

1 **Title:** Disruption of cross-feeding interactions by invading taxa can cause invasional meltdown
2 in microbial communities

3 **Running head:** Invasional meltdown via disrupted cross-feeding

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13

13 **Abstract**

14 The strength of biotic interactions within an ecological community affects the
15 susceptibility of the community to invasion by introduced taxa. In microbial communities, cross-
16 feeding is a widespread type of biotic interaction that has the potential to affect community
17 assembly and stability. Yet, there is little understanding of how the presence of cross-feeding
18 within a community affects invasion risk. Here, I develop a metabolite-explicit model where
19 native microbial taxa interact through both cross-feeding and competition for metabolites. I use
20 this model to study how the strength of biotic interactions, especially cross-feeding, influence
21 whether an introduced taxon can join the community. I found that stronger cross-feeding and
22 competition led to much lower invasion risk, as both types of biotic interactions lead to greater
23 metabolite scarcity for the invader. I also evaluated the impact of a successful invader on
24 community composition and structure. The effect of invaders on the native community was
25 greatest at intermediate levels of cross-feeding; at this “critical” level of cross-feeding,
26 successful invaders generally causing decreased diversity, decreased productivity, greater
27 metabolite availability, and decreased quantities of metabolites exchanged among taxa.
28 Furthermore, these changes resulting from a successful primary invader made communities
29 further susceptible to future invaders. The increase in invasion risk was greatest when the
30 network of metabolite exchange between taxa was minimally redundant. Thus, this model
31 demonstrates a case of invasional meltdown that is mediated by initial invaders disrupting the
32 metabolite exchange networks of the native community.

33 **Keywords:** invasion, cross-feeding, invasional meltdown, community ecology, microbial
34 ecology, biotic resistance

35

35 **Introduction**

36 Cross-feeding, wherein one individual consumes a metabolic product of a different
37 individual, is ubiquitous in microbial communities [1]. Stable cross-feeding relationships evolve
38 spontaneously even when a single strain of bacteria is grown in the lab; in a well-studied
39 example where a single genotype of *E. coli* is grown in glucose, a second genotype capable of
40 consuming acetate, a waste product, eventually evolves and coexists alongside the original
41 genotype [2]. In this case, a mutation allowing an *E. coli* cell to consume the unexploited acetate
42 resource confers a fitness advantage. The evolution of novel bacterial genotypes capable of
43 cross-feeding has been observed and reproduced under a variety of laboratory conditions [3-5],
44 demonstrating the widespread prevalence of cross-feeding even in simple microbial
45 communities. However, cross-feeding is not well studied in the context of theoretical community
46 assembly models, perhaps because many of these models were developed with macro-ecological
47 systems in mind, where cross-feeding is comparatively rare.

48 The simple example of the spontaneous evolution of cross-feeding in a culture of *E. coli*
49 demonstrates how cross-feeding can alter community structure. The number of functionally
50 distinct taxa in this case increases from one to two, and the total density of cells may increase as
51 the acetate-consuming genotype is able to subsist on a resource that would otherwise not be
52 consumed. Thus, diversity, productivity (cell density), and metabolite concentrations would all
53 be affected by the establishment of this cross-feeding relationship. Empirical studies have found
54 that cross-feeding is a vital process in determining what populations can persist within microbial
55 communities [6]. A single bacterial strain can produce dozens of metabolic byproducts capable
56 of sustaining other strains [7]. Therefore, in more complex communities, there is vast potential
57 for cross-feeding between bacteria [8]; the number of possible cross-feeding relationships

58 increases with the number of taxa present in the community and the number of nutrients
59 provided in the environment. Thus, cross-feeding has the potential to alter community structure
60 across a broad range of microbial ecosystems, and these structural changes may have cascading
61 effects on community stability and function.

62 Incorporating cross-feeding into mathematical models can be computationally
63 challenging, which may account for why much of the theoretical development of this topic has
64 been recent. Incorporating cross-feeding into models introduces many additional parameters, as
65 these models must track the concentrations of each metabolite in the environment and within
66 cells, in addition to the exchanges of each metabolite between cells. Previous theoretical models
67 studying the effects of cross-feeding on community assembly have largely focused on whether
68 communities with cross-feeding are stable and how these relationships affect the diversity of
69 communities (e.g. [6, 9, 10]). For example, in classical ecological models, there is a paradigm
70 that only one consumer can persist for each resource present in an ecological community [11].
71 However, recent theoretical models have found that cross-feeding can dramatically increase the
72 diversity of taxa, even in a homogenous environment [6, 10, 12]. Furthermore, multiple different
73 types of models have found that introducing cross-feeding into communities can result in a new
74 stable community composition [13-15]. However, fewer studies have examined how the strength
75 of cross-feeding relationships alters other emergent properties, such as susceptibility to invasion.

76 The model presented here uses a metabolite-explicit mathematical simulation to study
77 how cross-feeding between microbial taxa affects the ability of an introduced taxon to invade the
78 community. First, I study how cross-feeding alters the assembly of microbial communities
79 containing randomly generated taxa. Then, I investigate how cross-feeding networks and
80 community structure mediate the ability of an invader to join an established community. In the

81 case of a successful invader, I evaluate how the introduced taxon alters the composition and
82 cross-feeding network of the microbial community. Finally, I ask whether a successful primary
83 invader can lead to “invasional meltdown” by making the community more susceptible to future
84 invaders [16]. Thus, this modeling approach studies the interplay between community structure,
85 biotic interactions, and invasion history in determining the susceptibility of a microbial
86 community to invasion.

87

88 **Methods**

89 I constructed a mathematical model consisting of resident taxa, invading taxa, and the
90 metabolites required for cell reproduction. Taxa interact through competition for metabolites in
91 the environment and through cross-feeding of metabolites. Of all possible metabolites in the
92 model (m), each taxon requires a randomly assigned unique subset of n metabolites for growth,
93 giving each a distinct ecological niche. At the beginning of each model run, x native taxa were
94 introduced into the community. For example, for the models presented here, there were 20 native
95 taxa, each with an abundance of 50, at the start of the simulation. There were 8 possible
96 metabolites, and each taxon required 5 of those 8 metabolites for reproduction. Thus, there were
97 a total of 8 choose 5 (equal to 56) distinct niches that taxa could occupy. From these 56 niches,
98 20 niches were randomly assigned to the native taxa, and one was assigned to the invasive taxon;
99 this yielded 56 choose 20 (upwards of 100 trillion) combinations of possible metabolite
100 requirements for the native taxa. Each taxon also excretes a subset of q metabolites, which do not
101 overlap with its n required metabolites. The “input” metabolites are a set of n metabolites that
102 entered the environment at the beginning of each time step, and one of the x native taxa had
103 metabolite requirements that matched the input metabolites.

104 Cross-feeding in the model was implemented as one taxon directly transferring its
 105 excreted metabolites to another taxon that required those metabolites. All possible unidirectional
 106 metabolite transfers were identified by looking at which metabolites were excreted and required
 107 by all taxa; a random fraction (given by the cross-feeding parameter p) of these possible
 108 metabolite transfers were implemented as cross-feeding relationships in the model. The cross-
 109 feeding step occurred separately from competitive uptake of metabolites from the environment.
 110 Other parameters in the simulation model include the average competition coefficient (c),
 111 variability in competition coefficients among taxa (v), an input rate for metabolites (i), and a
 112 flushing rate for metabolites and cells (f). Competition coefficients for native taxa were drawn
 113 from a normal distribution with mean c and a standard deviation of v . The initial abundance of all
 114 native taxa when initializing the model was 50, and this was also the abundance at which the
 115 invader was introduced.

116

117 **Table 1: Input Parameters and Measured Outputs for Cross-feeding Model**

| Input Parameters | Value |
|--|---|
| Maximum number of taxa in community (x) | 20 |
| Number of possible metabolites (m) | 8 |
| Number of metabolites required by each taxon (n) | 5 |
| Number of metabolites excreted by each taxon (q) | 3 |
| Flushing rate of cells and metabolites (f) | 0.1 |
| Metabolite input rate (i) | 200 per timestep for each metabolite |
| Proportion of direct cross-feeding relationships (p) | 0.0 to 0.5 in increments of 0.01 |
| Mean competition coefficient of native taxa (c) | 0.5 to 0.8 in increments of 0.01 |
| Standard deviation of competition coefficients (v) | 0.3 * mean competition coefficient (c) |
| Measured Model Outputs | Definition |
| Persistence of invader | An invasion was deemed successful if the invader had an abundance greater than 1 at model equilibrium |
| Total individuals | Sum of all individuals from all taxa at equilibrium |
| Taxa coexisting | Number of taxa with at least 1 |

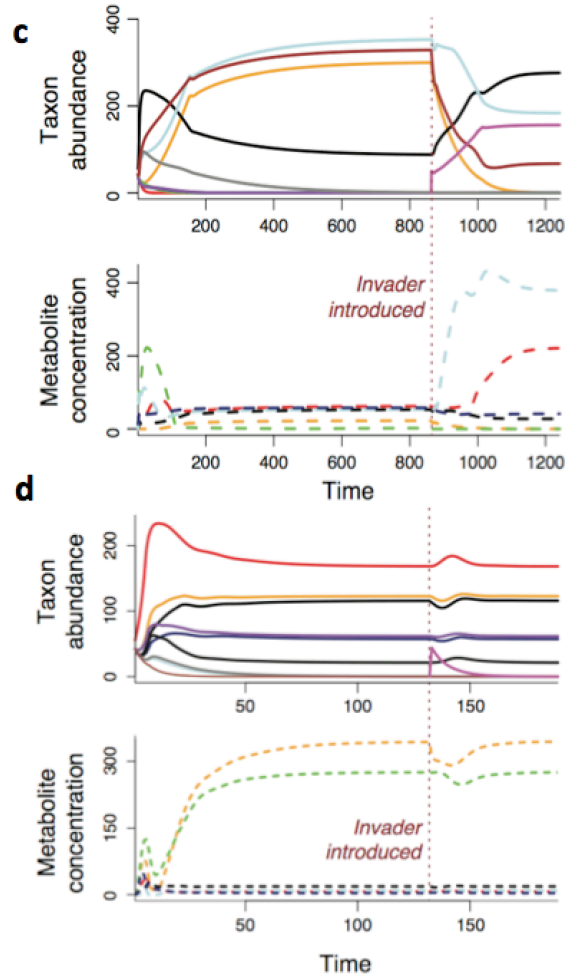
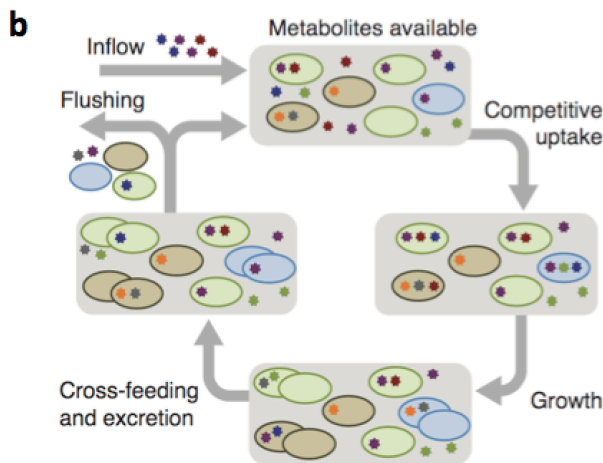
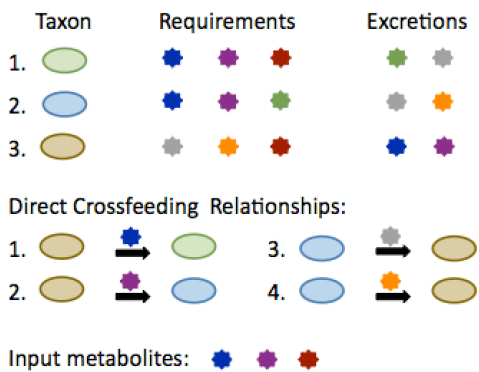
| | |
|------------------------------|---|
| | individual present at equilibrium |
| Metabolites at equilibrium | Sum of all metabolites present in the environment at equilibrium |
| Metabolites traded | Sum of all metabolites directly exchanged through cross-feeding |
| Flows per taxon | Number of direct cross-feeding relationships per taxon |
| Redundancy of limiting flows | Average number of cross-feeding relationships that provide the growth-limiting nutrient to each taxon |

118

119 Each time step begins with input metabolites entering the environmental pool (Fig. 1b).
120 Taxa then compete for these metabolites, with uptake rates governed by their competition
121 coefficients, which quantify scavenging efficiency. Each individual is able to store 1 unit of each
122 required metabolite, and reproduction occurs when the individual procures 1 unit of all its
123 required metabolites. Metabolite uptake from the environment is allocated proportionally among
124 taxa in accordance with each taxon's demand for the metabolite; demand for a metabolite is
125 calculated as the number of individuals needing the metabolite multiplied by their respective
126 competition coefficients. If there is metabolite scarcity (meaning that total demand for
127 metabolites exceeds availability of metabolites), metabolites are allocated among taxa in
128 proportion to the demand of each taxon. I assume for simplicity that metabolite uptake amongst
129 individuals in a population is arranged to maximize biomass production [17]. Population growth
130 is limited by whatever metabolite is most scarce in the population. The reproducing individuals
131 (those having acquired all necessary metabolites) also excrete one unit of each of the metabolites
132 in their excretion profile. If these individuals are from taxa participating in cross-feeding, the
133 excreted metabolites are preferentially available to the recipient taxon; in this case, the
134 metabolites are directly transferred to the recipient without being available for competitive
135 uptake. If the reproducing taxon has more than one cross-feeder, the excreted metabolites are

136 divided equally among the recipient taxa. Any excreted metabolites that are not part of cross-
 137 feeding relationships enter the environmental pools of metabolites. Thus, this model also allows
 138 for “indirect” cross-feeding, wherein taxa can consume metabolites from the environment that
 139 were produced by a different taxon. However, the term “cross-feeding” in this paper refers to
 140 direct metabolite transfers between taxa. Finally, a proportion f of individuals and environmental
 141 metabolites are flushed from the system.

a Model Specifications



142
 143 **Fig. 1: Design and output of simulation model studying invaders in microbial communities.**
 144 **Panel (a) gives model specifications for a simplified version of the cross-feeding model,**
 145 **containing three taxa, which is depicted in panel (b). Panel (b) shows the processes that**
 146 **occur during each timestep of the model. Different metabolites are represented by**

147 **differently colored stars. Different taxa are represented by differently colored ovals. When**
148 **a cell acquires one unit of each of its required metabolites, it reproduces and also excretes**
149 **its given metabolites. In this example, the native community of three taxa has reached**
150 **equilibrium. Panels (c) and (d) show results of model simulations, tracking both taxon**
151 **abundances and the concentration of each metabolite in the environment through time.**
152 **Panel (c) shows a successful invasion, where the invading taxon (pink line) persists in the**
153 **community, whereas in (d), the invader is excluded from the community. Red lines indicate**
154 **the time point when the invader is introduced.**

155

156 The invader was introduced after the community of resident taxa equilibrated (Fig 1c,
157 Fig. 1d). Equilibrium was determined as when the maximum change in any taxon's population
158 was less than 0.001 between time steps. The invader had a fixed competition value of 0.9 in all
159 simulations (generally larger than that of native taxa), and did not have any cross-feeding
160 relationships. The lack of cross-feeding relationships is the primary way in which the invader
161 differs from native taxa. There are multiple reasons why invasive taxa were not allowed to cross-
162 feed in the model. First, I reasoned that cross-feeding relationships often need time to develop
163 (e.g. time for proper spatial configuration [18], construction of nanotubes [19], or within-host
164 coevolution [20]), and that an invading taxon would therefore have no preexisting methods of
165 directly acquiring metabolites. Additionally, many studies of invasive taxa have concluded that
166 invasive taxa differ from native taxa in their biotic interactions (as reviewed in [28]). The lack of
167 cross-feeding relationships for invaders differentiates the biotic interactions of invaders from
168 those of native taxa. Finally, the invader was given a relatively high competition coefficient
169 because strong competitive ability can be another characteristic trait of invasive taxa [21].

170 After the invader was added, the simulation continued until the community again reached
171 equilibrium. A successful invader changes the abundances of native taxa by introducing
172 additional competition for metabolites. After the model equilibrates, a second invader with a
173 different, randomly chosen metabolite profile was added, and again the model was run until
174 equilibrium. At each of these three equilibria (without invader, after the first invader, and after
175 the second invader), I recorded properties of the community and properties of the cross-feeding
176 network established between community members (Fig. 1b). The community-level outcomes
177 recorded were persistence of the invaders, total individuals in the community, number of taxa
178 present in the community, and the number of metabolites in the environment at equilibrium
179 (Table 1). Successful invaders were counted in the total number of individuals and total number
180 of taxa. The network-level outcomes recorded were the number of metabolites traded during
181 each time step, the average number of cross-feeding relationships (abbreviated in figures as
182 “flows” of metabolites) for each taxon, and the average number of cross-feeding relationships
183 (again, metabolite “flows”) providing each taxon’s growth-limiting nutrient (Table 1). Finally, I
184 also tested whether the second invader could persist in the absence of the first invader by
185 resetting the community to its first equilibrium and adding only the second invader. I evaluated
186 these model outputs while changing the proportion of cross-feeding relationships and the degree
187 of competition present between taxa.

188 Parameter values used in model simulations can be found in Table 1. Any randomly
189 generated competition values below 0.1 were set to 0.1, as to minimize the outcome that no taxa
190 were able to persist in the community. Results were qualitatively similar regardless of the
191 number of taxa used in the simulation (x), so long as there were sufficiently many taxa (at least
192 8-10). I generated 5000 simulated communities for each combination of competition coefficient

193 and cross-feeding proportion, resulting in a total of 7,905,000 simulated communities. Thus,
194 there are 5000 values of each model output for each set of parameters evaluated. In a small
195 fraction of runs (3.5%, on average), the model resulted in a stable limit cycle or did not
196 equilibrate within 40,000 time steps, and these runs were discarded.

197

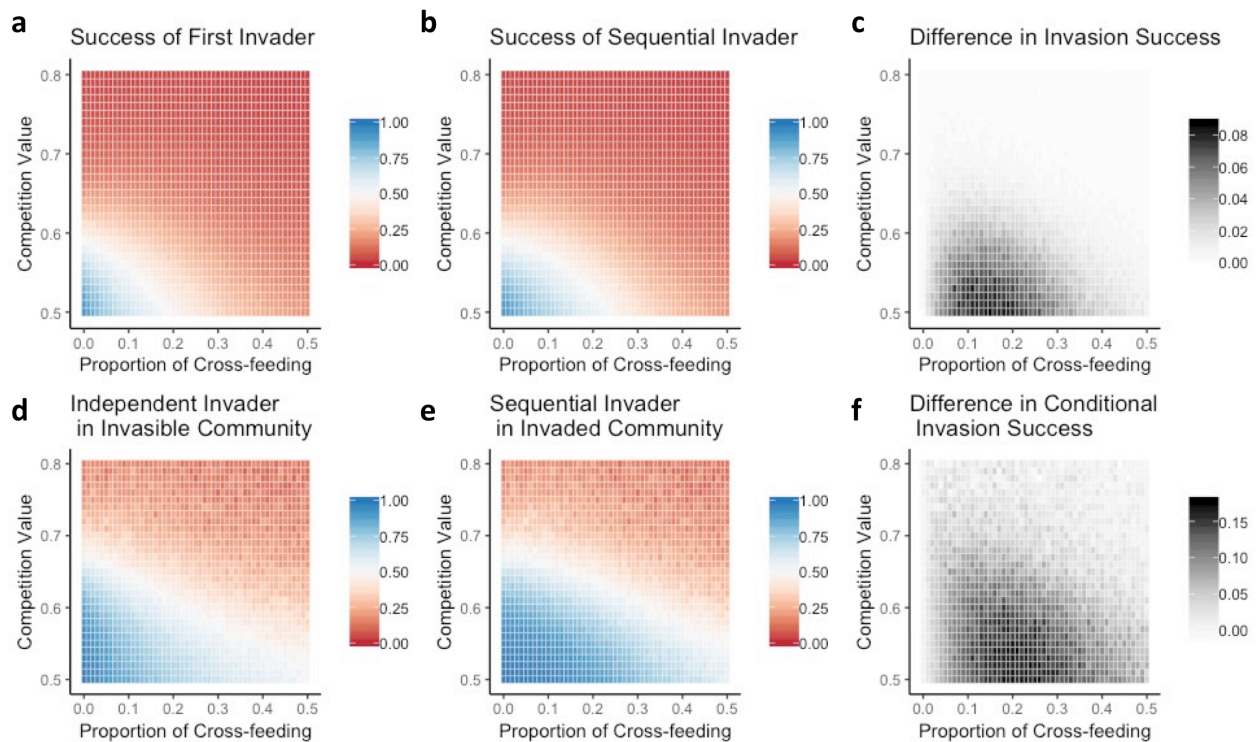
198 **Results**

199 The proportion of possible cross-feeding relationships present in a community strongly
200 influenced community structure and connectivity of the metabolite exchange network. Higher
201 prevalence of cross-feeding was related to increased diversity, increased productivity (more
202 individuals in the community), fewer metabolites in the environment, and increased metabolite
203 exchange between individuals.

204 Biotic interactions between taxa within the native microbial community were strong
205 determinants of whether an invading taxon could persist in the community. Invasive taxa were
206 most successful when both competition and cross-feeding within the resident community were
207 weak (Fig. 2a). When competition and cross-feeding were at their lowest values, invaders were
208 successful in nearly every community, whereas invaders succeeded less than 1% of the time in
209 communities with the maximum competition and cross-feeding values. Secondary invaders
210 (those introduced at model equilibrium after the first invader) were more successful than primary
211 invaders across all values of competition and cross-feeding (Fig. 2b). The largest discrepancy
212 between the success of secondary invaders versus the success of primary invaders was at
213 intermediate cross-feeding values (Fig. 2c).

214 I then evaluated whether communities that were susceptible to a primary invader were
215 more susceptible to other invaders (Fig. 2 bottom row). I tested the ability of the same

216 independent invader (meaning, with the same metabolic profile) to join a community either in
217 the absence of a primary invader (Fig. 2d) or after a primary invader had previously established
218 in the community (Fig. 2e). In both cases, communities that could be invaded by one type of
219 invader were also more susceptible to a different type of invader. However, the presence of a
220 primary invader within a community increases susceptibility to a secondary invasion, and this
221 invasional meltdown was most likely to occur at intermediate levels of cross-feeding (Fig. 2f).

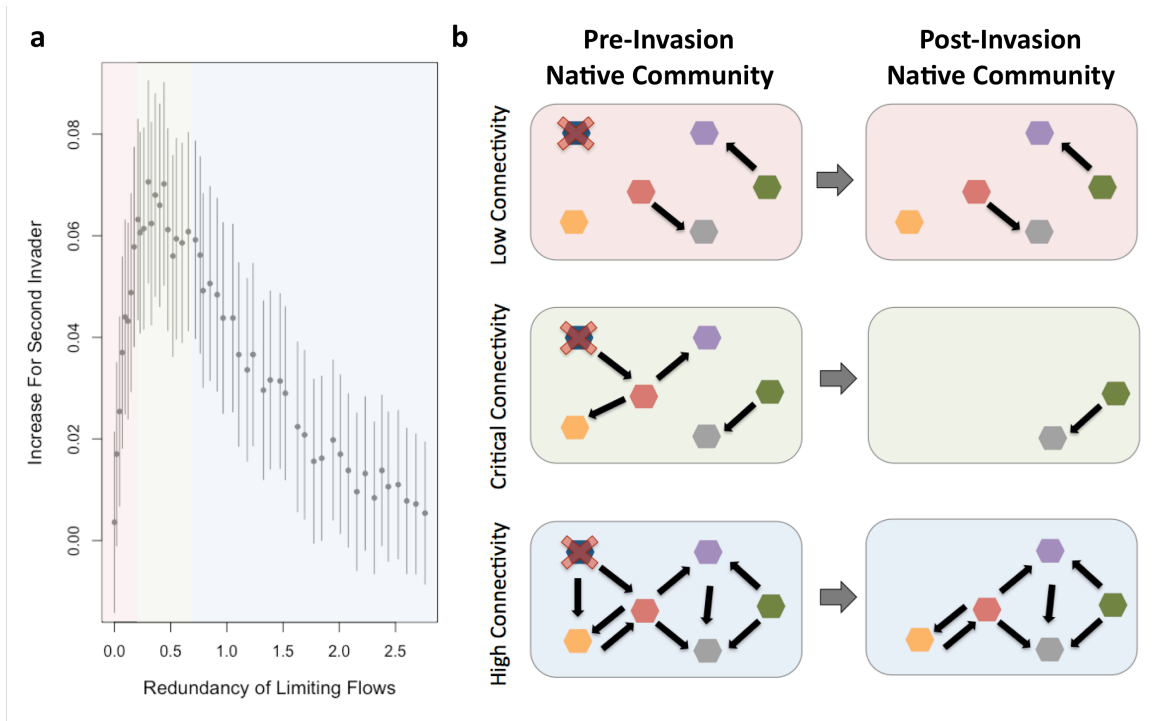


222

223 **Fig. 2: Primary and secondary invasion success across all communities (top row) and**
224 **within communities susceptible to invasion (bottom row). Primary invaders (i.e. the first**
225 **invader introduced) are highly successful when cross-feeding and competition are low, but**
226 **quickly become less successful as the strength of either of these interactions increases (a). A**
227 **sequential invader (introduced after the primary invader) is more successful than the**
228 **primary invader (b). The difference in invasion success (success of sequential invader**

229 **minus success of primary invader) is greatest at intermediate levels of cross-feeding and**
230 **low levels of competition (c). I isolated communities susceptible to the primary invader, and**
231 **tested whether a different invader would be able to succeed there (d). Communities that**
232 **were invisable by one invader were generally much more susceptible to a different invader.**
233 **However, those same communities were more susceptible to a sequential invader, in**
234 **comparison to a primary invader (e). Again, the difference in invasion success as a result of**
235 **the presence of the first invader was greatest at intermediate levels of cross-feeding (f).**

236 After observing that the invasional susceptibility increased most at intermediate degrees
237 of cross-feeding, I looked for a mechanism that might cause this pattern. I compared the
238 difference in susceptibility with the average redundancy in the cross-feeding relationships
239 providing each taxon's limiting resource (Fig. 3a). The limiting nutrient is an important quantity
240 to track in this model, because reducing the supply of the limiting nutrient hinders population
241 growth, whereas this is not necessarily true for non-limiting metabolites. As the redundancy of
242 the limiting resource flows increases, it is less likely removing a single taxon will lead to an
243 absence of a limiting nutrient for another taxon (Fig 3a, Fig. 3b). I found that increased
244 susceptibility to invasion was most common when the average number of taxa providing each
245 limiting resource was less than 1 (Fig. 3a). Thus, communities with high redundancy in
246 metabolite exchanges were strongly protected from both primary and secondary invaders.



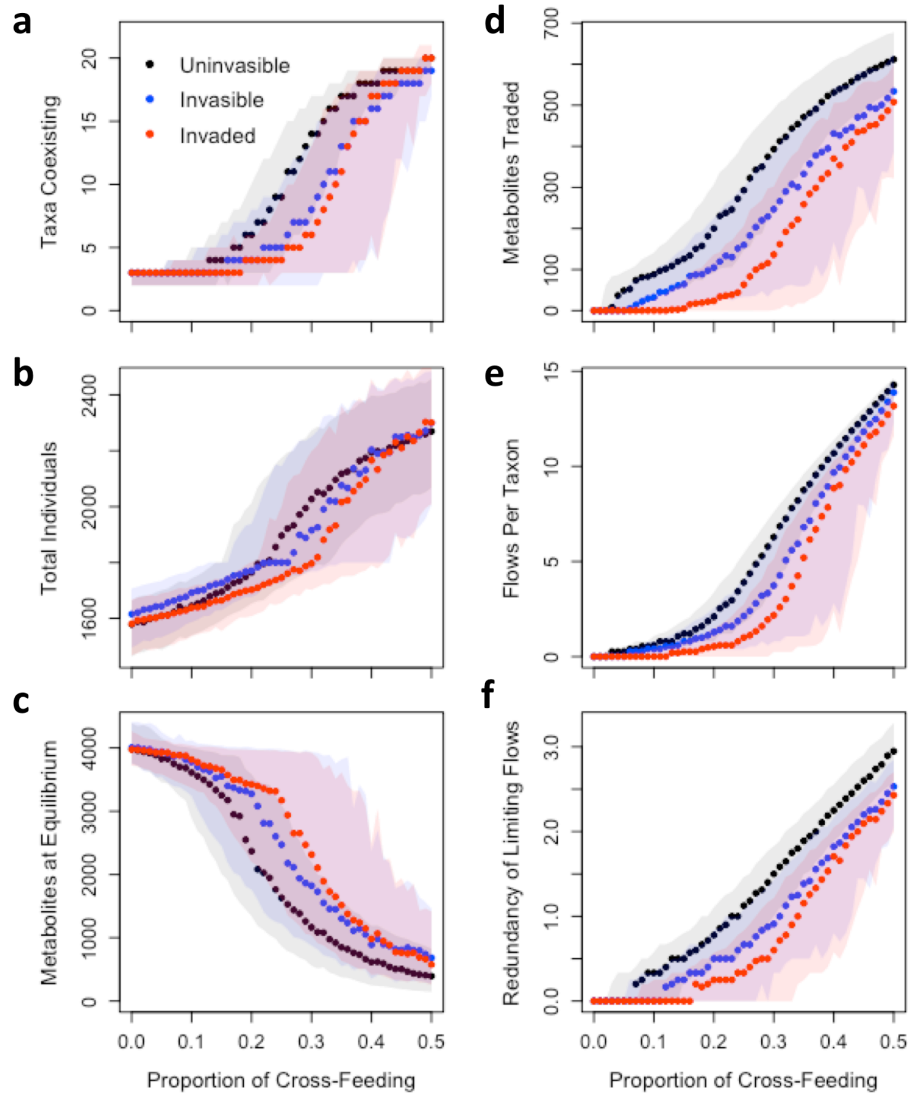
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248 **Fig. 3: The difference in success between a first invader and a secondary invader is greatest**
249 **when there are intermediate amounts of metabolite exchanges (a). The redundancy of**
250 **limiting flows measures the average number of cross-feeding relationships that provide**
251 **each taxon with its growth-limiting metabolite. Bars show 95% confidence intervals for the**
252 **difference in invasion success between a first and second invader, calculated using the**
253 **standard error for binomially distributed variables. Shaded areas approximate regions of**
254 **low cross-feeding connectivity (red), intermediate or “critical” connectivity where the**
255 **invasion difference is highest (green), high connectivity (blue). Panel (b) gives an example**
256 **of how invaders have maximal impact at critical connectivity. This illustration shows the**
257 **same community with different cross-feeding dependencies. If a single native taxon is**
258 **competitively excluded after invasion (blue taxon with red X), it is likely that no other taxa**
259 **are singularly dependent on this taxon at low or high connectivity (red and blue shaded**
260 **communities, respectively). However, at critical connectivity, removing a single taxon**

261 **causes all taxa with downstream dependencies to also become extinct (green communities).**
262 **The data in panel (a) are from simulations where the mean competitive values of the native**
263 **communities are fixed at 0.55.**

264

265 To further investigate why invasional meltdown was strongest at intermediate levels of
266 cross-feeding, I compared the community and network structures of uninvincible, invincible, and
267 invaded communities across different values of cross-feeding. If any of these community or
268 network properties contributed to increased susceptibility to invasion, the property should be
269 different between uninvincible and invincible communities. Furthermore, properties affecting
270 invasion risk should also be impacted by the presence of a successful invader (because these
271 communities were shown to be more invincible). I found this pattern to some degree in all six of
272 the simulation properties studied (Fig 4).



273

274 **Fig. 4: Properties of uninvasive (black), invulnerable (blue), and invaded (red) communities**

275 **assembled under differing levels of cross-feeding. Each panel shows one aspect of the**

276 **community structure (left column) or metabolite exchange network (right column). Solid**

277 **points with shaded envelopes show the median values and interquartile range. Properties**

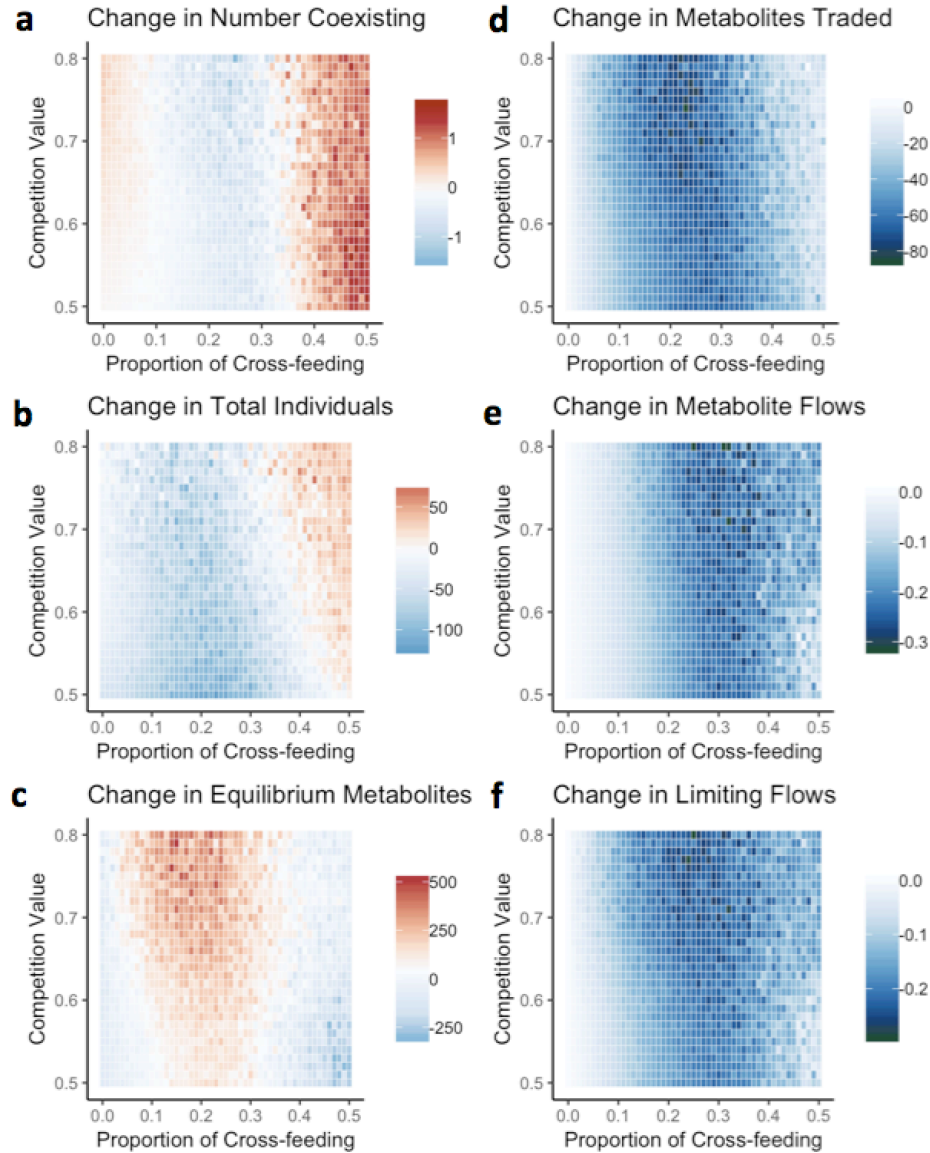
278 **that contribute to invasional meltdown would be expected to be different between**

279 **uninvasive and invulnerable communities, and should be further shifted after a successful**

280 **invasion. All communities shown have the same mean competition coefficient ($c = 0.55$).**

281

282 There were consistent differences in community properties between uninvasible and
283 invasible communities (Fig. 4 left column), although metabolite network properties showed more
284 pronounced differences between uninvasible and invasible communities (Fig. 4 right column).
285 Uninvasible communities were generally more diverse than invasible communities. An invading
286 taxon could reduce overall diversity if the invader caused the loss of more than one taxon from
287 the original community (Fig. 4a). However, at high levels of cross-feeding, a successful invader
288 generally did not displace any taxa. It was also possible for an invader to lead to increased
289 diversity by excreting novel metabolites into the environment, thereby creating new niches for
290 taxa to occupy. In this case, native taxa that were previously counted as absent (having a
291 population of less than 1) increased in abundance to join the community. Invasible and
292 uninvasible communities did not consistently differ in their total number of individuals, though
293 invasible communities at moderate levels of cross-feeding generally had fewer individuals (Fig.
294 4b). However, the number of metabolites present at equilibrium was consistently different
295 between uninvasible, invasible, and invaded communities; uninvasible communities had relative
296 metabolite scarcity, and invaded communities had comparatively high metabolite availability
297 (Fig. 4c). Additionally, uninvasible communities had the largest amount of metabolites
298 exchanged through cross-feeding, whereas invaded communities had the lowest amount of
299 exchanged metabolites (Fig. 4d). Two other measures of the strength of the cross-feeding
300 network, the number of metabolite flows per taxon (Fig. 4e) and the number of flows providing
301 the limiting metabolite (Fig. 4f) also showed that a successful invader weakened cross-feeding
302 networks. Similarly, communities that assembled with higher redundancy in metabolite
303 exchanges were less invasible (Fig 4e and Fig 4f).
304



305

306 **Fig. 5: Changes in community and network properties as a result of a successful invasion.**

307 **Each panel shows the median change in community (left column) or metabolite exchange**

308 **network (right column) properties when comparing the pre-invasion community to the**

309 **post-invasion community. Warm colors indicate increases, whereas cool colors indicate**

310 **decreases. Across all properties considered, there were strong changes at intermediate**

311 **levels of cross-feeding.**

312

313 Finally, I evaluated how strongly an invader altered a community as a function of the
314 degree of competition and cross-feeding present in the native community. Across all metrics
315 studied, I found that an invader had the strongest effect on community structure (Fig. 5 left
316 column) and networks of metabolite exchange (Fig. 5 right column) at intermediate levels of
317 cross-feeding. However, each of the six metrics had subtle differences in how invasion impacted
318 them at different competition and cross-feeding values. For changes in community diversity, taxa
319 were generally excluded at intermediate levels of cross-feeding, but added at higher levels of
320 cross-feeding; this was consistent across all competition values (Fig. 5a). Similarly, most
321 communities showed declines in the density of individuals after invasion (Fig. 5b), but these
322 losses of individuals were more extreme at low competition values. Conversely, at high
323 competition and cross-feeding values, there was generally a gain in the total number of
324 individuals after invasion. The total number of metabolites in the environment at equilibrium
325 increased after invasion at high levels of competition (Fig. 5c). However, metabolite
326 concentrations generally decreased at high and low levels of cross-feeding, especially when
327 competition values were also low.

328 The networks of metabolite exchanges between taxa were overwhelmingly weakened by
329 the introduction of an invader (Fig. 5 right column). All of the network properties showed
330 decreases in connectivity/redundancy at intermediate levels of cross-feeding, regardless of the
331 strength of competition. Additionally, the small impact on cross-feeding networks at low values
332 of cross-feeding stemmed primarily from the fact that there was a minimal established network
333 in this parameter range, and thus the maximum possible disruption to the network was small.
334 However, there were fine-scale differences in how invaders affected these three aspects of cross-
335 feeding networks. The number of metabolites traded (Fig. 5d) was negatively affected at the

336 lowest threshold of cross-feeding, but was minimally affected at very high levels of cross-
337 feeding. Furthermore, the number of metabolite flows providing limiting nutrients was affected
338 at a lower threshold of cross-feeding than the total number of metabolite flows, confirming that
339 quantities of limiting and non-limiting metabolites affected taxa differently.

340

341 **Discussion**

342 These studies of invasion within simulated microbial communities show that cross-
343 feeding is a strong determinant of microbial community assembly and of the potential for new
344 taxa to enter the community. Stronger biotic interactions between resident taxa, whether from
345 cross-feeding or competition, resulted in lower rates of invasion (Fig. 2). After accounting for the
346 effects of interactions within communities, invasion was more likely when metabolites were
347 abundant and diversity was low (Fig. 4). However, network properties were more reliable
348 indicators of invasibility than community structure; there was strong differentiation between
349 invulnerable and vulnerable communities based on the number of metabolites exchanged and the
350 redundancy of flows providing limiting nutrients, with invulnerable communities having weaker
351 cross-feeding networks (Fig. 4). Invading taxa had the greatest impact on the resident
352 communities at intermediate levels of cross-feeding and competition (Fig. 5). In this case,
353 invasion was somewhat common (~20-50% success rate, Fig. 2), and caused declines in diversity
354 and productivity of the community, leading to more unused metabolites. Additionally, all aspects
355 of the cross-feeding network were weakened. However, it was possible for invaders to increase
356 overall diversity, and this result was most common at the highest levels of cross-feeding (Fig. 5).

357 This study demonstrates that invasional meltdown can occur as a result of initial invaders
358 disrupting the cross-feeding network of a native set of taxa, thereby making the community more

359 susceptible to another invader. Invasional meltdown, defined here as an increased success rate of
360 a secondary invader, was observed across all parameter space evaluated, but was strongest at
361 intermediate levels of cross-feeding (Fig. 3). Thus, there was a critical level of cross-feeding at
362 which communities were most prone to undergo dramatic shifts, if disturbed (Fig. 5). Critical
363 connectivity occurs when there are many taxon contingencies but minimal redundancy, such that
364 a disruption in the network has a cascading effect of removing taxa/individuals (Fig. 3). When an
365 invader is added to a community, it can directly exclude individuals by increasing competition
366 for a metabolite to sufficiently high levels that the resident taxa cannot persist. If a competitively
367 excluded taxon provided limiting metabolites to other taxa, those taxa could be secondarily
368 excluded as a result of the loss of their requisite cross-feeding relationships (Fig. 3). This
369 community collapse does not occur at sufficiently low or high levels of network connectivity. At
370 low levels of cross-feeding, the pre-existing cross-feeding network is minimal, so there is both a
371 low probability of an invader disrupting a chain of cross-feeding relationships (Fig. 3b).
372 Conversely, at high metabolic connectivity, the cross-feeding network is redundant, so multiple
373 taxa provide the same function; thus, high levels of cross-feeding protect against the domino
374 effect of species loss (Fig. 3b).

375 Invasive taxa often differ from native taxa in their interactions with other organisms [22].
376 In many cases, these altered biotic interactions contribute to the success of the invader ([23, 24]).
377 The assumption in this model that invaders cannot cross-feed is the primary way in which the
378 invasive taxa are differentiated from native taxa. Although the invaders' competition coefficients
379 were relatively high, they were still within the range of values that could be assigned to native
380 taxa. This lack of cross-feeding by the invader proved crucial to the phenomenon of invasional
381 meltdown; when allowing the invader to have the same cross-feeding dynamics as the native

382 taxa, there was no increased susceptibility to future invasion after a primary invasion (Figs. S1).
383 Furthermore, a successful invasion under these circumstances was less disruptive to overall
384 community structure (Figs. S2 and S3). Thus, these sensitivity analyses show that even a single
385 taxon that does not participate in cross-feeding strongly affects the entire microbial community.
386 However, the model was much less sensitive to assumptions about how cross-feeding was
387 implemented among native taxa, as results were qualitatively similar when native taxa were
388 allowed to be differentially good or poor at obtaining metabolites through cross-feeding (Figs.
389 S4, S5, S6). Thus, the conclusions from this study apply primarily to cases where the invader is
390 not well integrated into metabolite exchanges among the native community. Future models might
391 use different criteria to differentiate an invader from a native taxon, such as specifying unique
392 metabolite requirements for the invader.

393 This study adds to the long history of theoretical literature investigating how the strength
394 of biotic interactions affects community structure and stability. The idea that strong interspecies
395 interactions within native communities can mediate susceptibility to invading taxa has become
396 known as “biotic resistance” [25]. Biotic resistance can occur through many mechanisms
397 (reviewed in [22]), including eliminating open niches through strong competition between
398 resident taxa [23]. Additionally, the strength of biotic interactions between the native community
399 and an invading taxon can determine the outcome of an invasion [24]. However, few studies
400 have investigated cross-feeding as a mechanism of biotic resistance, perhaps because cross-
401 feeding is relatively uncommon outside of microbial communities. In this study, the strength of
402 biotic interactions was related to both invasion risk and the magnitude of the effects of a
403 successful invader. However, the probability of invasion (Fig. 2) and the consequences of

404 invasion (Fig. 5) were decoupled, in the sense that they were maximized at different strengths of
405 biotic interactions.

406 The organization of microbial communities differs from the organization of macro-scale
407 communities [26], and the prevalence of cross-feeding in microbial communities may be one
408 reason why these communities are structurally distinct. First, cross-feeding is one possible
409 contributing factor to the high diversity of microbial communities, frequently referred to as the
410 “paradox of the plankton” [27]. The paradox arises because more taxa coexist than there are
411 nutrients in the system, which violates the competitive exclusion principle [28] and the rule that
412 only one taxon should persist per possible limiting resource [11]. One feature that distinguishes
413 these results from some previously published cross-feeding studies is that the number of taxa in
414 this study can exceed the number of metabolites. In the simulations here, multiple taxa can
415 procure the same limiting metabolite from different sources, which gives an example of how
416 competitive exclusion may be avoided. Additionally, cross-feeding has been shown to enable the
417 stable coexistence of microbial communities grown in the lab. In the Long Term Evolution
418 Experiment, multiple genotypes of *E. coli* have coexisted in a homogeneous culture [29], with
419 evidence of cross-feeding between some genotypes [5]. Cross-feeding had previously been
420 proposed as a mechanism for the repeated co-occurrence of taxa across varied environments
421 [30], and these models agree with this possibility. Similarly, a recent study of naturally occurring
422 marine microbial communities showed that collections of taxa synchronously rose and fell in
423 abundance at the daily time scale, with biotic interactions between taxa as one proposed
424 mechanisms of the cohesive dynamics of these subcommunities [31]. Thus, there is growing
425 evidence that microbial communities contain modules of taxa with linked abundance patterns,
426 and the basis for these subcommunities is metabolic contingency via cross-feeding.

427 In addition to linking cross-feeding to changes in community structure, this study further
428 found that cross-feeding can alter community function. Previous empirical studies have similarly
429 suggested that cross-feeding dependencies shape emergent functions of communities. For
430 example, cross-feeding can lead to succession of taxa within a microbial community [32],
431 thereby altering the metabolic capacity of a community and the potential for degrading
432 compounds in the environment [33]. Another way that biotic interactions might impact
433 community functionality can be observed when two microbial communities intermix. Instead of
434 resembling a proportional mixture of the starting communities, the community that emerges after
435 mixing often resembles one of the initial communities more strongly; the correlated successes of
436 taxa at the community scale has been termed “community coalescence” [34]. Furthermore, the
437 initial community that is dominant in the resulting mixture often has disproportionate
438 contribution to overall community function and metabolism [35]. One proposed mechanism for
439 this cohesiveness is that established interactions, such as cross-feeding, reinforce community
440 structure [36]. In this case, biotic interactions link the success of co-dependent taxa, and these
441 modules/subcommunities of taxa collectively displace one another. This hypothesis about the
442 cohesive force of cross-feeding also agrees with the observation that communities comprised of
443 highly interconnected taxa have greater compositional stability [37]. In this framework, the
444 presence of cross-feeding would both cause many taxa to show similar abundance patterns
445 through time and would buffer against compositional change within the community. In a review
446 of the prevalence and characteristics of microbial invasions, Litchman [21] proposed that low
447 metabolic diversity or poor resource use efficiency may increase the susceptibility of a
448 community to invasion. This work suggests that cross-feeding underlies these characteristics of
449 resource use and niche availability to shape emergent community functions.

450

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