

# Analysis of stability to cheaters in models of antibiotic degrading microbial communities

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## Abstract

Antibiotic resistance carried out by antibiotic degradation has been suggested recently as a new mechanism to maintain coexistence of microbial species competing on a single limiting resource, even in well-mixed homogeneous environments. Species diversity and community stability, however, critically depend on resistance against social cheaters, mutants that do not invest in production, but still enjoy the benefits provided by others. Here we investigate how different mutant cheaters affect the stability of antibiotic producing and degrading microbial communities. We consider two cheater types, production and degradation cheaters. We generalize the mixed inhibition-zone and chemostat models introduced previously (Kelsic et al., 2015) to study the population dynamics of microbial communities in well-mixed environment, and analyze the invasion of different cheaters in these models. We show that production cheaters, mutants that cease producing antibiotics, always destroy coexistence whenever there is a cost of producing these antibiotics. Degradation cheaters, mutants that lose their function of producing extracellular antibiotic degrading molecules, induce community collapse only if the cost of producing the degradation factors is above

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a critical level. Our analytical studies, supported by numerical simulations, highlight the sensitivity of antibiotic producing and degrading communities to loss-of-function mutants.

*Keywords:* rock-paper-scissors, social parasite, evolutionary instability, antibiotic-mediated microbiome, degradation resistance

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## 1 **1. Introduction**

2       Unraveling mechanisms that maintain high genetic and functional diversity  
3 of microbial communities has become one of the most challenging problems in  
4 theoretical and evolutionary ecology (Costello et al., 2012; Morris et al., 2012;  
5 Cordero and Polz, 2014). A great variety of bacteria form stable communi-  
6 ties in relatively homogeneous environments, competing for only a few limiting  
7 resources (Hibbing et al., 2010), seemingly contradicting with the competitive  
8 exclusion principle, which states that the number of species cannot be higher  
9 than the number of limiting resources (Gause, 1934).

10       In bacteria, the most common forms of interactions are carried out by  
11 molecules secreted into the extracellular environment, such as exoenzymes to  
12 digest nutrients (Arnosti, 2011), iron scavenging siderophores (Ross-Gillespie  
13 et al., 2009), signaling molecules (Miller and Bassler, 2001), virulence factors  
14 (Hacker and Carniel, 2001), antibiotics (Bernier and Surette, 2013), or antibiotic  
15 degrading molecules (Wright, 2005). Via these molecules, microorganisms can  
16 be in competitive, antagonistic, or cooperative relationships (West et al., 2001;  
17 Coyte et al., 2015). Interestingly, these molecules are public goods, meaning  
18 that not only the producers, but all nearby individuals can enjoy the benefits  
19 delivered by them (West et al., 2001). Cheaters, individuals that do not produce  
20 such molecules and hence pay no cost of production, can also enjoy these ben-  
21 efits. Thus cheaters have higher fitness and can outcompete producers, leading  
22 to the loss of the diversity by ceasing the production of the public good (West  
23 et al., 2001). These antagonistic interactions carried out by the extracellular  
24 antibiotics make cyclic competition dominance possible, for example, among

25 antibiotic sensitive, producer, and resistant types. Since producing of an an-  
 26 tibiotic and being resistant to it are both costly, the resistant strain wins over  
 27 the producer, similarly the sensitive wins over the resistant, and the producer  
 28 can take over the sensitive population. This 'rock-paper-scissors' interaction  
 29 cycle is the simplest example of cyclical competition dominance network, where  
 30 each species is superior to one, but inferior to another (Fig. 1.a). Coexis-  
 31 tence of species in such cyclical interaction networks is documented in spatially  
 32 structured environments, in which interaction and dispersion are limited to the  
 33 immediate neighbors of the focal individual (Kerr et al., 2002; Czárán et al.,  
 34 2002; Károlyi et al., 2005; Müller and Gallas, 2010), but coexistence is much  
 35 less prevalent in unstructured environments where individuals mix intensively  
 (Kerr et al., 2002; Károlyi et al., 2005).

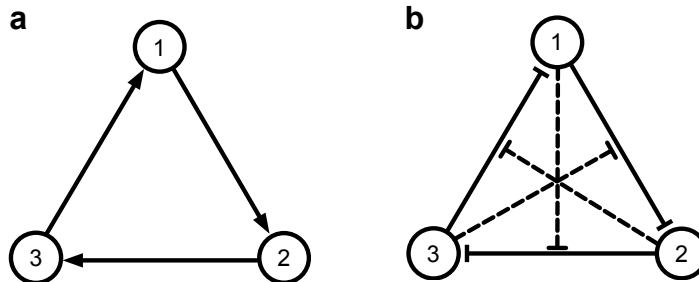


Figure 1: Cyclical competition dominance of three species. (a) Topology of a general 'rock-paper-scissors' type interaction. Here species 1 wins over species 2, species 2 wins over species 3, and species 3 wins over species 1, as indicated by the arrows. (b) The interaction topology where each species inhibits another by producing antibiotic (solid lines) and decomposes antibiotic produced by that species (dotted lines) according to a cyclical interaction topology.

36

37 Recently, Kelsic et al. (2015) (KEA) employed theoretical models to demon-  
 38 strate that bacterial species with different antibiotic production, intrinsic re-  
 39 sistance, and extracellular degradation factors can coexist even in well-mixed  
 40 microbial communities competing for one common limiting factor. Including  
 41 degradation resistance has a key role in their model, since excreting antibiotic  
 42 degrading molecules can weaken the inhibitory interaction between other species  
 43 thus balance the fitnesses through the community. Their study focuses mainly

44 on three species systems, in which species produce one type of antibiotics and  
45 reduce the effect of another type via degrading molecules (Fig. 1.b). The au-  
46 thors showed that coexistence of species in this system is robust to variation  
47 of model parameters even in well-mixed environment. They further demon-  
48 strated that analogous systems with four or five species producing 4-6 different  
49 antibiotics and degradation factors can have coexistence, although robustness  
50 is significantly less prevalent in these richer communities (Kelsic et al., 2015).  
51 However, the explanatory power and significance of degradation resistance in  
52 explaining microbial diversity largely depends on whether these communities  
53 prove to be resistant to the invasion of mutants, mainly against the invasion of  
54 social cheaters. A community is defined to be resistant or robust to the invasion  
55 of a mutant if its species composition does not change significantly after the  
56 invasion. That is, the mutant will be present in the community only transiently,  
57 and after its disappearance, the community returns to its pre-invasion state.

58 In the following, we study the generalized versions of KEA’s so-called mixed  
59 inhibition-zone and chemostat models (Kelsic et al., 2015), and show analytically  
60 that bacterial communities, independently of the interaction topology, are not  
61 robust against the invasion of social cheaters. More precisely, we show that  
62 mutant cheaters, loosing the costly function of antibiotic production, destroy any  
63 diverse community either in one step, or following a cascade of invasion steps.  
64 The other type of social cheaters considered in the model, the mutants loosing  
65 their functions of producing extracellular antibiotic degrading molecules have  
66 less dramatic effect on community stability, but species diversity still declines  
67 after the invasion of such mutants.

## 68 **2. Model description**

69 We assume that there are  $n_s$  phenotypically different species and  $n_a$  different  
70 antibiotics that can be produced by these species. A phenotype (or species) is  
71 defined by its relation to an antibiotic: it can produce, can be resistant to, or can  
72 be sensitive to the given antibiotic. Naturally, a species producing an antibiotic

73 is also resistant to it, where the resistance is carried out either by removing  
 74 antibiotic molecules from the cell via efflux mechanisms, or by neutralizing these  
 75 molecules within the cell (Kumar and Schweizer, 2005). Accordingly, a cell  
 76 producing an antibiotic  $l$  ( $P_l$ ) is also intrinsically resistant ( $R_l$ ) to this antibiotic.  
 77 Non-producing species can have two types of resistance: intrinsic resistance ( $R_l$ )  
 78 and degradation resistance ( $D_l$ ). Bacteria with degradation resistance produce  
 79 molecules and secrete to the extracellular matrix which diffuse and degrade the  
 80 target antibiotic molecules in a given neighborhood of the cell (Wright, 2005;  
 81 Bastos et al., 2015). Phenotypes which are not resistant to antibiotics  $l$  carried  
 82 out either by intrinsic or by degradation resistance, are considered sensitive  
 83 ( $S_l$ ) and the presence of this antibiotic in the locality reduces their fitnesses.  
 84 Thus, every species  $i = 1, 2, ..n_s$  is characterized by any of the four phenotypes  
 85  $P_l, R_l, D_l, S_l$  for each antibiotic  $l = 1, 2, ..n_a$ .

86 Let  $x_i$  be the abundance of species  $i$  per unit area, and assume that cells are  
 87 dispersed randomly on a two-dimensional surface. The fitness  $w_i$  of species  $i$  is  
 88 determined by its intrinsic replication rate  $g_i$  and the fraction of area  $1 - A_i^{(kill)}$   
 89 in which individuals of species  $i$  are not killed by antibiotics, that is

$$w_i = g_i(1 - A_i^{(kill)}). \quad (1)$$

90 Antibiotic  $l$  is effective within area  $K_l^{(P)}$  around the cell producing it and, sim-  
 91 ilarly, degrading molecules protect every sensitive cell within area  $K_l^{(D)}$  around  
 92 a cell producing this degrading molecule. A sensitive cell is killed if there is  
 93 at least one cell producing antibiotic  $l$  within its  $K_l^{(P)}$  neighborhood and there  
 94 is no bacterium producing degrading molecules for antibiotic  $l$  within its  $K_l^{(D)}$   
 95 neighborhood. Since the aim of this model is to show that coexistence is possi-  
 96 ble in unstructured environment, it is assumed that bacteria are dispersed  
 97 randomly, so the number of cells follows Poisson distribution within the defined  
 98 areas. Thus, the probability that at least one antibiotic producer cell is in the  
 99  $K_l^{(P)}$  neighborhood of a cell is  $1 - e^{-K_l^{(P)}x_p}$ , where  $x_p$  is the abundance of species  
 100 producing antibiotic  $l$ . This value gives the fraction of area in which sensitive  
 101 cells are killed except if they are protected by individuals producing degrading

102 molecules within area  $K_l^{(D)}$ . If the abundance of species producing degrading  
 103 molecules is  $x_d$ , then the probability of having no cells in this area is  $e^{-K_l^{(D)}x_d}$ .  
 104 So, species  $i$  is killed by antibiotic  $l$  in the fraction of area is as follows

$$A_{i,l}(x_d, x_p) = e^{-K_l^{(D)}x_d} \left(1 - e^{-K_l^{(P)}x_p}\right). \quad (2)$$

105 Since not only one species can produce antibiotics  $l$  or molecules degrading it,  
 106 the total area where at least one molecule of antibiotic  $l$  kills the sensitive species  
 107  $i$  is written as a product of the probabilities of all possible occurrences

$$A_{i,l}(x_1, x_2 \dots x_{i-1}, x_{i+1} \dots x_{n_s}) = A_{i,l}(\mathbf{x} \setminus x_i) = \prod_{j=1}^{n_s} e^{-\delta_{jl}K_l^{(D)}x_j} \left(1 - \prod_{j=1}^{n_s} e^{-\epsilon_{ijl}K_l^{(P)}x_j}\right), \quad (3)$$

108 where  $\delta_{jl} = 1$  if the  $j$ -th species degrades antibiotic  $l$ , otherwise  $\delta_{jl} = 0$ . Simi-  
 109 larly,  $\epsilon_{ijl} = 1$  if species  $i$  is sensitive to antibiotic  $l$  which is produced by species  
 110  $j$ , otherwise  $\epsilon_{ijl} = 0$  (for  $P$  and  $D$  type cells). Consequently, the fraction of  
 111 area where individuals of species  $i$  are not killed by any antibiotics of any other  
 112 species is

$$1 - A_i^{(kill)}(\mathbf{x} \setminus x_i) = \prod_{l=1}^{n_a} (1 - A_{i,l}(\mathbf{x} \setminus x_i)). \quad (4)$$

113 Thus, the fitness of species  $i$  will be

$$w_i = g_i \left(1 - A_i^{(kill)}(\mathbf{x} \setminus x_i)\right), \quad (5)$$

114 and the average fitness is

$$\bar{w} = \sum_{i=1}^{n_s} w_i x_i. \quad (6)$$

115 By knowing fitness functions for every species, the population dynamics of  
 116 the system can be described by the following discrete-time replication dynamics:

$$x_i(t+1) = \frac{c + w_i(t)}{c + \bar{w}(t)} x_i(t), \quad (7)$$

117 where the  $c > 0$  constant depends on the time unit (Weibull, 1997). For the  
 118 continuous time counterpart of the dynamics, see Appendix A.

119 We note here that KEA have pointed out previously, that the three-species  
 120 coexistence (see Fig 1.b) is robust if the areas of chemical activities ( $K_l^{(P)}$  and

121  $K_l^{(D)}$ ) and replication rates ( $g_i$ ) of all the three species are relatively similar.  
122 KEA have also shown that the same dynamics can be observed in the agent-  
123 based and the chemostat versions of the mixed inhibition-zone model (Kelsic  
124 et al., 2015). The detailed analyses of the generalized chemostat model can be  
125 found in Appendix C. They studied a system where  $K_l^{(P)} = K^{(P)}$  and  $K_l^{(D)} =$   
126  $K^{(D)}$  are constants for every antibiotic which assumption does not have to hold  
127 in our generalized model.

128 Besides the ecological stability of three species models, KEA investigated  
129 the invasion of "production cheaters", that is, the mutants which do not pro-  
130 duce antibiotics and "degradation cheaters" which do not produce degrading  
131 molecules. Losing these functions results in fitness increase for mutants, which  
132 is then translated into higher replication rates. Based on numerical simulations  
133 including cheaters in the community, they concluded that "These interactions  
134 enable coexistence that is robust to substantial differences in inherent growth  
135 rates and to invasion by 'cheating' species that cease to produce or degrade an-  
136 tibiotics." Our discussions with the authors clarified that they studied the evolu-  
137 tionary stability of this system in the spatially extended agent-based version of  
138 the mixed inhibition zone model, and analyzed it numerically for 3- and 4-species  
139 networks (Kelsic et al., 2015, 2016). They found that networks are resistant to  
140 both degradation and production parasites in these systems if the colonization  
141 radius is small enough. In the following sections, we show that cheater mutants  
142 crash such communities not only in the three-species 'rock-paper-scissors' in-  
143 teraction topology in the mixed inhibition model, but in the generalized mixed  
144 inhibition model, and similarly in the chemostat model with any interaction  
145 topology. In the discussion we explain briefly why the agent-based model with  
146 short range colonization behaves differently from the analytical model studied  
147 here.

### 148 **3. Results**

#### 149 *3.1. Evolutionary instability in the mixed inhibition-zone model: introducing* 150 *social cheaters*

151 Species having resistance  $D_l$  protect not only themselves but any other  
152 strains  $S_l$  in the neighborhood from the antibiotics, and similarly a strain  $P_l$   
153 producing antibiotic  $l$  generates empty space by killing sensitive individuals not  
154 only for itself but for non-producing strains  $R_l$  as well. Therefore these de-  
155 grading molecules and antibiotics are *public goods*, so strains not producing the  
156 costly degradation or antibiotic molecules have advantage over producers; thus  
157 these are *social cheaters* (Hardin, 1968; Cordero et al., 2012b). We consider two  
158 types of mutants, "production cheaters" that fail to produce antibiotics but re-  
159 tain intrinsic resistance to this antibiotic ( $P_l \rightarrow R_l$ ), and "degradation cheaters"  
160 that lose their resistance through antibiotic degradation and become suscepti-  
161 ble to the antibiotics ( $D_l \rightarrow S_l$ ). The benefit of non-producing extracellular  
162 materials results in higher replication rates for cheaters, that is the growth rate  
163 of mutant increases with  $(1 + \alpha)$ , where  $\alpha$  is an arbitrary, but generally small,  
164 positive number.

##### 165 *3.1.1. Invasion of antibiotic production cheaters*

166 Assume that an antibiotic production cheater evolves in a community in  
167 which  $n_s$  species are in a stable coexistence. (According to KEA, any type  
168 of species coexistence is possible from stable fixed points through limit cycles  
169 to chaotic behaviors. Our analysis remains valid for every type of dynamical  
170 coexistence.) Let us denote the mother species by  $m$ , and assume this species  
171 produces antibiotic  $l$ . The mutant  $m'$  of the mother loses the costly production  
172 of antibiotic  $l$  and consequently its replication rate increases as  $g_{m'} = g_m(1 + \alpha)$ .  
173 It follows from the definition of the model that the fitness function of species  $m$   
174 depends only on the abundances of the two types of species affecting survival:  
175 the species producing antibiotics for which the focal species is sensitive, and  
176 the species producing the molecules degrading this particular antibiotic (see



177 Eq. 3). Since  $m'$  remains sensitive to the same antibiotic as  $m$ , its replication  
 178 rate increases, but its fitness function does not change. Thus, the dynamics of  
 179 mother and mutant species are

$$x_m(t+1) = \frac{c + w_m(t)}{c + \bar{w}'(t)} x_m(t) \quad (8)$$

$$x_{m'}(t+1) = \frac{c + w_{m'}(t)}{c + \bar{w}'(t)} x_{m'}(t), \quad (9)$$

180 where  $\bar{w}'(t)$  is the average fitness in the population including the mutant. Di-  
 181 viding Eq. (8) by Eq. (9)

$$\frac{x_m(t+1)}{x_{m'}(t+1)} = \frac{c + w_m}{c + (1 + \alpha)w_m} \frac{x_m(t)}{x_{m'}(t)} \quad (10)$$

182 that is

$$\frac{x_m(t+1)}{x_{m'}(t+1)} = \left[ \frac{c + w_m(t)}{c + (1 + \alpha)w_m(t)} \right]^t \frac{x_m(0)}{x_{m'}(0)}. \quad (11)$$

183 Since  $0 < [c + w_m(t)]/[c + (1 + \alpha)w_m(t)] < 1$  for any  $c \geq 0$  then

184  $\lim_{t \rightarrow \infty} ([c + w_m(t)]/[c + (1 + \alpha)w_m(t)])^t = 0$  and consequently

$$\lim_{t \rightarrow \infty} x_m(t)/x_{m'}(t) = 0. \quad (12)$$

185 According to (12) three scenarios are possible: (i) both  $m$  and  $m'$  are selected  
 186 against in the community, but species  $m$  goes extinct faster than species  $m'$ ;  
 187 (ii) species  $m$  is selected against, and the invading mutant  $m'$  is getting fixed  
 188 in the community, but mutant  $m'$  triggers the loss of another species besides  
 189 the mother strain; (iii) species  $m$  is selected against, and species  $m'$  replaces it  
 190 in the community, so the number of coexisting species remains unchanged. In  
 191 case of scenarios (i) and (ii), the number of coexisting species decreases after  
 192 the invasion of the mutant. In scenario (iii) a non-producing cheater merely  
 193 replaces a producer.

194 Let us assume a sequence of production cheaters invading according to (iii).  
 195 The number of coexisting species doesn't change in this scenario, however if  
 196 there were  $n_a$  number of different antibiotics in the community then the num-  
 197 ber of antibiotics decreases to zero after at most  $n_a$  number of such a species  
 198 replacements. As a result, neither of the coexisting species produces antibi-  
 199 otics any more in this new community. However, survival of more than one

200 species becomes impossible in this situation, since the replication rate will be-  
 201 come  $w_i = g_i$  for every  $i$  as there are no more interactions between the species,  
 202 and thus only the species with the highest  $g_i$  will survive (survival of the fittest).  
 203 Consequently, in any of the above mentioned possible scenarios, species  $m$  (and  
 204 consequently the community) is *not resistant* against the invasion of mutant  $m'$   
 205 that has any replication benefit ( $\alpha > 0$ ) due to its loss of antibiotic producing  
 206 function. We show that continuous time replicator dynamics and the chemostat  
 207 model lead to completely similar results (see Appendix A and C for details).

### 208 3.1.2. Invasion of degradation cheaters

209 The other type of social cheater is the degradation cheater  $m'$ , which ceases  
 210 the production of degradation molecule synthesized by the mother species  $m$   
 211 against antibiotic  $l$ . By loosing this function,  $m'$  becomes sensitive to antibiotic  
 212  $l$  if it is present in the environment but its replication rate increases as  $g_m(1 + \alpha)$   
 213 at the same time. Thus, the equations of the mother and the mutant species  
 214 dynamics are

$$x_m(t+1) = \frac{c + w_m(t)}{c + \bar{w}'(t)} x_m(t) \quad (13)$$

$$x_{m'}(t+1) = \frac{c + (1 + \alpha)(1 - A_{m',l}(\mathbf{x} \setminus x_{m'}))w_m(t)}{c + \bar{w}'(t)} x_{m'}(t). \quad (14)$$

215 Dividing Eq. (13) by Eq. (14) we get

$$\frac{x_m(t+1)}{x_{m'}(t+1)} = \left[ \frac{c + w_m(t)}{c + (1 + \alpha)(1 - A_{m',l}(\mathbf{x} \setminus x_{m'}))w_m(t)} \right]^t \frac{x_m(0)}{x_{m'}(0)} \quad (15)$$

216 The fate of a mutant depends on the values of both  $\alpha$  and  $A_{m',l}(\mathbf{x} \setminus x_{m'})$ ,  
 217 thus the advantage of the invading mutant  $m'$  is insufficient yet. By defining  
 218  $A_{m',l}^{(max)} = \text{Max}\{A_{m',l}(\mathbf{x} \setminus x_{m'}) \mid x_i \in [0, 1], \sum_i x_i = 1\}$  a sufficient condition for  
 219 the invasion of mutant  $m'$  can be set. For  $\lim_{t \rightarrow \infty} x_m(t)/x_{m'}(t) = 0$  to be valid,  
 220 the expression in the square bracket on the right hand side of (15) must be in  
 221 the  $(0, 1)$  interval which leads to the following sufficient condition:

$$\alpha > \frac{A_{m',l}^{(max)}}{1 - A_{m',l}^{(max)}}. \quad (16)$$

222 Consequently, one of the above mentioned three possible scenarios describes  
 223 the fate of mutant  $m'$  in this case as well. However, besides the loss of species  
 224 diversity, according to the above described three invasion scenarios, it is possible  
 225 that the degradation-molecule producer and the sensitive mutant strains coexist.  
 226 To prove this we show that it is possible that  $m'$  invades the community where  
 227 type  $m$  is resident, but  $m$  invades the community where  $m'$  is resident. Let us  
 228 assume first that  $m$  is resident in a stably coexisting community. For the sake of  
 229 simplicity, we assume that coexistence is characterized by a stable fixed point,  
 230 denoted by  $\hat{\mathbf{x}}^{(1)}$ . The mutant  $m'$  emerges in small abundance, that is  $x'_m \ll \hat{x}_i^{(1)}$   
 231 for every  $i \neq m'$ ,  $\hat{x}_i^{(1)} > 0$ . Since  $x_i(t+1) = x_i(t)$  for every  $i$ ,  $\hat{x}_i^{(1)} > 0$  at the  
 232 equilibrium the abundance of the rare mutant  $m'$  increases in the community if  
 233 (cf. Eq. (14))

$$\frac{c + (1 + \alpha)(1 - A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'}))w_m(t)}{c + \bar{w}'(t)} > 1, \quad (17)$$

234 which leads to the condition

$$\alpha > \frac{A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})}{1 - A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})}. \quad (18)$$

235 Let us consider now  $m'$  as the resident species of the same community but  $m$  is  
 236 replaced by  $m'$  and thus  $m$  is the rare mutant. Let  $\hat{\mathbf{x}}^{(2)}$  denote the equilibrium  
 237 abundances before invasion, so the rare mutant  $m$  spreads if

$$\frac{c + \frac{w_{m'}(t)}{(1+\alpha)(1-A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'}))}}{c + \bar{w}'(t)} > 1, \quad (19)$$

238 (cf. Eq. (14) that is if

$$\alpha < \frac{A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'})}{1 - A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'})}. \quad (20)$$

239 Consequently, if  $A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'}) < A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})$  then both (18) and (20) can  
 240 be satisfied simultaneously, thus the rare  $m$  and  $m'$  mutants mutually invade  
 241 the communities in which the other is resident, which guarantees the coexis-  
 242 tence of these species. Naturally, this analysis assumes that beside species  $m$   
 243 and  $m'$  there is at least one another species that produces an antibiotic lethal

244 for species  $m'$ . Furthermore, it is assumed that residents  $m$  and  $m'$  are in co-  
245 existence with the same species, but their densities can be different. Identical  
246 conditions determine the invasion of mutants in a model based on continu-  
247 ous replicator dynamics (see Appendix B for details). Thus, according to our  
248 analytical investigation, degradation cheaters can coexist within the resident  
249 community, and can degrade resident community only if their replication rate  
250 is above a critical level. This level can be arbitrarily low or high depending on  
251 the parameters. In the next section, we will test the generality of our results  
252 using numerical investigations.

### 253 3.2. Numerical studies

254 Next, we run numerical investigations to test the effect of social cheaters, and  
255 for comparison we followed the methodology and parameters used by KEA in  
256 their simulations. In the first series of experiments we generated a statistically  
257 representative sample of ecologically stable communities of 3-5 coexisting species  
258 producing 2-5 different antibiotics, where the initially selected five species can  
259 be any of the four phenotypes  $(S_l, D_l, R_l, P_l)$  for each antibiotic  $l = 1, 2, \dots, 5$   
260 and the intrinsic replication rate for species  $i$  is:  $g_i = 1 + (i - 1) \cdot 0.005$ . The area  
261 of chemical activities were either  $K_l^{(P)} = K^{(P)} = 10$  and  $K_l^{(D)} = K^{(D)} = 3$  or  
262  $K_l^{(P)} = K^{(P)} = 30$  and  $K_l^{(D)} = K^{(D)} = 10$ . We randomly assembled communi-  
263 ties with five interacting species by assigning randomly selected phenotypes for  
264 each antibiotic  $l$  to each of the species. The initial abundances were  $1/n_s$  for  
265 each species. We repeated  $T = 10.000$  update steps according to Eq. (7) with  
266  $c = 0$  and determined the number of coexisting species and the type of equilib-  
267 rium at the end (fixed point, limit cycle or chaotic behavior). (We note that  
268  $c = 0$  is the standard parameter choice used by KEA as well, although  $c > 0$   
269 fits the mathematical deduction of the dynamics (Weibull, 1997). However, this  
270 modification does not alter the qualitative behavior of the model.) A species  
271 was considered to be extinct if its frequency went below  $0.01/n_s$  (Kelsic et al.,  
272 2015).

273 In agreement with Kelsic et al. (2015, Extended data Figure 8), we experi-

274 enced that only an extremely small fraction of possible interaction topologies  
275 were suitable to maintain complex communities. While three species remain  
276 in coexistence from the the initial five species networks in 1 out of  $10^2 - 10^3$   
277 randomly selected networks, five species could coexist only in 1 out of  $10^4 - 10^6$   
278 randomly selected networks on average (depending on the  $K^{(P)}$  and  $K^{(D)}$  pa-  
279 rameters). That is, in line with the Extended Data Figure 8 of Kelsic et al.  
280 (2015), we found that the fraction of stable communities decreases dramatically  
281 as the number of coexisting species increases.

282 After generating the sample of ecologically stable 3-5 species communities  
283 we tested the resistance of these communities against the production and degra-  
284 dation cheaters but only one function and only in one species could be lost at  
285 a time, thus either  $P \rightarrow R$  or  $D \rightarrow S$  mutants could emerge in the community  
286 for each possible case. The mutants with fitness of  $(1 + \alpha)g_i$  were introduced  
287 at the 10.000th time step with density of  $10^{-3}$ , and the density of the corre-  
288 sponding mother species was decreased by the same amount. After subsequent  
289 10.000 update steps the coexistence was monitored again, and we recorded the  
290 communities that could not resist invasion and hence diversity declined. We  
291 declared communities not being resistant to the invasion of mutants if at least  
292 one mutant type caused the number of coexisting species (with frequency higher  
293 than 0.01) to be smaller after  $T$  time steps compared to the number of species  
294 before the invasion. That is, we consider only the cases when the invasion of  
295 mutants decreases the number of coexisting species within one step (scenarios  
296 (i) and (ii)).

297 We tested the resistance of three, four, and five-species communities against  
298 the cheater mutants as the function of the  $\alpha$  growth-rate advantage of the mu-  
299 tants. There is a critical  $\alpha$  above which the fraction of unstable communities  
300 increases abruptly in a sigmoid manner (Fig. 2a). Species diversity declines  
301 dramatically in the majority of these communities even at as little as 0.1% rela-  
302 tive growth-rate advantage of mutants  $\alpha^* = \alpha/\bar{g}_i$  where  $\bar{g}_i$  is the average growth  
303 rate in the community. The rapid decline of diversity results in the exclusion  
304 of all but one species in most of the cases (around 70% of the outcomes in the

305 case of five species communities in Fig 1a). Production cheaters are responsible  
 for the decline of diversity in more than 99% of the cases.

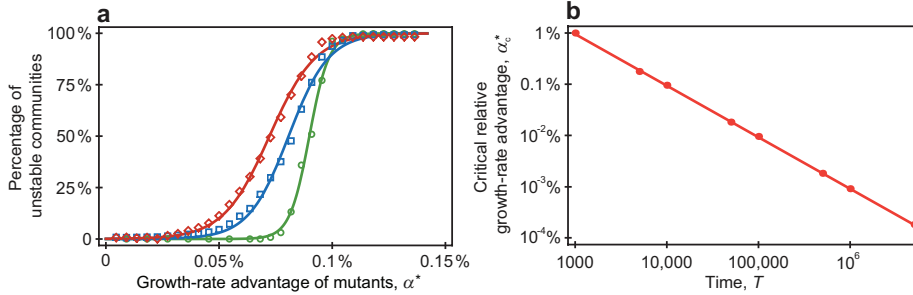


Figure 2: Measures of community instability fostered by cheater mutants. **(a)** The fraction of unstable communities increases in a sigmoid manner (depicted by colored lines) as the relative growth-rate advantage of cheater mutants increases. At 0.1% growth-rate advantage, the majority of the modeled communities become unstable. Statistics are based on  $10^3$  randomly selected communities composed of three (green circles), four (blue rectangles), and five (red diamonds) species. **(b)** The critical level of relative growth-rate advantage of mutants (where at least 99% of communities are not resistant to the invasion of at least one mutant type) decreases as the duration of simulations ( $T$ ) increases for  $10^3$  randomly selected interaction network topologies composed of 5 species. Parameters are:  $g_i = 1 + (i - 1) \cdot 0.05$ ,  $K_j^{(P)} = K^{(P)} = 30$ ,  $K_j^{(D)} = K^{(D)} = 10$ .

306

307 In our second analysis, we studied the dependence of community resistance  
 308 on simulation time. According to Eq. (11), it is straightforward to assume  
 309 that it takes more time to observe competitive exclusion if fitness differences  
 310 are smaller. To test this hypothesis, we repeated the numerical experiments  
 311 in five-species communities with parameters used in Figure 2a but for differ-  
 312 ent simulation times ( $T$ ), and measured the critical  $\alpha_c^*$ , that is the  $\alpha^*$  value  
 313 for which at least 99% of the communities proved to be unstable. As Figure  
 314 2b demonstrates,  $\alpha_c^*$  decreases continuously as the duration of the simulations  
 315 increases according to  $\alpha_c^* \propto T^{-1.05 \pm 0.01}$ . This relation is in concordance with  
 316 our analytical results, since the necessary condition to detect collapse of com-  
 317 munity is that  $x_m(t)/x_{m'}(t) \leq x_c$  where  $x_c$  is a critical frequency below which

318 the species is selected out by definition. It follows from Eq. (11) that

$$\ln(x_c) = T \ln \left( \frac{1}{1 + \alpha} \right). \quad (21)$$

319 For  $\alpha \ll 1$   $\ln[1/(1+\alpha)] \approx -\alpha$ , consequently  $\alpha \propto 1/T$  determines the relationship  
320 between these two variables in the extinction dynamics.

321 To investigate the different invasion scenarios discussed previously, we nu-  
322 merically analyzed the invasion dynamics of different production and degrada-  
323 tion cheaters in a community with the topology shown in Figure 3a. Note that  
324 in this case antibiotic production—sensitivity combinations are not cyclic as in  
325 Figure 1, but still each antibiotic is degraded by one of the species. This topol-  
326 ogy enables us to demonstrate all possible invasion events starting from the same  
327 community. We iterated the dynamics for 1000 time steps and then introduced  
328 mutants into the system. The number of coexisting species was monitored until  
329  $t = 2000$  (except in Fig. 4d in which case due to slow invasion dynamics the  
330 mutant was added at  $t = 2000$  and the simulation was terminated at  $t = 4000$ ).

331 Investigating the three invasion scenarios in the numerical model discussed  
332 previously (see Eq. (12) and afterwards) confirms that the invasion of mutants  
333 can (i) result in the extinction of both the mutant and the mother species (Fig.  
334 3b); (ii) result in the exclusion of mother species leading to a decrease in species  
335 diversity (Fig. 3c); and (iii) exclude the mother species but the mutant remains  
336 in coexistence with the other species (Fig. 3d).

337 Figure 3b shows the effect of the invasion of production cheater mutant  
338 for species 2 (mutant ceases producing the antibiotic that inhibits species 5).  
339 Although the invasion of this mutant is unsuccessful it triggers a community  
340 collapse and only one resident species (species 5 in this case) remains in the  
341 end. In Figure 3c the other possible production cheater mutant of species 2  
342 (mutant ceases producing the antibiotic that inhibits species 4) invades the  
343 system and reduces the number of coexisting species (to an odd number smaller  
344 than the original number of species; in our case to one).

345 Finally, in Figure 3d the same type of mutant with lower fitness advantage  
346 invades the community and replaces the mother species preserving the number of

347 coexisting species but reducing the number of interactions by one. In accordance  
348 with Eq. (12) and discussions afterwards, these results suggest that the invasion  
349 of cheater mutants can result in the loss of species diversity, antibiotic diversity,  
350 or both.

351 In case of degradation cheater invasion experiments (in model community  
352 with the same topology as in Fig. 3a) we found the four different outcomes in  
353 line with expectations from Eq. (16) and the discussion afterwards. In contrast  
354 to production cheater mutants, degradation cheaters cannot always invade the  
355 system, thus the community structure can remain intact, or the mutants can  
356 coexist with the original coalition (Fig. 4). In line with the first scenario of the  
357 production mutants, the degradation cheater (mutant of species 5) can destroy  
358 the coexistence and one of the original species survives (Fig. 4c), or the cheater  
359 (mutant of species 2) survives only after the community collapses (Fig. 4d).

#### 360 4. Discussion

361 Our results imply that the counteraction of antibiotic production by ex-  
362 tracellular antibiotic degradation does not in itself guarantee high diversity in  
363 antibiotic producing microbial communities. In particular, we pointed out that  
364 production cheaters with increased reproduction rate demolish the coexistence  
365 of interacting species in well-mixed models. According to our studies, three  
366 scenarios are possible: in two cases (scenarios (i) and (ii)) the invasion of pro-  
367 duction cheaters causes immediate decrease of the number of coexisting species.  
368 In scenario (iii) it takes more than one invasion events to decrease the number of  
369 coexisting species, but eventually a sequence of invasion events also leads to the  
370 decrease of species diversity. These results are valid for the mixed inhibition-  
371 zone model and the chemostat model with any interaction topology and even  
372 if the different antibiotics and degradation molecules have different diffusion  
373 abilities (different  $K_i^{(D)}$  and  $K_i^{(P)}$  parameters). It follows that the invasion  
374 success of production cheaters is independent of the model details. Our con-  
375 clusions remain valid for any other systems where the fitness of phenotype  $i$  is



376 described by  $g_i f_i(x_1(t), x_2(t), x_{i-1}(t), x_{i+1}(t), \dots)$ , where  $f_i(\mathbf{x} \setminus x_i)$  is an arbitrary  
377 continuous function and the replicator dynamic describes the selection among  
378 the different phenotypes (see Eqs. (9-12)). We found that the emergence of  
379 degradation cheaters causes less dramatic changes in the community; they are  
380 able to invade a stable community only if their fitness benefit is above a critical  
381 level, and in some cases the coexistence of mutant and resident types is possible  
382 after invasion.

383 Our numerical simulations show (in line with Kelsic et al. (2015) Extended  
384 Data Figure 8.) that the proportion of ecologically stable communities among  
385 randomly selected interaction topologies becomes negligibly low as the number  
386 of coexisting species increases to five or more. As in the current study the  
387 focus was on the evolutionary stability of microbial communities against invasion  
388 by cheaters, this aspect of ecological stability received less attention in our  
389 analyses. Similarly, in the study of KEA this behavior of the system did not  
390 receive sufficient attention. However, we would like to emphasize that it becomes  
391 increasingly unlikely that stable communities can emerge when the number of  
392 species increases. That is, besides the evolutionary instability, the robustness  
393 of ecological stability of these communities is also problematic in well-mixed  
394 models without additional mechanisms promoting diversity.

395 A more recent investigation by (Kelsic et al., 2016) pointed out that the  
396 spatially extended agent-based version of the mixed inhibition model exhibits  
397 resistance to invasion of cheaters. The crucial difference is that in this spatial  
398 extended model empty sites are colonized from a finite distance. A producer  
399 cell creates empty sites by killing sensitive cells in its neighborhood. Such cells  
400 have a greater chance for colonizing these empty sites than the non-producing  
401 cheaters being in the vicinity of the empty site. Thus producer cells have higher  
402 replication success than non-producers which can balance the higher per-capita  
403 replication rate of non-producer ones. The smaller the colonization distance  
404 the higher the benefit of producers compared to non-producers, and since the  
405 colonization distance tends to be infinite in the well-mixed models studied here  
406 this effect disappears.

407 We assumed in the analysis that the production of antibiotics and molecules  
408 degrading antibiotics is costly for the cells. In line with this assumption, there  
409 are numerous experiments demonstrating that the inactivation or loss of such  
410 genes have a significant positive effect on the fitness of such mutant types in a  
411 given environment (Lee and Marx, 2012; Koskiniemi et al., 2012; D’Souza et al.,  
412 2014). Moreover, other investigations reveal that such antibiotic resistance fac-  
413 tors can be the by-products of the general metabolism and thus the production  
414 costs are practically negligible (Melynk et al., 2014). In some cases, switching  
415 off such gene can even be beneficial for the cell due to pleiotropic effects of the  
416 regulating genes (Dandekar et al., 2012; Mitri and Foster, 2016). However, the  
417 high population size which is typical in bacterial communities enhances selection  
418 and thus it can dominate over genetic drift even for small fitness differences.

419 The mixed inhibition-zone and chemostat models consider the dynamics of  
420 well-mixed individuals producing diffusive antibiotics and degrading molecules.  
421 The assumptions behind these models enable us to handle the problem analyt-  
422 ically, however, these assumptions oversimplify some aspects of the dynamics.  
423 First and foremost a more realistic diffusion dynamics and chemical interactions  
424 among the dispersed molecules and cells are not taken into account. It is known  
425 from other studies that even minor modifications in the dynamics describing  
426 diffusion of public goods molecules, interaction of these molecules with cells,  
427 the non-linear relation between the molecule concentration and the fitness, and  
428 even timing of death and birth events in population dynamics can have signifi-  
429 cant effect on selection between producers and non-producers (Borenstein et al.,  
430 2013; Scheuring, 2014; Archetti, 2014).

431 Recent studies pointed out that the secreted extracellular molecules are not  
432 completely mixing public goods, because due to the restricted motion of cells and  
433 of molecules in real bacterial communities, only the immediate neighborhood of  
434 the producer is able to enjoy the benefits (Morris, 2015). As the close neighbors  
435 of the producer are most probably the clones of the producer, non-producers  
436 further away from the source can benefit much less. According to the exper-  
437 iments, these definite spatial effects establish density-dependent and negative

438 frequency-dependent selection which stabilizes the coexistence of the producers  
439 and social cheaters (Kerr et al., 2002; Cordero et al., 2012a; Drescher et al., 2014;  
440 Kümmerli et al., 2014; Morris, 2015). In addition, our results highlight that in-  
441 teractions of antibiotic production and attenuation are insufficient in effectively  
442 stabilizing bacterial communities in well-mixed environments. Presumably mi-  
443 croscale spacial structure of the habitat, negative frequency-dependent selection,  
444 pleiotropy, auxotrophy, and top down control by phages play more significant  
445 role in maintaining microbiome diversity (Cordero and Polz, 2014; Morris et al.,  
446 2012, 2014; Morris, 2015; Koskiniemi et al., 2012; D’Souza et al., 2014; Velend,  
447 2010; Ross-Gillespie et al., 2007, 2009; Dandekar et al., 2012; Mitri and Foster,  
448 2016; Kelsic et al., 2016).

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#### 454 **Appendix A. Continuous replicator dynamics: invasion of produc-** 455 **tion cheaters**

456 The continuous replication dynamics of bacterial strains is generally written  
457 as

$$\dot{x}_i(t) = (w_i(t) - \bar{w}(t))x_i(t), \quad (\text{A.1})$$

458 where  $w_i(t)$  and  $\bar{w}(t)$  are the fitness values of individuals and the population  
459 average as defined in the main text. Let us denote the mother and production  
460 cheater mutant with  $m$  and  $m'$ , respectively. Thus, the dynamics of these two  
461 types are

$$\dot{x}_m(t) = (w_m(t) - \bar{w}'(t))x_m(t) \quad (\text{A.2})$$

$$\dot{x}_{m'}(t) = ((1 + \alpha)w_m(t) - \bar{w}'(t))x_{m'}(t). \quad (\text{A.3})$$

462 Dividing the two equations by  $x_m(t)$  and  $x_{m'}(t)$ , respectively, and subtracting  
 463 Eq. (A.3) from Eq. (A.2), after some rearrangement we get

$$\frac{\dot{x}_m(t)}{x_m(t)} - \frac{\dot{x}_{m'}(t)}{x_{m'}(t)} = -\alpha w_m(t), \quad (\text{A.4})$$

464 which leads to

$$\frac{x_m(t)}{x_{m'}(t)} = e^{-\alpha \int_0^t w_m(\tau) d\tau}. \quad (\text{A.5})$$

465 Since  $w_m(t) > w_{min} > 0$ , where  $w_{min}$  is a constant, we have  $\lim_{t \rightarrow \infty} \int_0^t w_m(\tau) d\tau =$   
 466  $\infty$ . Therefore, equation (12), and consequently the three scenarios described in  
 467 the main text remain valid in continuous time dynamical systems as well.

## 468 **Appendix B. Continuous replicator dynamics: invasion of degrada-** 469 **tion cheaters**

470 In case of continuous replicator dynamics, the time evolution of  $m$  and  $m'$   
 471 species is

$$\dot{x}_m = (w_m(t) - \bar{w}(t)) x_m \quad (\text{B.1})$$

$$\dot{x}_{m'} = ((1 + \alpha)w_m(t)(1 - A_{m',l}(\mathbf{x} \setminus x_{m'})) - \bar{w}'(t)) x_{m'}, \quad (\text{B.2})$$

472 where  $m'$  denotes the degradation cheater. Following the algebraic steps de-  
 473 scribed in the previous subsection, we get

$$\frac{\dot{x}_m(t)}{x_m(t)} - \frac{\dot{x}_{m'}(t)}{x_{m'}(t)} = [1 - (1 + \alpha)(1 - A_{m',l}(\mathbf{x} \setminus x_{m'}))] w_m(t). \quad (\text{B.3})$$

474 The sign of the right hand side of (B.3) depends on  $\alpha$  and  $A_{m',l}(\mathbf{x} \setminus x_{m'})$ . As be-  
 475 fore, a sufficient condition for the invasion of mutant  $m'$  can be determined with  
 476 the help of the maximum value of  $A_{m',l}(\mathbf{x} \setminus x_{m'})$ : if  $\left[1 - (1 + \alpha)(1 - A_{m',l}^{(max)})\right] <$   
 477  $0$ , that is if

$$\alpha > \frac{A_{m',l}^{(max)}}{1 - A_{m',l}^{(max)}}. \quad (\text{B.4})$$

478 To determine the criterion of mutual invasibility, let us assume first that  
 479 type  $m$  is the resident species and type  $m'$  invades the community. For sake  
 480 of simplicity (as in the discrete model presented in the main text), we assume

481 that the dynamics of the resident population is in fixed point, the abundances  
 482 before invasion are denoted by  $\mathbf{x}^{(1)}$ . Mutant  $m'$  spreads if

$$\dot{x}_{m'}(t) = \left( (1 + \alpha)(1 - A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'}))w_m(t) - \bar{w}(t) \right) x_{m'}(t) > 0 \quad (\text{B.5})$$

483 which leads to

$$\alpha > \frac{A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})}{1 - A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})}. \quad (\text{B.6})$$

484 Let us consider now  $m'$  as the resident species in a community and  $m$  as the  
 485 rare mutant. Let  $\hat{\mathbf{x}}^{(2)}$  denote the equilibrium abundances before invasion, so  
 486 the rare mutant  $m$  spreads if

$$\dot{x}_m(t) = \left( \frac{w_{m'}(t)}{(1 + \alpha)(1 - A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'}))} - \bar{w}'(t) \right) x_m(t) > 0, \quad (\text{B.7})$$

487 which leads to the condition

$$\alpha < \frac{A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'})}{1 - A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'})}. \quad (\text{B.8})$$

488 Again, as in the discrete time dynamics, if  $A_{m',l}(\hat{\mathbf{x}}^{(2)} \setminus x_{m'}) < A_{m',l}(\hat{\mathbf{x}}^{(1)} \setminus x_{m'})$   
 489 then both (B.6) and (B.8) can be satisfied simultaneously, thus the rare  $m$   
 490 and  $m'$  mutants mutually invade each other which guarantees the coexistence  
 491 of these species. (Naturally, this analysis assumes that beside species  $m$  and  
 492  $m'$  at least one similar a species is present in the community which produces  
 493 antibiotic affecting species  $m'$ .)

## 494 Appendix C. Invasion of production cheaters in the chemostat model

495 Here we review the chemostat model version of microbial community with  
 496 interference competition. Following Kelsic et al. (2015), it is assumed that  
 497 bacteria compete for a common limiting resource  $z$  and there is a constant  
 498 dilution  $d$  from the chemostat. The dynamics of the resource is

$$\dot{z}(t) = (z_0 - z(t))d - \frac{\sum_{i=1}^{n_s} w_i(t)x_i(t)}{\mu}, \quad (\text{C.1})$$

499 where  $z_0d$  is the constant inflow into the chemostat,  $w_i(t)$  is the actual growth  
 500 rate of species  $i$  with concentration  $x_i$  and  $\mu$  is a conversion factor between

501 resource and species concentration. The species concentrations change according  
 502 to

$$\dot{x}_i(t) = (w_i(t) - d) x_i(t), \quad (\text{C.2})$$

503 with

$$w_i(t) = g_i \frac{z(t)}{k_z + z(t)} \prod_{j=1}^{n_a} e^{-\sigma_{i,j} K_j^{(P)} c_j(t)}, \quad (\text{C.3})$$

504 that is the growth rate  $w_i(t)$  is determined by the intrinsic growth rate  $g_i$ , the  
 505 concentrations of the resource and the antibiotics  $z(t)$  and  $c_j(t)$ , respectively.  
 506 The effect of  $z$  is saturated in line with the standard Michaelis-Menten kinetics  
 507 with half saturation constant  $k_z$  and the antibiotics cause exponential decay on  
 508 total growth rate,  $\sigma_{i,j} = 1$  if species  $i$  is sensitive to antibiotic  $j$  otherwise  $\sigma_{i,j} =$   
 509  $0$ . The concentration of the antibiotics changes because of the production, the  
 510 degradation, and the dilution of antibiotics, thus the dynamics can be written  
 511 as

$$\dot{c}_j(t) = \rho \sum_{i=1}^{n_s} \eta_{i,j} w_i(t) x_i(t) - K_j^{(D)} c_j(t) \sum_{i=1}^{n_s} \delta_{i,j} x_i(t) - d c_j(t), \quad (\text{C.4})$$

512 where  $\rho$  is the amount of antibiotics produced by unit concentration of cells,  
 513  $\eta_{i,j} = 1$  if antibiotic  $j$  produced by species  $i$ , otherwise  $\eta_{i,j} = 0$ . Similarly  
 514  $\delta_{i,j} = 1$  if species  $i$  produces degradation molecules for antibiotic  $j$ , otherwise  
 515  $\delta_{i,j} = 0$ . It follows from (C.1) and (C.2) that

$$\frac{d}{dt} \left( \sum_{i=1}^{n_s} \frac{x_i(t)}{\mu} + z(t) - z_0 \right) = -d \left( \sum_{i=1}^{n_s} \frac{x_i(t)}{\mu} + z(t) - z_0 \right), \quad (\text{C.5})$$

516 thus after a transient time

$$z(t) = z_0 - \sum_i \frac{x_i(t)}{\mu}. \quad (\text{C.6})$$

517 Therefore (C.1) can be eliminated when we study the stationary solutions of  
 518 the system by substituting (C.6) into (C.3) (Kelsic et al., 2015).

519 Let us assume that dynamics of a bacterial community is described by (C.1-  
 520 C.4), and a species  $m$  is a member of a community ( $\bar{x}_m > 0$  in the stationary  
 521 state), and produces at least one type of antibiotic. The mutant  $m'$  species  
 522 loses the production of this antibiotic, thus it has an increased growth rate

523 ( $g_{m'} = (1 + \alpha)g_m, \alpha > 1$ ) as above. Thus, the difference of relative growth rates  
 524 of  $m$  and  $m'$  species is

$$\frac{\dot{x}_m(t)}{x_m(t)} - \frac{\dot{x}_{m'}(t)}{x_{m'}(t)} = w_m(t) - w_{m'}(t) = -\alpha \frac{z(t)}{k_z + z(t)} \prod_{j=1}^{n_a} e^{-\sigma_{m,j} K_j^{(P)} c_j(t)}. \quad (\text{C.7})$$

525 Our aim here is to show that  $z(t)/(k_z + z(t)) \prod_j e^{-\sigma_{m,j} K_j^{(P)} c_j(t)} > W_0 > 0$  if  
 526  $t > t_c$  which guarantees that  $\lim_{t \rightarrow \infty} x_m(t)/x_{m'}(t) = 0$ . It follows from (C.2)  
 527 that  $x_i(t) \geq 0$  if  $x_i(0) > 0$  and thus because of (C.6)  $z(t) \leq z_0$  and  $x_i < \mu z_0$  for  
 528 every  $i$ . Therefore,  $w_i(t) < g_i z_0 / (k_z + z_0)$  and the right hand side of (C.4) can  
 529 be estimated above with

$$\dot{c}_j(t) < \rho \mu \frac{z_0^2}{k_z + z_0^2} n_s g_{\max} - \left( K^{(D)} \mu z_0 n_s + d \right) c_j(t) = \alpha_1 - \alpha_2 c_j(t) \quad (\text{C.8})$$

530 where  $g_{\max} = \max\{g_i, i = 1, \dots, n_s\}$ ,  $\sum_{i=1}^{n_s} \eta_{i,j}$  and  $\sum_{i=1}^{n_s} \eta_{i,j}$  can be estimated  
 531 above by  $n_s$ . Here  $\alpha_1, \alpha_2$  are positive constants. By introducing function  $C(t)$   
 532 in such a way that its derivative estimates over  $\dot{c}(t)$ , we get

$$\dot{c}_j(t) < \dot{C}_j(t) = \alpha_1 - \alpha_2 C(t) \quad (\text{C.9})$$

533 This estimation is valid as the ordering between derivatives guarantees  $C(t) >$   
 534  $c(t)$  if  $t > t^*$ . It is easy to show that  $\lim_{t \rightarrow \infty} C_i(t) = C^*$  where  $C$  is a finite  
 535 positive constant, thus  $\lim_{t \rightarrow \infty} c_i(t) \leq C^*$  for every  $i$ . Similarly, knowing that  
 536  $\sum_{i=1}^{n_s} x_i / \mu \leq z_0$  and using the estimation introduced above Eq. (C.1) can be  
 537 estimated below with

$$\dot{z}(t) \geq \dot{Z}(t) = (z_0 - Z(t))d - g_{\max} \frac{z_0}{\mu(k_z + z_0)} Z(t), \quad (\text{C.10})$$

538 Since  $\lim_{t \rightarrow \infty} Z(t) = Z^* > 0$ , thus  $\lim_{t \rightarrow \infty} z(t) \geq Z^*$ . That is,  $z/(k_z +$   
 539  $z) \prod_j e^{-\sigma_{i,j} K_i^{(P)} c_j(t)} > Z^* / (k_z + Z^*) \prod_j e^{-\sigma_{i,j} K_i^{(P)} C^*} = W_0 > 0$  for every  $t$  greater  
 540 than a critical time  $t_c$ . Thus

$$\lim_{t \rightarrow \infty} x_m(t)/x_{m'}(t) = 0 \quad (\text{C.11})$$

541 as in the mixed inhibition model. We note here that the calculation remains  
 542 valid if we use any monotonously decreasing function to model the effect of the  
 543 antibiotic.

544 **References**

- 545 Archetti, M., 2014. Stable heterogeneity for the production of diffusible factors  
546 in cell populations. *PLOS One* 9, DOI: 10.1371/journal.pone.0108526.
- 547 Arnosti, C., 2011. Microbial extracellular enzymes and the marine carbon cycle.  
548 *Ann. Rev. Mar. Sci.* 3, 405–425.
- 549 Bastos, M. C., Coelho, M. L., Santos, O. C., 2015. Resistance to bacteriocins  
550 produced by gram-positive bacteria. *Microbiology* 161, 683–700.
- 551 Bernier, S. P., Surette, M. G., 2013. Concentration-dependent activity of an-  
552 tibiotics in natural environments. *Front. in Microbiol.* 4, 20:1–14.
- 553 Borenstein, D. B., Meir, Y., Shaevitz, J. W., Wingreen, N. S., 2013. Non-local  
554 interaction via diffusible resource prevents coexistence of cooperators and  
555 cheaters in a lattice model. *PLOS One* 8, DOI:10.1371/journal.pone.0063304.
- 556 Cordero, O. X., Polz, M. F., 2014. Explaining microbial genomic diversity in  
557 light of evolutionary ecology. *Nat. Rev. Microbiol.* 12, 263–273.
- 558 Cordero, O. X., Ventouras, L., DeLong, E. F., Polz, M. F., 2012a. Public good  
559 dynamics drive evolution of iron acquisition strategies in natural bacterio-  
560 plankton populations. *Proc. Natl. Acad. Sci. USA* 109(49), 120059–120064.
- 561 Cordero, O. X., Wildschutte, H., Kirkup, B., Proehl, S., Ngo, L., Hussain,  
562 F., Le Roux, F., Mincer, T., Polz, M. F., 2012b. Ecological populations of  
563 bacteria act as socially cohesive units of antibiotic production and resistance.  
564 *Science* 337, 1228–1231.
- 565 Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J. M., Relman,  
566 D., 2012. The application of ecological theory toward an understanding of the  
567 human microbiome. *Science* 336, 1255–1262.
- 568 Coyte, K. Z., Schluter, J., Foster, K. R., 2015. The ecology of the microbiome:  
569 Networks, competition, and stability. *Science* 350, 663.



570 Czárán, T. L., Hoekstra, R. F., Pagie, L., 2002. Chemical warfare between  
571 microbes promotes biodiversity. *Proc. Natl. Acad. Sci. USA* 99, 786–790.

572 Dandekar, A. A., Chugani, S., Greenberg, E. P., 2012. Pleiotropy and the low  
573 cost of individual traits promote cooperation. *Science* 338, 264–266.

574 Drescher, K., Nadell, C. D., Stone, H. A., Wingreen, N. S., Bassler, B. N., 2014.  
575 Solutions to the public goods dilemma in bacterial biofilms. *Curr. Biol.* 24,  
576 50–55.

577 D’Souza, G., Waschina, S., Pande, S., Bohl, K., Kaleta, C., Kost, C., 2014. Less  
578 is more: Selective advantages can explain the prevalent loss of biosynthetic  
579 genes in bacteria. *Evolution* 68, 2559–2570, doi:10.1111/evo.12468.

580 Gause, G. F., 1934. *The struggle for existence*. Baltimore, MD: Williams &  
581 Wilkins.

582 Hacker, J., Carniel, E., 2001. Ecological fitness, genomic islands and bacterial  
583 pathogenicity. *EMBO Rep.* 2, 376–381.

584 Hardin, G., 1968. The tragedy of the commons. *Science* 162, 1243–1248.

585 Hibbing, M. E., Fuqua, C., Parsek, M. R., Peterson, S. B., 2010. Bacterial com-  
586 petition: surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.*  
587 8, 15–25 doi:10.1038/nrmicro2259.

588 Károlyi, G., Neufeld, Z., Scheuring, I., 2005. Rock-scissors-paper game in chaotic  
589 flow: The effect of dispersion on the cyclic competition of microorganisms. *J.*  
590 *Theor. Biol.* 236, 12–20.

591 Kelsic, E. D., Zhao, J., Vetsigian, K., Kishony, R., 2015. Counteraction of an-  
592 tibiotic production and degradation stabilizes microbial communities. *Nature*  
593 521, 516–519.

594 Kelsic, R. D., Vetsigian, K., Kishony, R., 2016. Evolutionary stability of micro-  
595 bial communities with antibiotic degrading species. *bioRxiv*, 1–4.  
596 URL <http://dx.doi.org/10.1101/045732>

597 Kerr, B., Riley, M. A., Feldman, M. W., Bohannan, B. J. M., 2002. Local  
598 dispersal promotes biodiversity in real-life game of rock-paper-scissors. *Nature*  
599 418, 171–174.

600 Koskiniemi, S., Sun, S., Berg, O. G., Anderson, D. I., 2012. Selection-driven  
601 gene loss in bacteria. *Plos Genet.*, e1002787.

602 Kumar, A., Schweizer, H. P., 2005. Bacterial resistance to antibiotics: Active  
603 efflux and reduced uptake. *Adv. Drug Del. Rev.* 57, 1486–1513.

604 Kümmerli, R., T., S. K., Waldvogel, T., McNeill, K., Ackermann, M., 2014.  
605 Habitat structure and the evolution of diffusible siderophores in bacteria.  
606 *Ecol. Lett.* 12, 1536–1544.

607 Lee, M. C., Marx, C. J., 2012. Repeated, selection-driven genome reduction of  
608 accessory genes in experimental populations. *Plos Genet.* 8, e1002651.

609 Melnyk, A. H., Wong, A., Kassen, R., 2014. The fitness costs of antibiotic  
610 resistance mutations. *Evol. Appl.* 8, 273–283.

611 Miller, M. B., Bassler, B. L., 2001. Quorum sensing in bacteria. *Ann. Rev.*  
612 *Microbiol.* 55, 165–199.

613 Mitri, S., Foster, K. R., 2016. Pleiotropy and the low cost of individual traits  
614 promote cooperation. *Evolution* 70, 488–494, DOI: 10.1111/evo.12851.

615 Morris, J. J., 2015. Black queen evolution: the role of leakiness in structuring  
616 microbial communities. *Trends in Genetics* 31, 475–482.

617 Morris, J. J., Lenski, R. E., Zinser, E. R., 2012. The black queen hypothesis:  
618 evolution of dependencies through adaptive gene loss. *mBio* e00036-12.

619 Morris, J. J., Papoulis, S. e., Lenski, R. E., 2014. Coexistence of evolving bac-  
620 teria by shared black queen function. *Evolution* 68, 2960–2971.

621 Müller, A. P. O., Gallas, J. A. C., 2010. How community size affects survival  
622 chances in cyclic competition games that microorganisms play. *Phys. Rev. E*  
623 82, 052901.

- 624 Ross-Gillespie, A., Gardner, A., Buckling, A., West, S. A., Griffin, A. S., 2009.  
625 Density dependence and cooperation: theory and a test with bacteria. *Evo-*  
626 *lution* 63, 2315–2325.
- 627 Ross-Gillespie, A., Gardner, A., West, S. A., Griffin, A. S., 2007. Frequency  
628 dependence and cooperation: theory and a test with bacteria. *Am. Nat.* 170,  
629 331–342.
- 630 Scheuring, I., 2014. Diffusive public goods and coexistence of cooperators and  
631 cheaters on a 1d lattice. *PLOS One* 9, DOI:10.1371/journal.pone.0100769.
- 632 Velend, M., 2010. Conceptual synthesis in community ecology. *Quart. Rev. Biol.*  
633 85, 183–206.
- 634 Weibull, J. W., 1997. *Evolutionary Game Theory*. The MIT Press Cambridge,  
635 Massachusetts, London, England.
- 636 West, S. A., Griffin, A. S., A., G., Diggle, S. P., 2001. Social evolution theory  
637 for microorganisms. *Nat. Rev. Microbiol.* 4, 597–607.
- 638 Wright, G. D., 2005. Bacterial resistance to antibiotics: enzymatic degradation  
639 and modification. *Adv. Drug Deliv. Rev.* 57, 1451–1470.

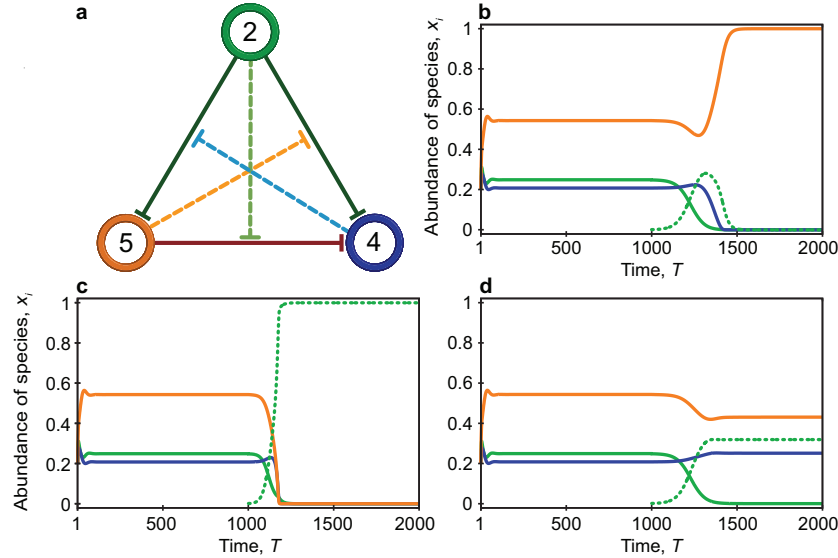


Figure 3: Invasion dynamics of different production cheaters in a model community. (a) The interaction topology of the model community. Each species produces different antibiotics, and species numbering represents the increments in reproduction rates as described in Methods. Species 2 is not affected by any antibiotic, species 5 is inhibited by antibiotic produced by species 2, and species 4 is inhibited by two different antibiotics produced by species 2 and 5. Three different scenarios of production cheater mutant (depicted by dashed lines) invasions: (b) both the introduced mutant and the corresponding mother species go extinct after the invasion of production cheater mutant for species 2 (that ceases producing the antibiotic that inhibits species 5, depicted by the green dashed line), (c) the invasion of production cheater mutant of species 2 (that ceases producing the antibiotic that inhibits species 4, depicted by the green dashed line) results in the exclusion of the mother type and triggers further species loss, and finally (d) the production cheater mutant of species 2 (that ceases producing the antibiotic that inhibits species 4, depicted by the green dashed line), similarly as in the previous numerical experiment, but with lower fitness advantage, replaces the mother lineage. Parameters are the same as in Fig. 2,  $\alpha = 0.05$  for (b,d),  $\alpha = 0.1$  for (c). Orange, green, blue solid lines correspond to species 5, 2, 4, respectively. Dashed line denotes the actual mutant colored similarly as its mother species.

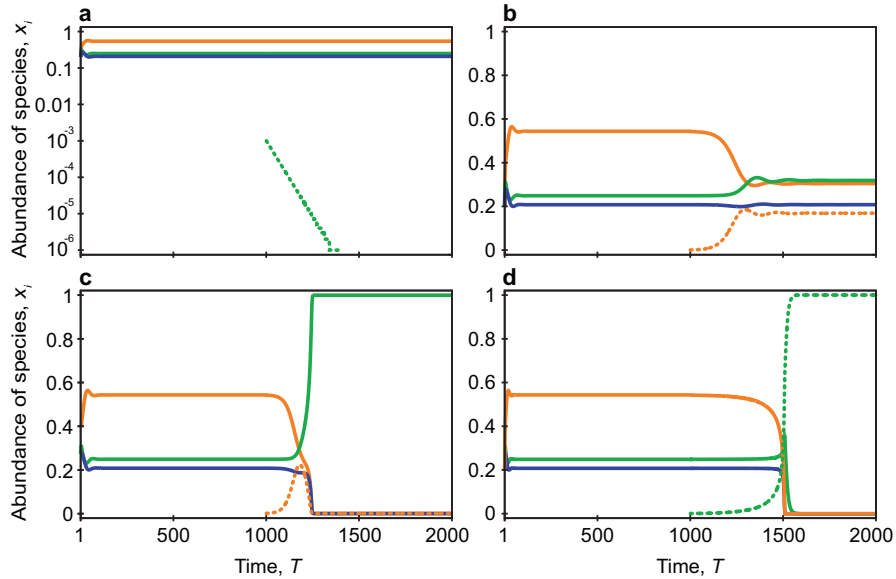


Figure 4: Four different scenarios for the invasion of degradation cheater mutants (dashed lines) in model communities depicted by Figure 3a. **(a)** Unsuccessful invasion of the degradation mutant of species 2 (that ceases to produce the factor degrading the antibiotic produced by species 5, depicted by the green dashed line), where the resident community remains unchanged after the invasion attempt. **(b)** Successful invasion of degradation mutant of species 5 (that ceases to produce the factor degrading one of the antibiotics produced by species 2, depicted by the orange dashed line), leading to the coexistence of all species, the residents and the mutant. **(c)** The invasion of degradation mutant of species 5 fails, but triggers species extinctions in the community, and one resident species survives in the end. **(d)** The mutant of species 2 successfully invades a stable community and excludes all other species. Parameters and color coding are the same as in Figure 3,  $\alpha = 0.05$  for **a** and **b**,  $\alpha = 0.08$  for **c**, and  $\alpha = 0.1$  for **d**.