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

Our results indicate that co-selection varies in direction and strength depending on the supplied limiting resource. This primary resource, in turn, has been shown to shape the structure and interactions of the communities [30]. We observe that, when top-down co-selection is weak, bottom-up co-selection can be very strong, with positive co-selection being far more common than negative co-selection. We then turn to a microbial consumer-resource model (microCRM) [29, 31, 32] that is able to capture the dynamics of microbial communities dominated by metabolic interactions, as is the case for the ones assembled in our experimental conditions [29, 30]. We show that the empirically observed trends in ecological co-selection are reproduced with minimal model assumptions, and that tuning the complexity of the metabolic interactions in our *in silico* communities can modulate the recurrence of top-down or bottom-up co-selection. Our findings indicate that collective interactions play an important role at dictating community structure during coalescence.

Results & Discussion

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

Top-down ecological co-selection

If communities being coalesced were highly cohesive from the top-down, the dominant species would co-select the rarer members of its community during coalescence (Figure 1e, left panels). In this scenario, we would expect the outcome of community coalescence to be predicted by which of the two dominants is most competitive in pairwise competition. To test this hypothesis, we performed all pairwise competitions between dominant species in glutamine and citrate environments by mixing them 1:1 on their native media and propagating the cultures for seven serial transfers, roughly 42 generations (Methods: Coalescence, competition and invasion experiments). We then performed all possible pairwise community coalescence experiments by mixing equal volumes of the communities and propagating the resulting cultures for seven extra transfers (Figure 1f), and we quantified coalescence outcomes using the similarity between the coalesced and the invasive communities (Methods: Metrics of community distance). The frequencies of all species in both community-community and dominant-dominant competitions were determined by 16S Illumina sequencing (Methods: Determination of community composition by 16S sequencing).

We found that, for communities assembled in the glutamine environment, the pairwise competitive ability of an invasive dominant is only weakly predictive of the performance of the invasive community in coalescence (as quantified by the relative Bray-Curtis similarity between the coalesced and invasive communities), but this correlation was stronger for the citrate communities (Figure 2a, $R^2 = 0.15$, $p < 0.05$ for

glutamine and $R^2 = 0.57$, $p < 10^{-4}$ for citrate). Alternative quantifications of community distance yielded similar results, with weaker effects when the metric used accounted only for the presence/absence of specific species and not for their relative abundance in the communities (Figure S2). All these metrics include the presence of the dominant species themselves. To better disentangle the effect that these dominants have on the other members of their communities, we repeated the analysis this time excluding the dominant species from the compositional data, finding that our results still hold (Figure S3). Furthermore, we observed that, in the glutamine communities, the relative frequency of a dominant against another in head-to-head pairwise competition is barely predictive of its relative frequency against that same other dominant when the cohorts are present too, i.e. after coalescence (Figure S4, $R^2 = 0.04$, $p > 0.05$). On the other hand, pairwise competition between dominants of the citrate communities is highly correlated with the relative abundance of said dominants in community coalescence (Figure S4, $R^2 = 0.83$, $p < 10^{-8}$).

Together, these results suggest that the strength of top-down co-selection depends on the primary resource supplied to the coalesced communities. Communities assembled with citrate as the primary supplied resource display a strong degree of top-down co-selection, with the fates of the sub-dominant species determined to a large extent by dominant-dominant pairwise competition. This competition is, in turn, only weakly affected by the presence of the cohorts. For glutamine communities, although some level of top-down co-selection is consistent with our data, the cohorts do not appear to be passively responding to their dominants. Instead, evidence suggests that the cohorts might be playing an active role in community coalescence. This finding led us to investigate the potential role of bottom-up ecological co-selection (Figure 1e, right panels), i.e. whether in some cases the dominants may be co-selected for or against by their cohorts.

Bottom-up co-selection during community coalescence

To study the effect of bottom-up co-selection, we compared the invasion success of the dominant from one of the communities when invading the other community in isolation versus when doing so accompanied by its cohort. In situations where positive bottom-up ecological co-selection is strong, we expect to see dominants invading more effectively with their cohorts than by themselves (Figure 2b, green shaded region). On the other hand, a high degree of negative bottom-up co-selection would result in dominants reaching higher invasion success alone than in the presence of their cohorts (Figure 2b, red shaded region). Alternatively, if both forms of bottom-up co-selection were weak, we would see a similar invasion success of the dominant regardless of whether it is invading with its cohort (Figure 2b, gray shaded region).

We performed a new round of invasion experiments where each dominant invaded every other community in isolation (Methods: Coalescence, competition and invasion experiments). The dominant-invaded communities were then propagated for seven serial transfers, and the final community compositions were determined by 16S Illumina sequencing. We compared the relative abundances reached by the dominants in these experiments with the relative abundances of the same dominants in community coalescence (i.e. when they invade with their cohorts). Figure 2b shows that, for communities assembled in citrate environments, the cohorts had little effect and the dominants were able to invade with comparable success regardless of being accompanied by them. This was expected since we had seen that coalescence between citrate communities tended to be determined by top-down co-selection. However, glutamine communities displayed a different behavior. A large number of dominants (roughly 50%) that were not able to invade by themselves (or could only do so at very low final relative abundances, below 0.1) were able to reach much higher frequencies when they invaded together with their cohorts, which indicates that positive bottom-up co-selection is common in our experiments. In contrast, negative co-selection appears to be rare and no instances of it were observed in our experiments.

Simulations of community coalescence with a consumer-resource model

We ran a set of simulations of community coalescence to better understand the mechanisms of ecological co-selection in our experiments. We used a microbial consumer-resource model (microCRM) [29, 31] implemented in the Community Simulator package for Python [32]. We chose this modeling framework because communities assembled under our experimental conditions (natural microbiomes re-assembled into multispecies communities through serial growth-dilution cycles in synthetic minimal media with a single carbon source) have been shown to be sustained by dense metabolic cross-feeding networks [29], for which the microCRM provides a good description. We implemented a new feature in the package that makes

individual species able to secrete different sets of byproducts when consuming a same resource (Methods: Simulations). We also used sparse metabolic matrices, equivalent to assuming that species secrete only a few types of byproducts when consuming a resource. These assumptions are supported by the experimental observation that individual species can indeed secrete different sets of few metabolites to the environment when consuming a primary resource [37, 38]. We reason that these choices are necessary to potentially generate community cohesiveness: if the secretions of all species were roughly similar, the effect of interchanging members of a community would be minimal. But in practice, species with a history of coexistence make up cohesive communities with highly specific cross-feeding structures [29, 30]. In short, we replicated our experimental protocol *in silico* by generating a library of species that we divided into two pools. Each pool was used to seed a collection of invasive and resident communities, that were then allowed to stabilize through 20 growth-dilution cycles. Stable communities were then used to reproduce our coalescence and dominant-dominant competition experiments, as well as the invasion experiments from the dominants alone. A full description of our modeling framework can be found in the Methods: Simulations section.

We carried out two sets of simulations under two different regimes. In one case, we generated a library of 30 resources divided into 3 resource classes (Methods: Simulations). This corresponds to a situation where the digestion of a resource can yield a wide variety of secreted byproducts. We refer to this scenario as the *complex resource* regime. Our second set of simulations was performed under a *simple resource* regime, where we generated a library of only 15 resources belonging to a same class. For the simple resource, we found that coalescence outcomes were highly correlated with the competitive ability of the community dominants in pairwise competition (Figure 2c), indicating a large degree of top-down positive co-selection. These dominants were able to reach similar fractions in the final communities when invading alone with respect to when invading with their cohorts (Figure 2d), suggesting that bottom-up co-selection is weak in this scenario. On the other hand, simulations under the complex resource regime evidenced weak top-down co-selection but common and strong bottom-up recruitment (Figure 2e-f), with negative co-selection remaining rare. Simulations suggest that community cohesiveness is dependent on the metabolic interactions among species, which is in line with previous findings [29, 30]. These interactions are determined by the metabolic profile of the primary resource in the conditions of our simulations and, in turn, modulate the strength and direction of ecological co-selection.

Conclusion

Understanding the mechanisms underlying the responses of microbial communities to invasions is an essential but poorly understood question in microbial ecology [8]. Theory has suggested that communities may exhibit an emergent cohesiveness [9, 15, 20, 21], leading to members of the same community recruiting one another during community-community invasions. Our results provide direct experimental evidence of ecological co-selection in a large number of community coalescence experiments, and highlight the critical role played by the rarer, sub-dominant species in the generation of community cohesiveness. Our data suggests that the strength and direction of ecological co-selection is modulated by the supplied primary carbon source, which in turn shapes the cross-feeding network that dictates the structure of our communities [29, 30]. These observations, together with previous experimental results in different systems [24] as well as theoretical predictions [9, 19–23], suggest that collective interactions arising from metabolic feedbacks between microbes and the environment should be generically expected to produce ecological co-selection during community coalescence.

Further work is required to clarify the relationship between metabolic interactions, community structure, cohesiveness and co-selection. The experimental system that we introduced in this work can be easily expanded so that large numbers of community coalescence experiments can be carried out in parallel. It thus represents a promising tool to explore the properties of microbial community coalescence in high throughput and test quantitative theories about its role in microbiome assembly.

Methods

Stabilization of environmental communities in simple synthetic environments

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

Isolation of dominant species

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

Coalescence, competition and invasion experiments

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

Determination of community composition by 16S sequencing

The sequencing protocol was identical to that described in [29]. Community samples were collected by spinning down at 3500rpm for 25min in a bench-top centrifuge at room temperature; cell pellets were stored at −80°C before processing. To maximize Gram-positive bacteria cell wall lysis, the cell pellets were re-suspended and incubated at 37°C for 30min in enzymatic lysis buffer (20mM Tris-HCl, 2mM sodium EDTA, 1.2% Triton X-100) and 20mg/mL of lysozyme from chicken egg white (Sigma-Aldrich). After cell lysis, the DNA extraction and purification was performed using the DNeasy 96 protocol for animal tissues (Qiagen). The clean DNA in 100µL elution buffer of 10mM Tris-HCl, 0.5mM EDTA at pH 9.0 was quantified using Quan-iT PicoGreen dsDNA Assay Kit (Molecular Probes, Inc.) and normalized to 5ng/µL in nuclease-free water (Qiagen) for subsequent 16S rRNA Illumina sequencing. 16S rRNA amplicon library preparation was performed following a dual-index paired-end approach [39]. Briefly, PCR amplicon libraries of V4 regions of the 16S rRNA were prepared sing dual-index primers (F515/R805), then pooled and sequenced using the Illumina MiSeq chemistry and platform. Each sample went through a 30-cycle PCR in duplicate of 20µL reaction volumes using 5ng of DNA each, dual index primers, and AccuPrime Pfx SuperMix (Invitrogen). The thermocycling procedure includes a 2min initial denaturation step at 95°C, and 30 cycles of the following PCR scheme: (a) 20-second denaturation at 95°C, (b) 15-second annealing at 55°C, and (c) 5-minute extension at 72°C. The duplicate PCR products of each sample were pooled, purified, and normalized using SequalPrep PCR cleanup and normalization kit (Invitrogen). Barcoded amplicon libraries were then pooled and sequenced using Illumina Miseq v2 reagent kit, which

generated 2×250bp paired-end reads at the Yale Center for Genome Analysis (YCGA). The sequencing reads were demultiplexed on QIIME 1.9.0 [40]. The barcodes, indexes, and primers were removed from raw reads, producing FASTQ files with both the forward and reverse reads for each sample, ready for DADA2 analysis [36]. DADA2 version 1.1.6 was used to infer unique biological exact sequence variants (ESVs) for each sample and naïve Bayes was used to assign taxonomy using the SILVA version 123 database [41, 42].

Metrics of community distance

Beta-diversity indexes between the invasive and coalesced communities or the resident and coalesced communities were computed using various similarity metrics. For two arbitrary communities with ESV abundances represented by the vectors $\mathbf{x} = (x_1, x_2, \cdots, x_N)$ and $\mathbf{y} = (y_1, y_2, \cdots, y_N)$ (where x_i and y_i represent the relative abundance of the ith ESV in each community respectively and N is the total number of ESVs), the Bray-Curtis similarity $BC(\mathbf{x}, \mathbf{y})$ is calculated as [43]

$$BC(\mathbf{x}, \mathbf{y}) = \sum_i \min(x_i, y_i) \tag{1}$$

The Jensen-Shannon similarity $JS(\mathbf{x}, \mathbf{y})$ is defined as one minus the Jensen-Shannon distance (which is, in turn, the square root of the Jensen-Shannon divergence [44])

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

where $\mathbf{m} = (\mathbf{x} + \mathbf{y})/2$ and KL denotes the Kullback-Leibler divergence [45]

$$KL(\mathbf{x}, \mathbf{y}) = \sum_i x_i \log_2\left(\frac{x_i}{y_i}\right) \tag{3}$$

The Jaccard similarity is given by $J(\mathbf{x}, \mathbf{y})$ [46]

$$J(\mathbf{x}, \mathbf{y}) = \frac{|\,\mathbf{x} \cap \mathbf{y}\,|}{|\,\mathbf{x} \cup \mathbf{y}\,|} \tag{4}$$

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

For all the metrics above, we quantify the relative similarity between the invasive and the coalesced communities using relative metrics (Q):

$$Q(\mathbf{x}_\mathrm{I}, \mathbf{x}_\mathrm{R}, \mathbf{x}_\mathrm{C}) = \frac{F(\mathbf{x}_\mathrm{I}, \mathbf{x}_\mathrm{C})}{F(\mathbf{x}_\mathrm{I}, \mathbf{x}_\mathrm{C}) + F(\mathbf{x}_\mathrm{R}, \mathbf{x}_\mathrm{C})} \tag{5}$$

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

Simulations

We used the Community Simulator package [32] and included new features for our simulations. In the package, species are characterized by their resource uptake rates ($c_{i\alpha}$ for species i and resource α), and they all share a common metabolic matrix $\mathbf{D}$. The element $D_{\alpha\beta}$ of this matrix represents the fraction of energy in the form of resource α secreted when resource β is consumed. Here we implemented a new operation mode in which species can secrete different metabolites (and/or in different abundances) when consuming a same resource. Experimental observations support the idea of distinct species producing different sets of byproducts when feeding off the same primary resource [37, 38]. We call $D_{i\alpha\beta}$ to the fraction of energy in the form of resource α secreted *by species* i when consuming resource β —note that now $D_{i\alpha\beta}$ need not be equal to $D_{j\alpha\beta}$ if $i \neq j$, unlike in the original Community Simulator. In the package's underlying Microbial Consumer Resource Model [29, 31], this just means that the energy flux $J^{\mathrm{out}}_{i\beta}$ now takes the form

$$J^{\mathrm{out}}_{i\beta} = \sum_\alpha D_{i\beta\alpha} l_\alpha J^{\mathrm{in}}_{i\alpha} \tag{6}$$

The documentation for the Community Simulator contains detailed descriptions of the model, parameters and package use. For the updated package with the new functionality, see Data & code availability.

For our simulations, we first generated a library of 2400 species divided into three specialist families of 800 members each and a generalist family of 240 members. We split this library into two non-overlapping pools of 1320 species each. We randomly sample 50 species from each pool in equal ratios to seed 100 resident and 100 invasive communities respectively. We then let grow and diluted the communities serially, replenishing the primary resource after each dilution. We repeated the process 20 times to ensure generational equilibrium is achieved [29]. We then performed the *in silico* experiments by using the generationally stable communities to seed 100 coalesced communities that we again stabilize as described previously. Similarly, we identified the dominant (most abundant) species of every resident and invasive community to carry out pairwise competition and single invasion simulations.

Two sets of simulations were carried out, corresponding to a *simple* or *complex* externally replenished resource respectively (see details in main text). For the *simple* resource scenario, we generated a library of 15 resources belonging to a same class. For the *complex* resource scenario, the library contained 30 resources subdivided into 3 classes (each being preferentially consumed by one of the three species families). Our aim with this approach was to examine the implications of allowing for different degrees of density in the metabolic networks sustaining our communities. Most other parameters were set to the defaults of the original Community Simulator package, with the only exception of the maintenance costs (m) which are set to zero for all species (as cell death is negligible through the duration of our growth cycles) and the sparsity of the metabolic matrices (s) which is set to 0.9 to generate significant variability in the secretion fluxes across different species (see main text).

Data & code availability

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

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Figures

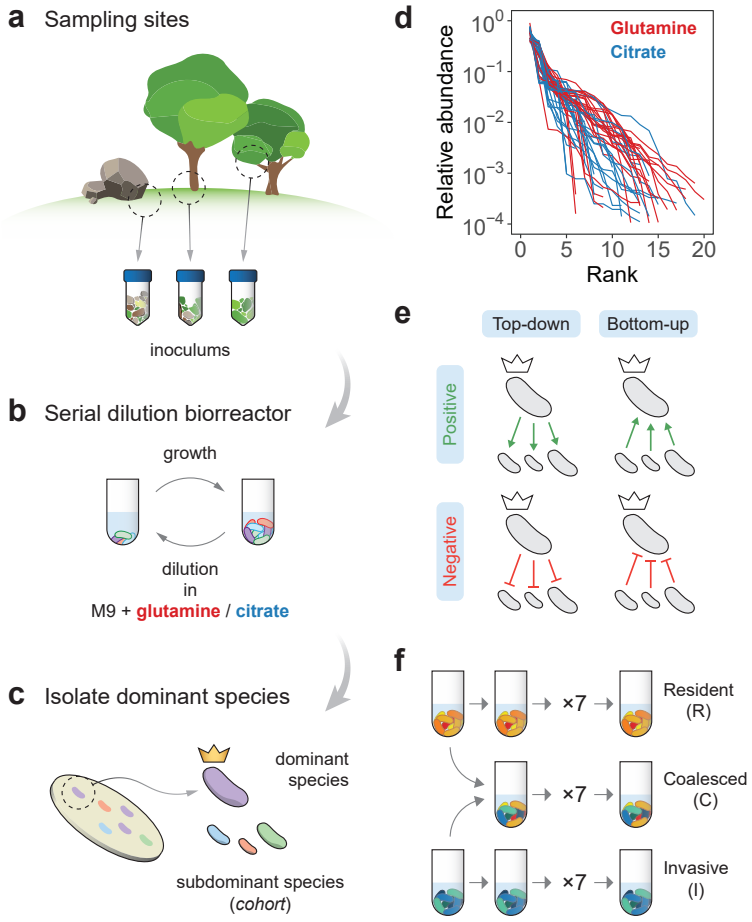

Figure 1. Overview of the experimental protocol. a. Environmental samples collected from eight different locations were used to inoculate our communities. **b.** Communities were stabilized in serial batch culture bioreactors [29] in minimal synthetic media with glutamine or citrate as the only supplied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

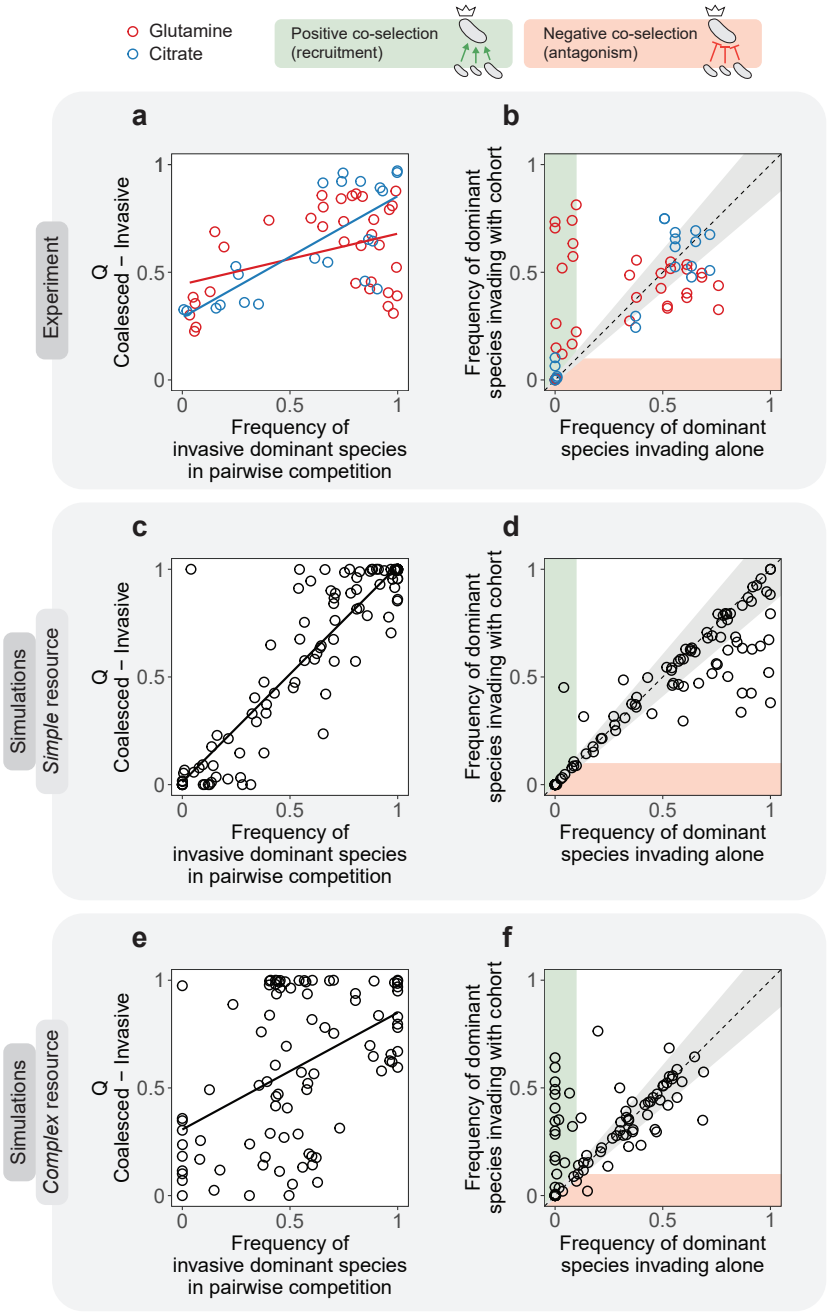

Figure 2. Co-selection in microbial community coalescence. a. Coalescence outcomes are quantified by the relative Bray-Curtis similarity (Q) between the coalesced and invasive communities. These outcomes are predicted by the pairwise competition between the invasive and resident dominant species ($R^2 = 0.15$, $p < 0.05$ for glutamine and $R^2 = 0.57$, $p < 10^{-4}$ for citrate). A higher correlation is consistent with a scenario of top-down positive co-selection where dominants recruit their cohorts for the final coalesced community. Two biological replicates per experiment are plotted individually. **b.** We represent the frequency reached by the invasive dominant species when they invade the resident communities on their own versus when they are in the company of their cohort. Three scenarios are possible: green and red shaded areas represent limit cases of positive (recruitment) or negative (antagonism) bottom-up co-selection, gray area corresponds to situations where invasive dominant species can invade with equal success regardless of the presence of their cohorts. Data shows that positive co-selection is common, whereas antagonistic co-selection is rare in our experiments. Two biological replicates per experiment are plotted individually. **c-d.** Simulations of community coalescence with a consumer-resource model and a metabolically simple supplied resource yield a strong degree of top-down positive co-selection ($R^2 = 0.81$, $p < 10^{-15}$) and few instances of bottom-up co-selection. **e-f.** Simulations with a metabolically complex resource result in weaker top-down co-selection ($R^2 = 0.22$, $p < 10^{-5}$) but frequent bottom-up positive co-selection.

Supplementary Figures

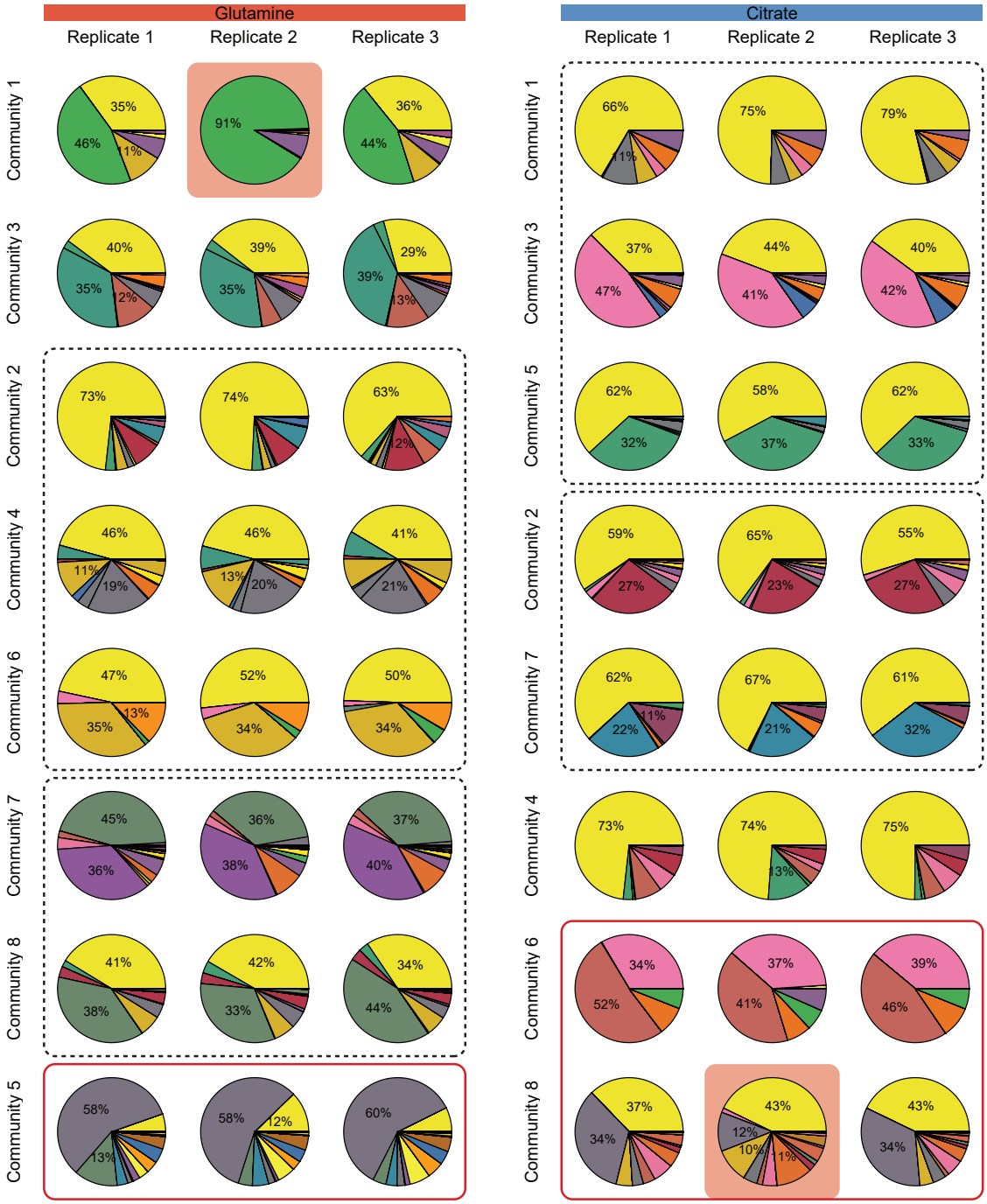

Figure S1. Community compositions after seven additional transfers without coalescence. Each color of the pie plots corresponds to a different exact sequence variant (Methods: Determination of community composition by 16S sequencing). Replicate 2 of community 1 from glutamine, as well as replicate 2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

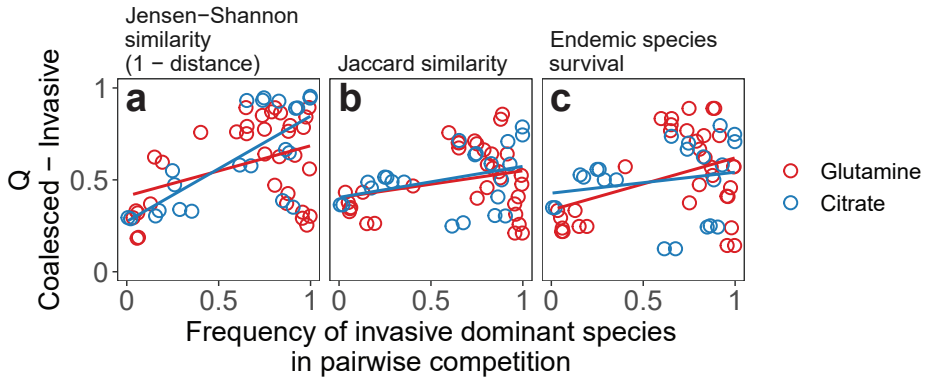

Figure S2. Alternative metrics of community distance. Quantifying coalescence outcomes using different metrics of community similarity (Methods: Metrics of community distance) gives similar results to those shown in Figure 2a. Metrics that account for the relative species abundances (Bray-Curtis or Jensen-Shannon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

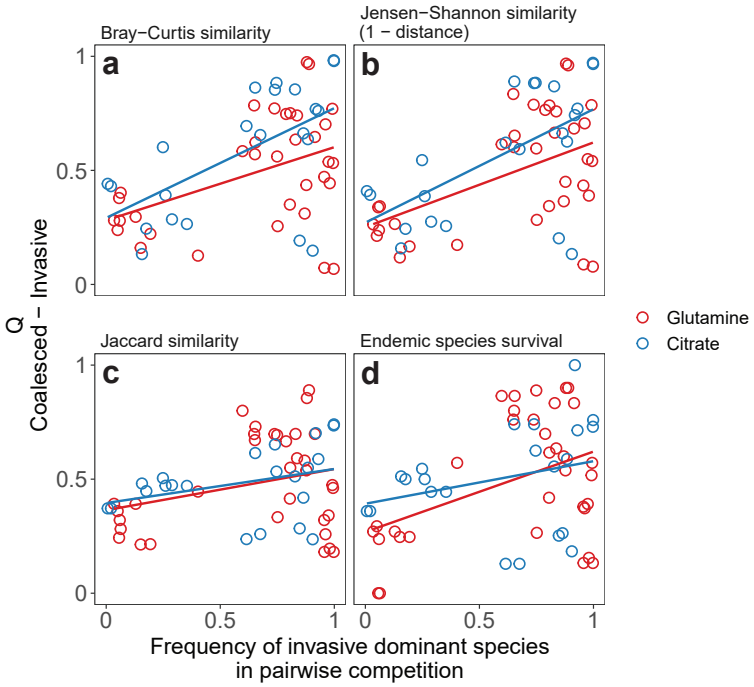

Figure S3. Dominant species have limited effects on coalescence outcomes quantification. We repeated the analyses shown in Figure 2a and Figure S2, but this time we removed the dominants from the compositional data prior to quantifying community distances. The trends observed before are maintained. **a.**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

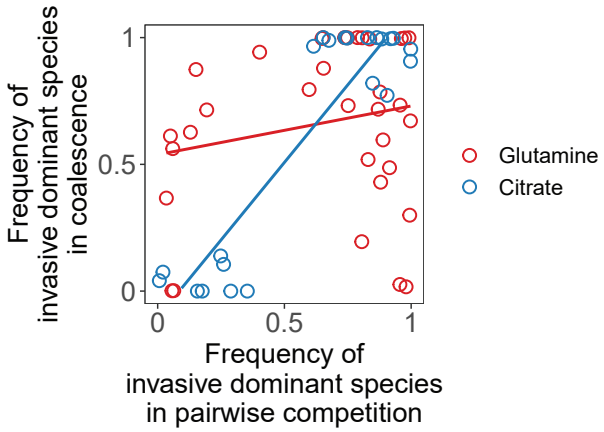

Figure S4. Pairwise competition of dominants with or without their cohorts. In the horizontal axis, we plot the relative frequency of the invasive dominant species in head-to-head pairwise competition. In the vertical axis, we plot the same relative frequency when competition takes place in the presence of the cohorts, i.e. during community coalescence. $R^2 = 0.04$, $p > 0.05$ for glutamine and $R^2 = 0.83$, $p < 10^{-8}$ for citrate.