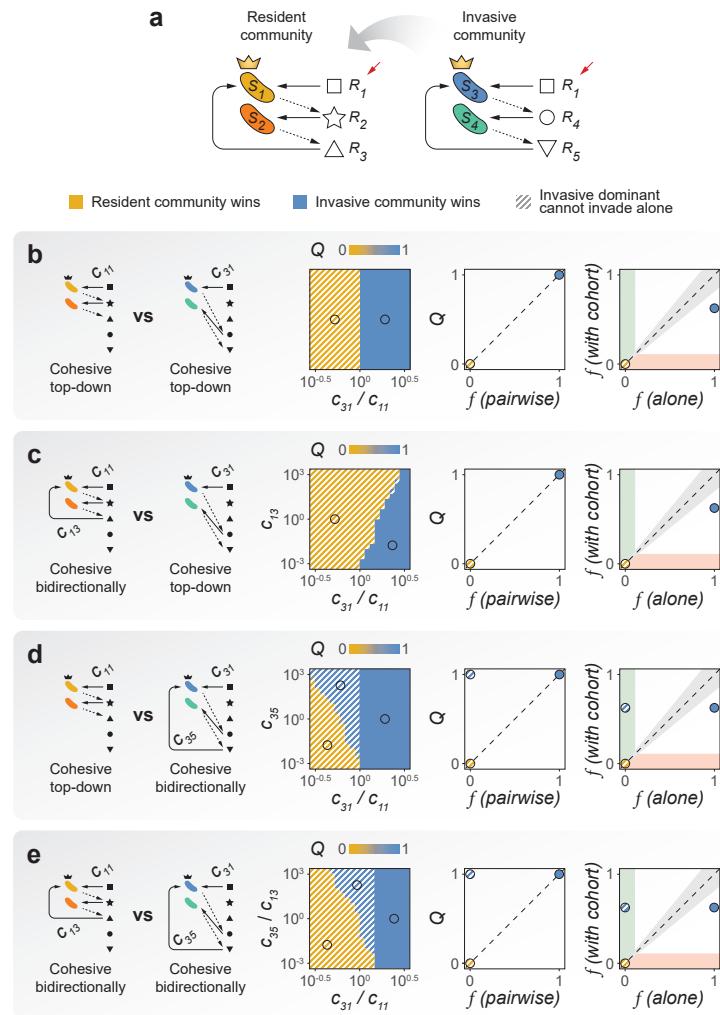


485 **Figure 2. Top-down co-selection in microbial community coalescence.** **a.** Pairwise competition of dominants with or
 486 without their cohorts. In the horizontal axis, we plot the frequency of the invasive dominant species in head-to-head pairwise
 487 competition with the resident dominant. In the vertical axis, we plot the same relative frequency when the two species compete
 488 in the presence of their cohorts, i.e. during community coalescence. $R^2 = 0.04$, $p > 0.05$ for glutamine (red) and $R^2 = 0.83$,
 489 $p < 10^{-8}$ for citrate (blue). **b.** Coalescence outcomes are quantified by the relative Bray-Curtis similarity (Q) between the
 490 coalesced and invasive communities. These outcomes are predicted by the pairwise competition between the invasive and
 491 resident dominant species. Left panel (red): glutamine communities, $R^2 = 0.15$, $p < 0.05$. Middle panel (blue): citrate
 492 communities, $R^2 = 0.57$, $p < 10^{-4}$. A high correlation is consistent with a scenario of strong top-down positive co-selection
 493 where dominants recruit their cohorts for the final coalesced community. Two biological replicates per experiment are plotted
 494 individually. Right panel (black): simulations with a Microbial Consumer-Resource Model are able to capture these trends
 495 ($R^2 = 0.22$, $p < 10^{-5}$).



497

Figure 3. Trade offs between bottom-up and top-down ecological co-selection. **a.** We hypothesize that three scenarios are possible regarding bottom-up co-selection: sub-dominant species could co-select for (green) or against (red) their dominant in coalescence, or they could have no effect in the invasion success of the dominant taxa (gray). **b.** Simulations with a Microbial Consumer-Resource Model suggest that positive bottom-up co-selection is common and can be very strong, whereas negative bottom-up co-selection is rare. We plot the frequency reached by the invasive dominants when invading the resident communities in isolation versus the same frequency when invading together with their cohorts, i.e. in community coalescence. Points in the green/red area represent instances where the invasive dominant is able to invade with higher/lower success when accompanied by its cohort, evidencing positive/negative bottom-up co-selection. Points around the diagonal (gray area) correspond to cases where the success of the invasive dominant is only weakly affected by the presence or absence of its cohort. **c.** We divided the data from our simulations into two sets according to whether positive or no bottom-up co-selection was observed (that is, whether points fell into the green or gray areas of panel b). Here we reproduce the plots in Figure 2b for each set, representing the result of the dominant head-to-head pairwise competition versus the outcome of community coalescence. Left panel: when positive bottom-up co-selection is strong, head-to-head pairwise competition of dominants is poorly predictive of coalescence outcomes ($R^2 = 0.00, p > 0.05$). Right panel: on the other hand, when bottom-up co-selection is weak coalescence outcomes are more strongly dictated by the result of the dominant-dominant competition ($R^2 = 0.34, p < 10^{-6}$). **d.** Experiments show that in our conditions, positive bottom-up co-selection is indeed more frequent and strong than negative bottom-up co-selection. **e.** We reproduce the plots in panel c for our experimental data. This is equivalent to recreating Figure 2b, this time splitting our data by the strength of bottom-up co-selection instead of by the carbon source provided to the communities. Like simulations predicted, instances of community coalescence displaying strong positive bottom-up co-selection yield a poor correspondence between dominant pairwise competition and coalescence outcomes (left panel, $R^2 = 0.07, p > 0.05$) and vice-versa (right panel, $R^2 = 0.37, p < 10^{-4}$).



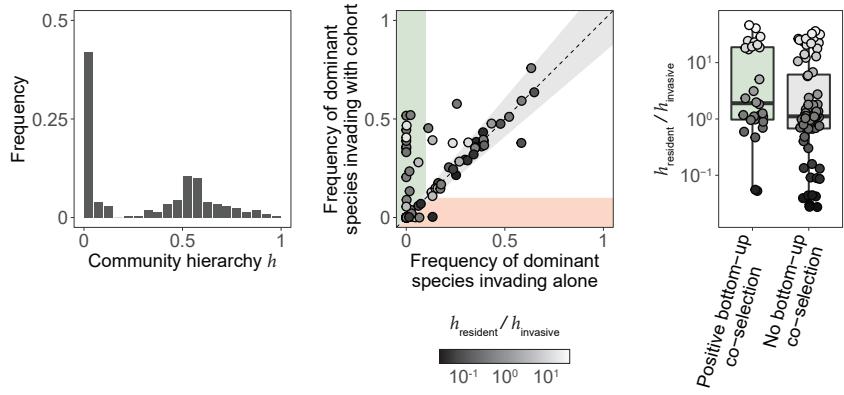
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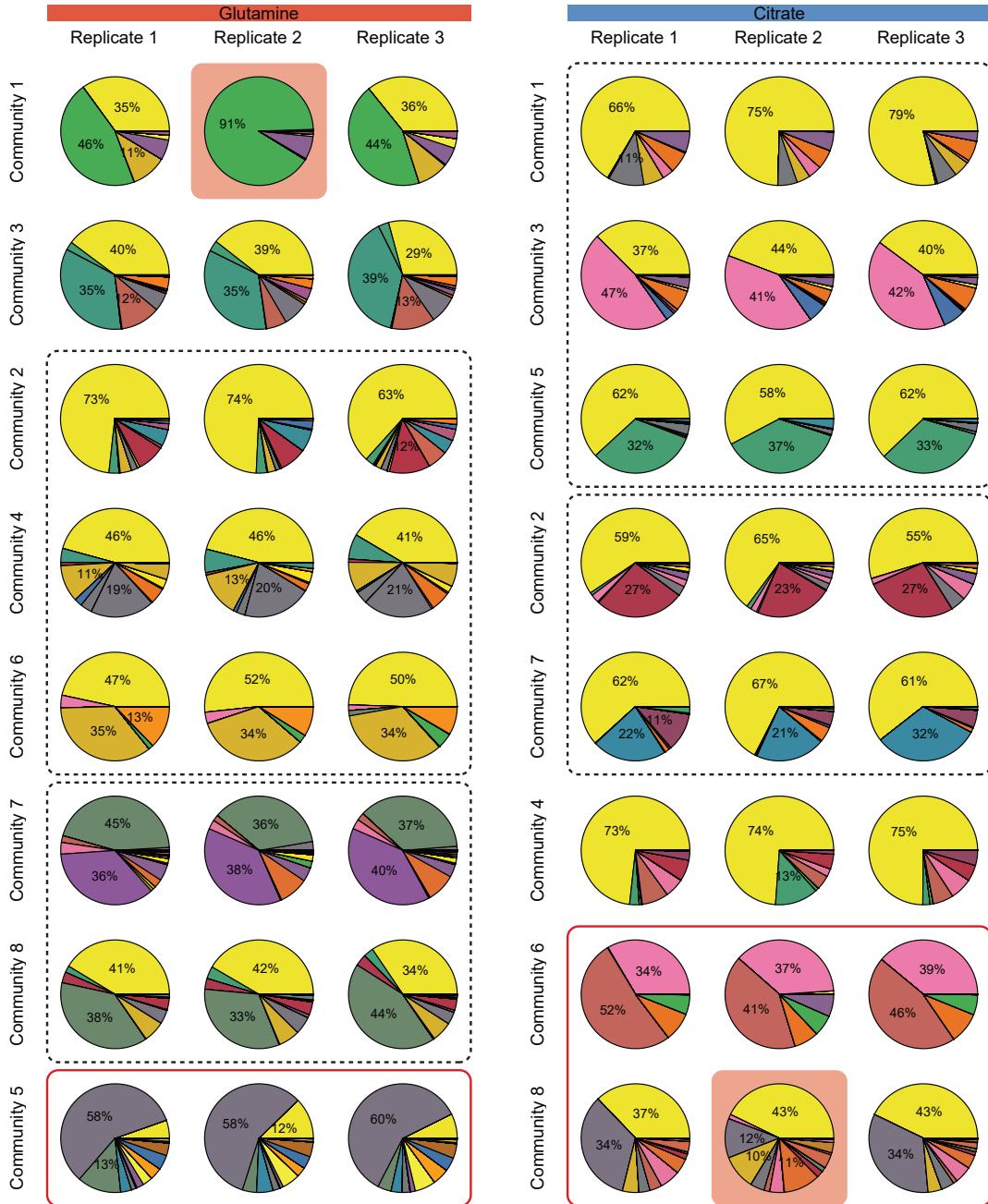
Figure 4. A minimal model of community coalescence. a. Blablabla... **b.** Blablabla...

523

524 **Figure 5. Community hierarchies determine coalescence outcomes.** **a.** Blablabla... **b.** Blablabla... **c.** Blablabla... ($p =$
525 0.083).

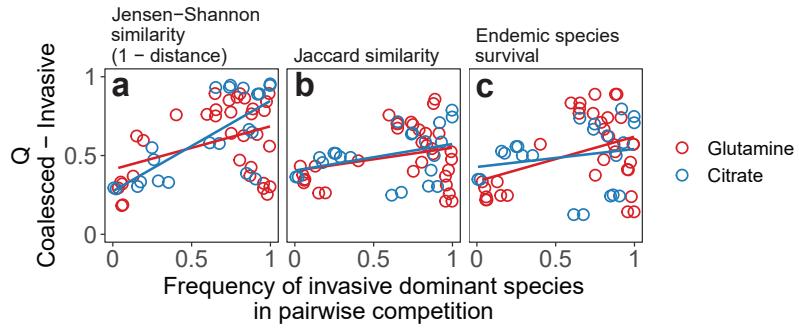


527 **Supplementary Figures**



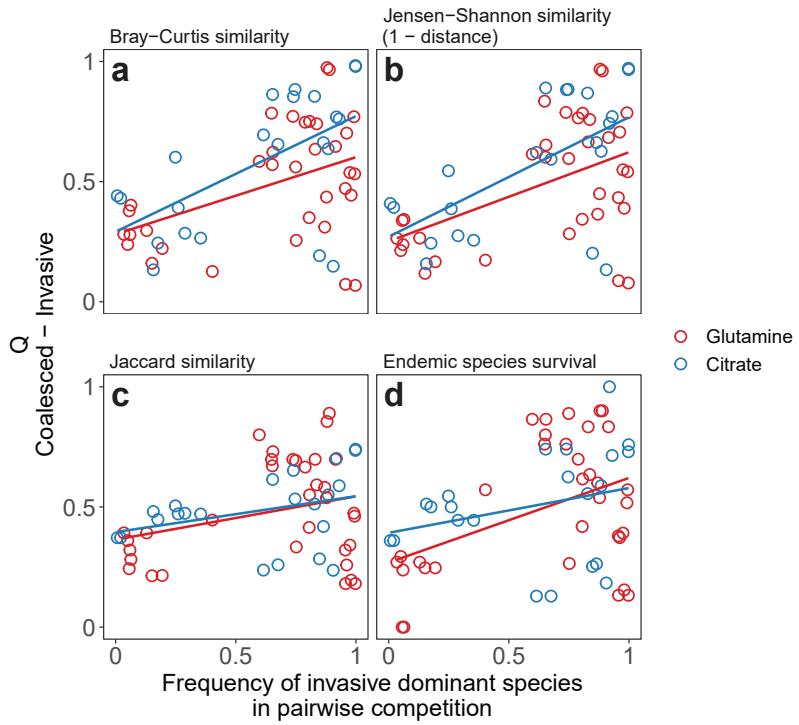
528

529 **Figure S1. Community compositions after seven additional transfers without coalescence.** Each color of the pie plots
 530 corresponds to a different exact sequence variant ([Methods: Determination of community composition by 16S sequencing](#)).
 531 Replicate 2 of community 1 from glutamine, as well as replicate 2 of community 8 from citrate (highlighted)
 532 were removed based on their dissimilarity to the other two replicates (details in code for data analysis, see [Data & code availability](#)). Communities
 533 clustered in dashed boxes shared the same dominant species as revealed by sequencing data. For communities enclosed
 534 in red boxes, sequencing data showed that the species isolated by plating was not detectable in the community after seven
 535 additional transfers (i.e. the dominant was incorrectly identified) and were therefore excluded from downstream analyses.
 536



537

538 **Figure S2. Alternative metrics of community distance.** Quantifying coalescence outcomes using different metrics of commu-
 539 nity similarity (Methods: Metrics of community distance) gives similar results to those shown in Figure 2a. Metrics that account
 540 for the relative species abundances (Bray-Curtis or Jensen-Shannon similarities) yield higher correlations than less quantitative
 541 metrics that only account for species presence/absence (Jaccard similarity or the fraction of endemic invasive species persisting
 542 in the coalesced community). **a.** Relative Jensen-Shannon similarity ($R^2 = 0.15$, $p < 0.05$ for glutamine and $R^2 = 0.53$,
 543 $p < 5 \times 10^{-4}$ for citrate) **b.** Relative Jaccard similarity ($R^2 = 0.08$, $p > 0.05$ for glutamine and $R^2 = 0.13$, $p > 0.05$ for citrate)
 544 **c.** Relative survival of invasive endemic species after coalescence ($R^2 = 0.16$, $p < 0.05$ for glutamine and $R^2 = 0.04$, $p > 0.05$
 545 for citrate).



547

548 **Figure S3. Dominant species have limited effects on coalescence outcomes quantification.** We repeated the analyses shown
 549 in [Figure 2a](#) and [Figure S2](#), but this time we removed the dominants from the compositional data prior to quantifying community
 550 distances. The trends observed before are maintained. **a.** Relative Bray-Curtis similarity ($R^2 = 0.20$, $p < 0.01$ for glutamine
 551 and $R^2 = 0.34$, $p < 0.005$ for citrate) **b.** Relative Jensen-Shannon similarity ($R^2 = 0.24$, $p < 0.005$ for glutamine and $R^2 = 0.36$,
 552 $p < 0.005$ for citrate) **c.** Relative Jaccard similarity ($R^2 = 0.09$, $p > 0.05$ for glutamine and $R^2 = 0.11$, $p > 0.05$ for citrate) **d.**
 553 Relative survival of invasive endemic species after coalescence ($R^2 = 0.18$, $p < 0.05$ for glutamine and $R^2 = 0.08$, $p > 0.05$ for
 554 citrate).