

# 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

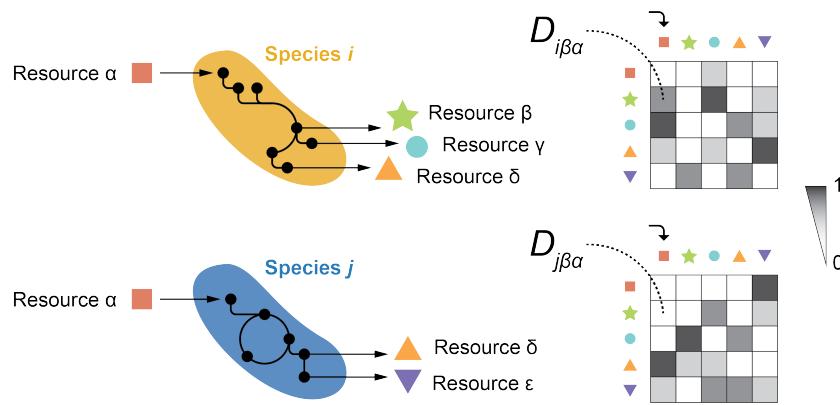
102 the pairwise competitions between dominants alone. We again noticed differences between glutamine and citrate  
103 communities: for the former, the pairwise competitive ability of an invasive dominant is only weakly predictive of  
104 the performance of the invasive community in coalescence ([Figure 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 ( $w_\alpha$  for  
 140 resource  $\alpha$ ), 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 ( $l_\alpha$  for resource  $\alpha$ ). The element  $c_{ia}$  of the  
 142 consumer preference matrix represents the uptake rate of resource  $\alpha$  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  $\beta$  that is secreted  
 147 by species  $i$  when it metabolizes resource  $\alpha$ . 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  $\alpha$ -th resource (denoted as  $R_\alpha$ ):

$$152 \frac{dN_i}{dt} = g_i N_i \left[ \sum_\alpha (1 - l_\alpha) w_\alpha c_{i\alpha} R_\alpha - m_i \right] \quad (1)$$

$$153 \frac{dR_\alpha}{dt} = - \sum_j N_j c_{j\alpha} R_\alpha + \sum_j \sum_\beta N_j c_{j\beta} R_\beta \left[ l_\beta D_{j\beta\alpha} \frac{w_\beta}{w_\alpha} \right] \quad (2)$$

154 These equations can take slightly different forms in certain cases, e.g. if the primary resource is supplied con-  
 155 tinuously instead of at the beginning of each growth cycle [31, 32], but they are a good approximation for the  
 156 community dynamics between consecutive serial dilutions in our setup. Here, we assembled *in silico* com-  
 157 munities by randomly sampling a set of species from a pool, then integrating equations 1 and 2, diluting the  
 158 final abundances, replenishing the primary resource, and repeating the process until generational equilib-  
 159 rium was achieved (Methods: Simulations). Coalescence simulations were carried out following the same logic,  
 160 this time seeding the coalesced communities by mixing the invasive and resident ones instead of sampling  
 161 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 of  
212  $R_1$  metabolism, the dominants secrete a single byproduct ( $R_2$  and  $R_4$  respectively) off which the sub-dominants ( $s_2$   
213 and  $s_4$  respectively) can feed. Finally, these sub-dominants secrete an additional resource ( $R_3$  and  $R_5$  respectively)  
214 that can again be metabolized by the respective dominants. This is the simplest structure where both top-down and  
215 bottom-up cohesiveness can emerge, with their relative strengths being determined by how effectively the species  
216 can utilize each other's metabolic byproducts. Several parameters can modulate the strength and direction of com-  
217 munity cohesiveness, and even in this simple model there can be complex interactions between them. For instance,  
218 a sub-dominant that is very efficient at metabolizing the dominant's secretions will therefore be a strong producer  
219 of secondary byproducts, in turn increasing the metabolic flow towards the dominant itself. To keep both com-  
220 munities symmetrical and maintain tractability of the model's behavior, we chose to make the two sub-dominant  
221 species equally able consumers of the byproduct of their respective dominants ([Methods: Minimal model](#)). We  
222 also made it so species secrete only one type of byproduct each, i.e. the metabolic matrix is binary in this case (see  
223 [Box 1](#)). The secretions of the resident and invasive communities are non-overlapping: this ensures that coalescence

224 always results in one of the two communities taking over and completely excluding the other one, which facilitates  
225 the interpretation of the model's outcomes.

226 With these constraints, the model is specified by four rates: the resident dominant's uptake rate of the primary  
227 resource ( $c_{11}$ ) and of the secondary byproduct secreted by  $s_2$  ( $c_{13}$ ), as well as the invasive dominant's uptake  
228 rate of the primary resource ( $c_{31}$ ) and of the secondary byproduct secreted by  $s_4$  ( $c_{35}$ ). Analogous to what we  
229 did for our initial experiments and simulations of community coalescence, we first stabilized each community  
230 separately through serial growth-dilution cycles, and then mixed them 1:1 and stabilized again until generational  
231 equilibrium was achieved. Equations 1 and 2 in Box 1 describe the dynamics of this system between consecutive  
232 dilutions with the corresponding choices for the parameter values (Methods: Minimal model). We ran simulations  
233 for both community-community competitions and invasions from the invasive dominant alone. We considered  
234 four different scenarios illustrated in Figure 4b-e: 1) both communities are cohesive from the top-down but not  
235 from the bottom-up, 2) the resident community is cohesive both from the top-down and from the bottom-up while  
236 the resident community is cohesive from the top-down only, 3) the resident community is cohesive from the top-  
237 down only while the invasive community is cohesive both from the top-down and from the bottom-up, and 4) both  
238 communities are cohesive from the top-down and from the bottom-up. The outcomes in each case are discussed  
239 below.

240 **Top-down resident versus top-down invasive.** This is the simplest scenario. When both communities are co-  
241hesive strictly from the top-down ( $c_{13} = 0$  and  $c_{35} = 0$ ), coalescence outcomes are determined by which dominant  
242 is a better consumer of the primary resource. The most competitive dominant excludes the least competitive one  
243 ( $s_1$  excludes  $s_3$  if  $c_{11} > c_{31}$  and vice-versa, Figure 4b) and, because the cross-feeding networks of the invasive  
244 and resident communities are non-overlapping, it co-selects its sub-dominant exclusively. The relative similarity  
245 between the invasive and the coalesced communities ( $Q$ , see Methods: Metrics of community distance) can only  
246 be 0 (if the resident dominant "wins" competition for  $R_1$ ) or 1 (if the opposite happens). The invasion success of  
247 the invasive dominant ( $s_3$ ) is unaffected by the presence or absence of its sub-dominant ( $s_4$ ) since it is not being  
248 cross-fed by it. Thus, again  $s_3$  is only able to invade alone when it outcompetes the resident dominant ( $s_1$ ) for the  
249 primary resource ( $c_{11} > c_{31}$ ).

250 **Bidirectional resident versus top-down invasive.** Blablabla...

251 **Top-down resident versus bidirectional invasive.** Blablabla...

252 **Bidirectional resident versus bidirectional invasive.** Blablabla...

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

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

255 We reason that the choice of species-specific metabolic architectures is necessary to potentially generate cohe-  
256 siveness at the community level during coalescence. If the secretions of all species were identical (or only slightly  
257 different), higher order cross-feeding effects would be very unspecific: the establishment of new invasive species  
258 –given that they could outcompete resident taxa within their metabolic niches, i.e. more effectively feed off the  
259 same resource or set of resources as them– would not alter (or only do so moderately) the metabolic flows through  
260 the rest of the community's cross-feeding network. On the other hand, said network could undergo a profound  
261 and further-reaching restructuring if the invasive species secreted very different sets of metabolites with respect  
262 to the resident ones, potentially disabling existing niches and/or enabling new ones where more invaders could  
263 be co-selected. For a similar reason, we argue that the sparsity of the metabolic matrix could also modulate the  
264 emergence of cohesiveness in the face of coalescence. A dense metabolic matrix corresponds to a situation where  
265 all species secrete a wide variety of byproducts. New-coming invasive species that secrete similar byproducts as  
266 resident ones –even if they do so in different relative amounts– might only induce moderate quantitative changes in  
267 the metabolic fluxes. But if the sets of secretions are qualitatively different, co-selection of species adapted to each  
268 of those sets becomes possible. These ideas are supported by experimental observations suggesting that species  
269 with a history of coexistence make up cohesive communities with highly specific cross-feeding configurations  
270 [28–30].

### 271 **Conclusions**

272 Understanding the mechanisms underlying the responses of microbial communities to invasions is an essential  
273 but poorly understood question in microbial ecology [8]. Theory has suggested that communities may exhibit an

274 emergent cohesiveness [9, 15, 20, 21], leading to members of the same community recruiting one another during  
275 community-community invasions. Our results provide direct experimental evidence of ecological co-selection in  
276 a large number of community coalescence experiments, and highlight the critical role played by the rarer, sub-  
277 dominant species in the generation of community cohesiveness.

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

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

296 **Methods**

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

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

311 **Isolation of dominant species**

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

318 **Coalescence, competition and invasion experiments**

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

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

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

348 **Metrics of community distance**

349 Beta-diversity indexes between the invasive and coalesced communities or the resident and coalesced communities  
 350 were computed using various similarity metrics. For two arbitrary communities with ESV abundances represented  
 351 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  
 352  $i$ th ESV in each community respectively and  $S$  is the total number of ESVs), the Bray-Curtis similarity  $BC(\mathbf{x}, \mathbf{y})$   
 353 is calculated as [44]

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

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

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

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

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

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

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

359 Additionally, we quantified coalescence outcomes by examining the fraction of the endemic cohort of the original  
 360 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  
 361 found in  $\mathbf{y}$ .

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

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

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

367 **Simulations**

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

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

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

378 For our simulations, we first generated a library of 2400 species divided into three specialist families of 800  
 379 members each and a generalist family of 240 members. We split this library into two non-overlapping pools of  
 380 1320 species each. We randomly sampled 50 species from each pool in equal ratios to seed 100 resident and 100  
 381 invasive communities respectively. We then let grow and diluted the communities serially, replenishing the primary  
 382 resource after each dilution. We repeated the process 20 times to ensure generational equilibrium was achieved  
 383 [29]. We then performed the *in silico* experiments by using the generationally stable communities to seed 100  
 384 coalesced communities that were again stabilized as described previously. Similarly, we identified the dominant

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

387 Most other parameters were set to the defaults of the original Community Simulator package, with the only  
388 exception of the maintenance costs ( $m$ ) which are set to zero for all species (equivalent to assuming cell death is  
389 negligible through the duration of our growth cycles) and the sparsity of the metabolic matrices ( $s$ ) which is set to  
390 0.9 to generate significant variability in the secretion fluxes across different species (see main text).

391 **Minimal model**

392 **Data & code availability**

393 Experimental data and code for the analysis, as well as code for the simulations and the updated Community  
394 Simulator package with instructions for enabling the new features are in [github.com/jdiazc9/coalescence](https://github.com/jdiazc9/coalescence).

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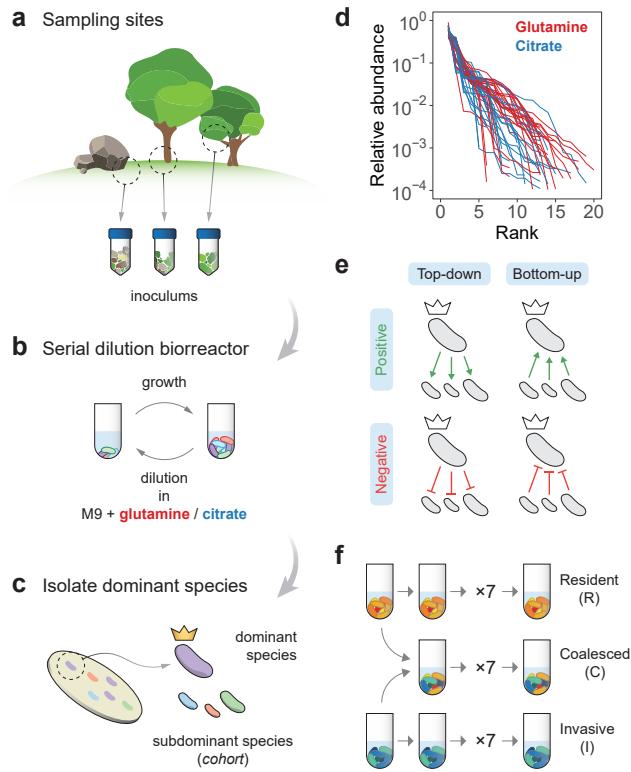
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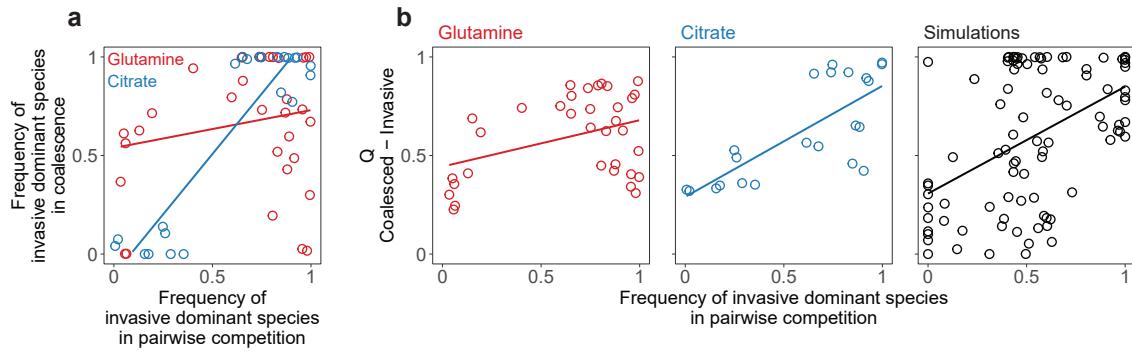
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503 **Figures**

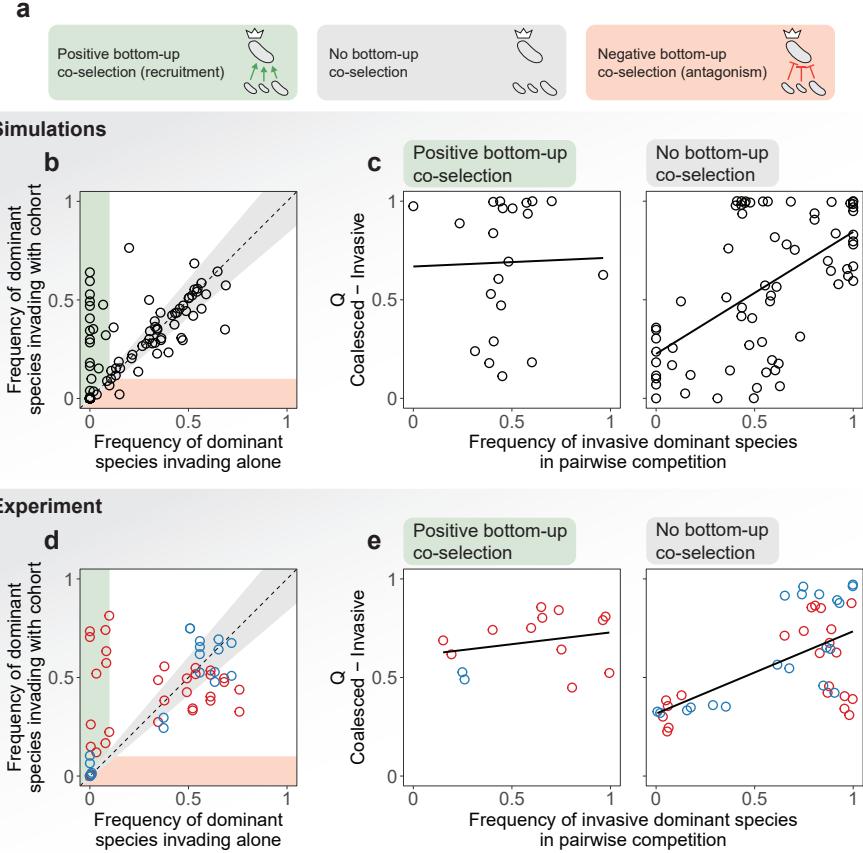
504



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

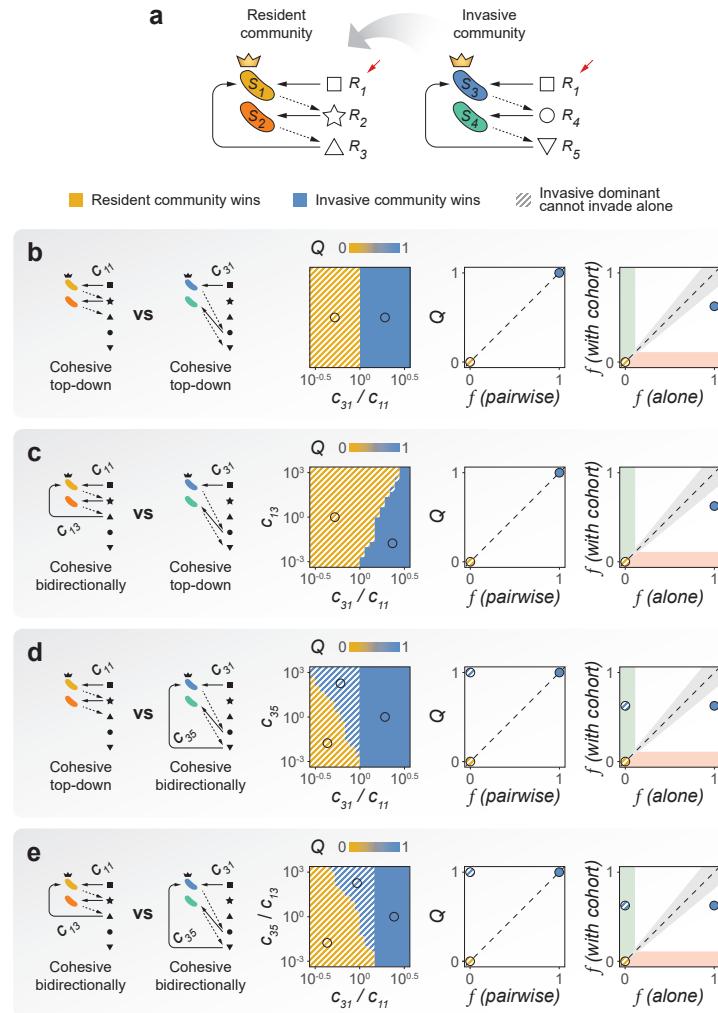


519 **Figure 2. Top-down co-selection in microbial community coalescence.** **a.** Pairwise competition of dominants with or  
 520 without their cohorts. In the horizontal axis, we plot the frequency of the invasive dominant species in head-to-head pairwise  
 521 competition with the resident dominant.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



531

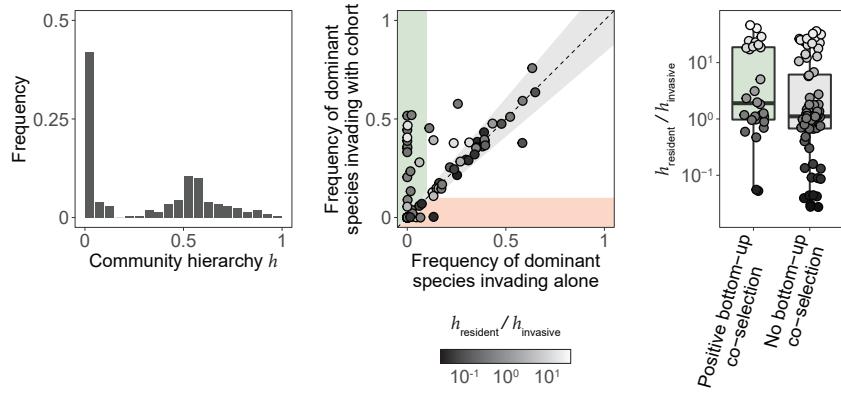
532 **Figure 3. Trade offs between bottom-up and top-down ecological co-selection.** **a.** We hypothesize that three scenarios are  
 533 possible regarding bottom-up co-selection: sub-dominant species could co-select for (green) or against (red) their dominant in  
 534 coalescence, or they could have no effect in the invasion success of the dominant taxa (gray). **b.** Simulations with a Microbial  
 535 Consumer-Resource Model: we plot the frequency reached by the invasive dominants when invading the resident communities  
 536 in isolation versus the same frequency when invading together with their cohorts, i.e. in community coalescence. Points in the  
 537 green/red area represent instances where the invasive dominant is able to invade with higher/lower success when accompanied  
 538 by its cohort, evidencing positive/negative bottom-up co-selection. Points around the diagonal (gray area) correspond to cases  
 539 where the success of the invasive dominant is only weakly affected by the presence or absence of its cohort. **c.** We divided  
 540 the data from our simulations into two sets according to whether positive or no bottom-up co-selection was observed (that is,  
 541 whether points fell into the green or gray areas of panel b). Here we reproduce the plots in Figure 2b for each set, representing  
 542 the result of the dominant head-to-head pairwise competition versus the outcome of community coalescence. Left panel: strong  
 543 positive bottom-up co-selection ( $R^2 = 0.00, p > 0.05$ ). Right panel: no bottom-up co-selection ( $R^2 = 0.34, p < 10^{-6}$ ). **d.** Experiments show that in our conditions, positive bottom-up co-selection is indeed more frequent and strong than negative  
 544 bottom-up co-selection. **e.** We reproduce the plots in panel c for our experimental data, i.e. we recreate Figure 2b but this  
 545 time splitting our data by the strength of bottom-up co-selection instead of by the carbon source supplied to the communities.  
 546 Left panel: strong positive bottom-up co-selection ( $R^2 = 0.07, p > 0.05$ ). Right panel: no bottom-up co-selection ( $R^2 = 0.37,$   
 547  $p < 10^{-4}$ ).



550

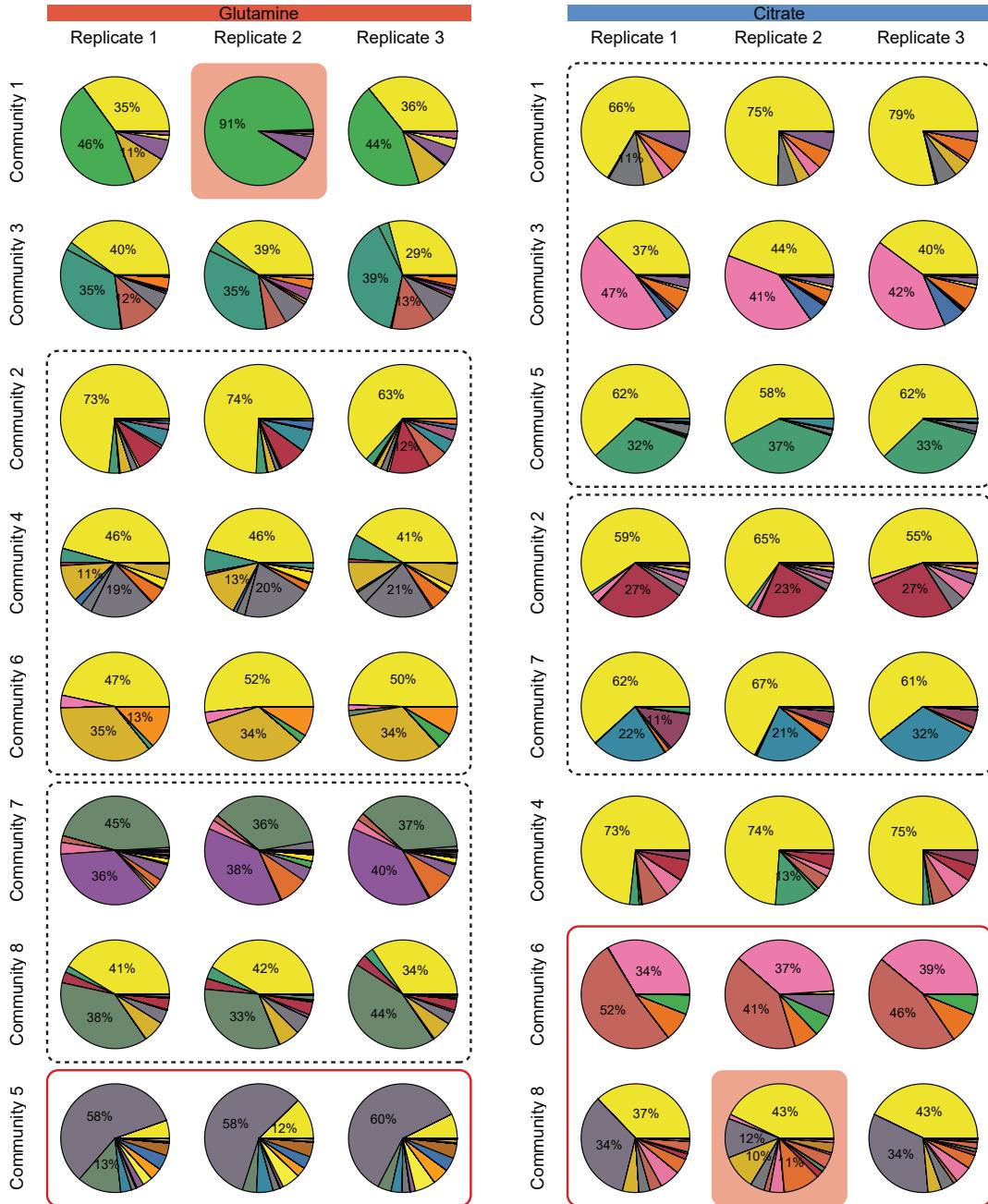
551 **Figure 4. A minimal model of community coalescence.** **a.** Illustration of the model structure and parameters. The primary  
 552 resource ( $R_1$ ) is replenished after each growth-dilution cycle (red arrows). Solid arrows indicate resource consumption,  
 553 dashed arrows represent resource secretion. **b-e.** Coalescence outcomes in the minimal model under different relations of cohesiveness  
 554 between the resident and the invasive communities.

556



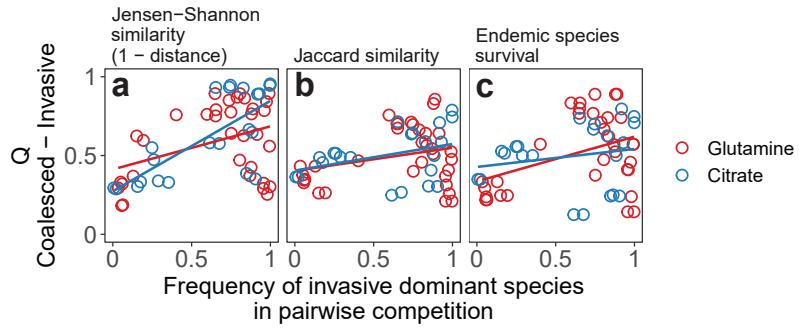
557 **Figure 5. Community hierarchies determine coalescence outcomes. a.** Blablabla... **b.** Blablabla... **c.** Blablabla... ( $p =$   
558 0.083).

560 **Supplementary Figures**



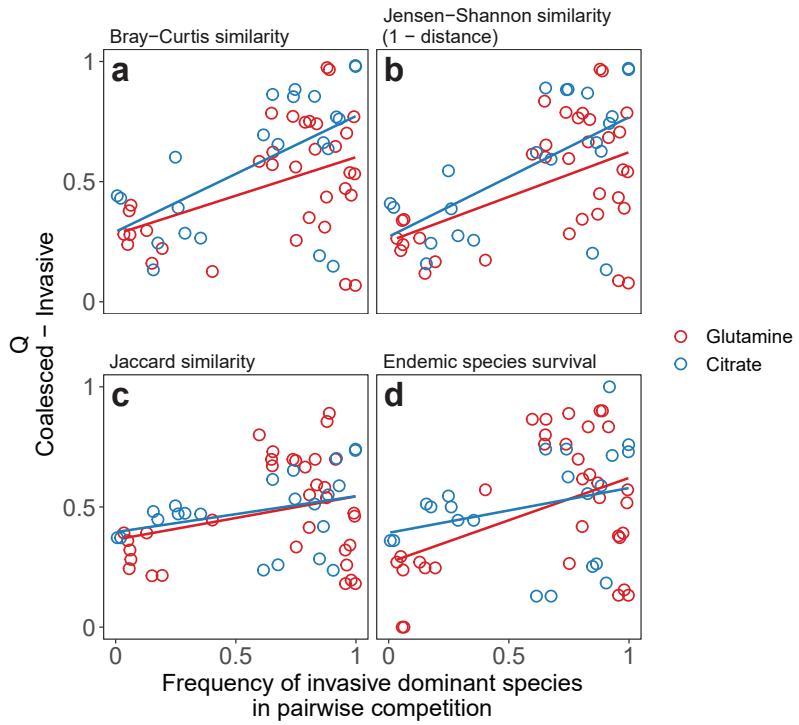
561

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



570

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



580

581 **Figure S3. Dominant species have limited effects on coalescence outcomes quantification.** We repeated the analyses shown  
 582 in [Figure 2a](#) and [Figure S2](#), but this time we removed the dominants from the compositional data prior to quantifying community  
 583 distances. The trends observed before are maintained. **a.** Relative Bray-Curtis similarity ( $R^2 = 0.20, p < 0.01$  for glutamine  
 584 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  $R^2 = 0.36,$   
 585  $p < 0.005$  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.**  
 586 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  
 587 citrate).