Recent work with microbial communities has demonstrated an adaptive response to artificial selection at the level of the ecosystem. The reasons for this response and the level at which adaptation occurs are unclear: does selection act implicitly on traits of individual species, or are higher-level traits genuinely being selected? If the ecosystem response is just the additive combination of the responses of the constituent species, then the ecosystem response could be predicted a priori, and the ecosystem-level selection process is superfluous. However, if the ecosystem response results from ecological interactions among species, then selection at a higher level is necessary. Here we perform artificial ecosystem selection experiments on an individual-based evolutionary simulation model of microbial ecology and observe a similar response to that seen with real ecosystems. We demonstrate that a significant fraction of artificially selected ecosystem responses cannot be accounted for by implicit lower-level selection of a single type of organism within the community, and that interactions among different types of organisms contribute significantly to the response in the majority of cases. However, when the ecological problem posed by the artificial ecosystem selection process can be easily solved by a single dominant species, it often is.

**Keywords:** multilevel selection, microbial ecology, evolution, ecosystem selection

Population-based modeling has been used to explore multilevel selection in metacommunities, where it was shown that higher-level selection pressures could have a strong effect on ecological dynamics (10). Generalized Lotka–Volterra equations were used to model communities inhabiting semi-isolated patches, and patch-level selection pressures created by the metacommunity structure were shown to cause the local patch communities to diverge to different equilibria than would otherwise have been reached. More recently, population-based simulations of artificial ecosystem selection have been reported (11–13). Responses to artificial ecosystem selection for diversity (11) and for maximization of an arbitrary linear function of species composition (12) were found in a system modeling competition among different species, also based on sets of Lotka–Volterra population equations. However, Lotka–Volterra models do not allow direct mutualisms or metabolic dependencies, do not consider energy and material flows, and are deterministic, making them significantly removed from the stochastic, mutualistic, and thermodynamically constrained natural world. More importantly, the interaction matrices used (10–13) were fixed for the duration of each ecosystem “generation,” meaning there was no possibility of individual-level adaptation. This limitation on the effects of selection at the individual level is problematic for any study of multilevel selection, where the central questions concern the interaction between selection pressures at lower and higher levels. Although theoretical models that incorporate individual-level adaptation have been used to study frequency-dependent effects such as resource competition (14), no such models have been applied to study higher-level selection in the present context.

Here we present a set of artificial ecosystem selection experiments performed on simulated microbial microcosms held in isolated containers. The selected ecosystems are evolving microbial communities interacting with their abiotic environment, with selection performed on properties of the coupled biotic-abiotic system. The model allows individual-level adaptation and natural selection pressure generated by ecological interactions to be incorporated alongside artificially imposed ecosystem-level selection on the effect of the community on its abiotic environment. Artificial ecosystem selection experiments are performed by using a method similar to Swenson et al. (1, 2), by using properties of the environment as a target. A response similar to that seen in the laboratory experiments is observed and found to be robust to different ecosystem transmission methods, to the time for which the ecosystems are allowed to develop between selection events, and to the mutation rate of individuals. We further show whether the ecosystem response can be decomposed into the independent responses of individual species or is a genuine community-level property. (Although the concept of a species is not well defined for microbes, we use the term here to refer to a clonal group of individuals.)
To address the question of how artificial selection produces a response in the selected ecosystems, we ask two main questions. First of all, was selection above the level of a single species (clonal group) necessary to achieve the observed response? This question is answered by searching for a species in the selected community that is alone responsible for generating the desired ecosystem property; if such a species exists, it could potentially have been found by lower-level artificial selection methods. Second, if higher-level selection is shown to be necessary, we ask whether the observed response results from the additive combination of a number of species, each making an independent contribution to the net response, or whether the observed response results from ecological interactions among species. If the former, the same response could in theory have been produced by carefully picking a complementary set of species based on their individual properties, and selection at the level of the ecosystem is superfluous (despite being the mechanism by which the community was assembled in this case). If the latter, the observed performance is a nonadditive function of the actions of the constituent species of the community (i.e., the community response is not equal to the sum of its parts) and is therefore not decomposable. In such cases we have the strongest argument for higher-level selection acting on traits above the level of the individual.

**Model Description.** The “Flask” model (15, 16) [see supporting information (SI) Text and SI Table 2] simulates a flask containing a neutral liquid matrix in which is suspended a microbial population. The composition of the liquid medium determines the environment of the microbes. Some of the chemicals present are “nutrients” that may be consumed as food and converted to biomass, whereas others are nonconsumable and form part of the abiotic environment. The environment is assumed to have properties such as temperature, pH, salinity, etc., that both affect and can be affected by microbial activity. Nonconsumable chemicals and physical properties of the flask environment are collectively referred to as “abiotic factors,” to distinguish them from nutrients.

There is a flow of liquid medium through each flask that occurs continuously at a prescribed rate. The inflow brings with it influxes of nutrients at fixed concentrations and steady inputs to abiotic factors, whereas the outflow removes fixed proportions of stored nutrients and abiotic factors. The liquid medium in each flask is assumed to be well mixed, so that in the absence of perturbation, the composition of the medium in each flask will reach a homogeneous steady state.

Microbes are modeled as simple organisms that consume and excrete nutrients and affect the levels of abiotic factors in their environment as a byproduct of metabolism. The precise ratios in which nutrients are consumed and excreted are genetically encoded for each individual, as are associated effects on abiotic factors and preferred abiotic conditions (i.e., the state of the abiotic environment in which growth rate is maximized). The amount of nutrients consumed by a microbe is constrained by availability, by a universal maximum consumption rate, and by the fit between the current state of the abiotic environment and the microbe’s preferences. Microbes affect the environmental levels of abiotic factors as a by-product of their metabolic actions, in proportion to the amount of biomass created.

Microbes grow by converting consumed nutrients to biomass and reproduce by splitting when their biomass reaches a fixed threshold. All nutrients have an equal value, and microbes have a universal standard conversion efficiency, so that the only determinants of differential growth rates among microbes are their genetically specified metabolisms. A microbe that grows at the maximum possible rate will reproduce approximately every 12 timesteps, but nutrient limitation and adverse abiotic conditions commonly cause much slower growth rates; the growth and reproduction of microbes mean that nutrient limitation is the normal ecosystem condition. Mutation may occur during each reproduction event by selecting a new random allele with probability $P_{\text{mut}}$ (experimentally varied in the range $[0,0.1]$) at each locus; otherwise each offspring microbe receives an identical copy of the parental genotype. Biomass is reduced at a fixed rate to represent the inevitable thermodynamic inefficiency of metabolism and the cost of maintaining cellular machinery. Microbes die if their biomass drops below a fixed threshold, which can happen in sustained periods of nutrient limitation. They may also die “from natural causes,” with a low probability at each timestep. This mechanism is a catch-all for death by predation, senescence, etc., and serves to thin out the microbial population in an unbiased way, thus promoting continuing competition and individual-level selection. When a microbe dies, it is assumed that its remaining biomass is washed out and lost from the system.

Growth of a population occurs only as a result of individual growth and reproduction and is not specified a priori as in more traditional population ecology models such as Lotka–Volterra systems. Flask ecosystem carrying capacities are determined by nutrient supply and typically measure in the hundreds for the parameters used. These quantities are small by comparison with real-world microbial populations, a constraint enforced by the demands of computational tractability, but the small population sizes (and high mutation rates) are reasonable if each individual in the model is considered to represent many genetically similar real-world individuals. The shared environment creates individual-level selection pressure on metabolic requirements and environmental preferences, but the nature of this selection pressure changes over time as microbial activity alters the environment. Ecological and evolutionary dynamics of these model ecosystems are discussed elsewhere (16).

**Artificial Selection Target.** The “phenotypic” ecosystem trait used for artificial selection is based on the levels of the abiotic factors in the flask environment. Basing the fitness of flask ecosystems on properties of the environment rather than the biotic population avoids any prespecification of the type of population that will provide a good solution to the evolutionary problem. An arbitrary target state of the abiotic environment is assigned, with the deviation error of the actual abiotic state of a flask from this target constituting its performance score, $\Phi$:

$$\Phi = \sqrt{\sum_{i=1}^{A} (\tilde{a}_i - \bar{a}_i)^2},$$

where $\tilde{a}_i$ is the target level for abiotic factor $a_i$, and $\bar{a}_i$ is the actual level of $a_i$, in the normalized state vector for the $A$ abiotic factors included in the model. Depending on the direction of artificial selection, the fitness of a flask ecosystem is based on maximizing or minimizing $\Phi$. In each artificial selection experiment, three lines were selected based on the same initial random population: The “high” line was selected to maximize $\Phi$, the “low” line was selected to minimize $\Phi$, and the “random” line (where the source ecosystem used to create the batch of ecosystems for each iteration was chosen at random) acted as a control. All lines consisted of a number of iterations of directed selection followed by an equivalent number of iterations of random selection, to allow study of the relaxation of the selected response. The different lines present different types of evolutionary “problem” for the artificial ecosystem selection process to “solve.” Because $\Phi$ measures the distance from a target, we may a priori say that the low line presents a more difficult problem than the high line, because converging on a target is more difficult than diverging from it; there are many ways to be far from a point in multidimensional space, but only one way to hit it. Furthermore, in a complex dynamic environment, holding an environmental variable close to a particular target level will often require correction in two direc-
tions. Because a single species can push any environmental variable in only one direction, at least two species may therefore be needed to provide the necessary opposing influences for the low line (target-seeking) problem. However, a variable can be moved away from a target by pushing in a single direction, so a single dominant species in the community may offer a good solution to the high line (target-avoiding) problem. Thus the high and low lines offer qualitatively different evolutionary problems that may demand qualitatively different ecosystem solutions.

Artificial ecosystem selection is an iterative process based on preferentially sampling from successive batches of flask ecosystems to create each succeeding batch. After a randomly seeded initial batch, at each iteration of the selection process, a new batch of flask ecosystems is created by inoculating sterile flasks with individuals from the fittest flasks of the previous iteration. A single inoculum of a fixed number of individuals is created by sampling at random from the source flasks, and identical copies of this inoculum are then used to seed the entire new batch of ecosystems. Two sampling methods are used: a propagule method, where the inoculum is drawn from a single source ecosystem, and a migrant pool method, where the inoculum takes individuals from several source ecosystems. The propagule method is analogous to asexual reproduction and should preserve ecological interactions among individuals. The migrant pool method is analogous to sexual reproduction and may contribute significantly to the selected ecosystem function. Therefore, whether any species taken from an artificially selected community can survive and persist in the context of a wild-type community gives performance that equals or exceeds that of the selected community.

### Results

A robust response to artificial selection is seen in our model ecosystems (e.g., Fig. 1). For an arbitrarily chosen target vector, in both the high and low selected lines, the normalized abiotic environment state vector quickly diverges from the randomly selected control line. The high line is selected to maximize \( \Phi \) (the distance of the actual abiotic environmental state from the target state), and there is a rapid initial increase in this distance followed by a leveling off. Similar behavior is displayed by the low line, except that the ecosystem-level selection in this case is for a decrease in \( \Phi \). When directed selection is removed (after 30 ecosystem selection iterations), the selected lines relax toward the nonselected condition (represented by the randomly selected control line ecosystems). The response to selection is very similar with both the propagule and the migrant pool sampling methods, and similar results are also achieved with different target vectors (SI Figs. 3 and 4).

We explored the effects on the observed response of different sampling methods, varying the microbial mutation rate, and the ecosystem propagation time (Table 1). Selected ecosystem scores deviate significantly from control line scores, showing the effect of artificial ecosystem selection. There are inverse relationships between the size of the response to artificial ecosystem selection and mutation rate, and between the size of the response and propagation time (Table 1; SI Figs. 5–7). The rate of relaxation when directed selection is removed is directly proportional to the individual-level mutation rate (SI Fig. 8a). No significant relaxation occurs when mutation rate is zero, indicating that fit ecosystems in this scenario undergo a stable transition in ecological organization, i.e., a switch to a high-fitness ecological equilibrium. Relaxation rate is unrelated to the frequency of ecosystem selection events (SI Fig. 8 b and c). Results from the migrant pool and propagule sampling methods are similar for equivalent \( P_{\text{mut}} \) and \( T_{\text{prop}} \). Perturbing the environmental fluxes (see SI Table 3) has a deleterious effect on performance, suggesting that both environment and community in general contribute significantly to the selected ecosystem function.

### Testing for Implicit Lower-Level Selection

We tested (see Methods) whether the observed response to artificial ecosystem selection could be due to implicit selection at a lower level, by examining whether any species taken from an artificially selected community could achieve or exceed the performance of the intact selected community, either when allowed to develop in isolation as a clonal monoculture population or when placed in the context of a wild-type community (by adding individuals of the test species to the associated control line community). These are attempts to falsify the hypothesis:

\( H_1: \) The adaptive response of the artificially selected ecosystems relies on the presence of multiple concurrently selected species.

With the observations:

\( O_1: \) A species from within the community exists that in monoculture gives performance that equals or exceeds that of the selected community.

\( O_2: \) A species from within the community exists that in the context of a wild-type community gives performance that equals or exceeds that of the selected community.

\( O_3: \) A species from within the community exists that both as a monoculture population and in the context of a wild-type community gives performance that equals or exceeds that of the selected community.

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**Table 1. Mean performance \( \Phi \) scores from artificial ecosystem selection experiments for control line, low-selected, and high-selected communities**

<table>
<thead>
<tr>
<th>Sampling</th>
<th>( T_{\text{prop}} )</th>
<th>( P_{\text{mut}} )</th>
<th>Runs</th>
<th>Control</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propague</td>
<td>2000</td>
<td>0.01</td>
<td>46</td>
<td>0.67</td>
<td>0.19</td>
<td>0.94</td>
</tr>
<tr>
<td>5000</td>
<td>0.01</td>
<td>57</td>
<td>0.66</td>
<td>0.16</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>10000</td>
<td>0.01</td>
<td>60</td>
<td>0.62</td>
<td>0.19</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>20000</td>
<td>0.01</td>
<td>75</td>
<td>0.71</td>
<td>0.29</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>0</td>
<td>43</td>
<td>0.56</td>
<td>0.28</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>0.03</td>
<td>87</td>
<td>0.62</td>
<td>0.25</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>0.05</td>
<td>42</td>
<td>0.56</td>
<td>0.32</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>5000</td>
<td>0.1</td>
<td>73</td>
<td>0.57</td>
<td>0.42</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1</td>
<td>483</td>
<td>0.63</td>
<td>0.27</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Migrant</td>
<td>5000</td>
<td>0.01</td>
<td>49</td>
<td>0.73</td>
<td>0.19</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Results for the propagule sampling method, with varying propagation time \( T_{\text{prop}} \) and mutation rate \( P_{\text{mut}} \), and for the migrant-pool sampling method at the default values.

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8920 | www.pnas.org/cgi/doi/10.1073/pnas.0610038104  Williams and Lenton
We tested all species from each of the selected communities to see whether they satisfied observations O1, O2, and O3. For each community, each observation was satisfied if at least one species met the designated criterion. At first glance, it might appear that O3 is superfluous and corresponds to the intersection of O1 and O2. However, it is possible for O1 and O2 to be satisfied by different species from the same community; O3 adds a further distinction in recording the number of cases where the same species that satisfied O1 also satisfied O2.

We tested every ecosystem that was artificially selected by using the propagule sampling method (483 in each line, giving 966 in total) (Fig. 2). These results ignore differences in propagation time of the propagule sampling method (483 in each line, giving 966 in total). These results show that the majority of ecosystems (91% cases with the propagule sampling method, 100% with the migrant pool method) artificially selected for high Φ contain a single species that can outperform the intact community in some context. However, in ecosystems selected for low Φ, no single better species could be found in a significant proportion of cases (43% with propagule sampling, 27% with migrant pool sampling), showing that multiple species were involved in performing the selected function in these cases.

**Testing for Selection Acting on Interactions.** We sought to establish whether the response to artificial ecosystem selection depended on interactions among different species by measuring the fitness score (Φ) for each species in each selected community when grown as a monoculture, then taking the sum of the contributions of all member species, weighted by the fraction of the community made up of that type (see Methods). This gives the expected score for the community in the absence of interactions between species (ΦE), which can be compared with the actual observed score (ΦO). Any significant difference between these values implies the presence of interactions among species in determining community performance. This method is similar to tests for biodiversity effects in determining the overall yield of plant communities (e.g., ref. 17). Formally, we attempted to falsify the hypothesis:

H2: The observed response to artificial ecosystem selection is due to selection acting on interactions among species.

With the observation:

O4: The expected performance in the absence of interactions among species is not significantly different from, or is better than, the observed performance of the intact community.

For ecosystems selected with propagule sampling, O4 is satisfied (hence interactions between species are insignificant or deleterious) in the minority of cases for both high-selected (38%) and low-selected (28%) ecosystems. For ecosystems selected with the migrant pool method, O4 is satisfied even less often (12% cases in both high and low lines). Hence beneficial interactions play a significant role in the function of the majority of artificially selected ecosystems.

**Combined Tests.** All combinations of O1–O4 are summarized in Fig. 2. If we look for cases where multiple species and beneficial interactions between them are an essential part of the selected community function (i.e., we reject any cases where any of O1, O2, or O4 is satisfied, noting that O3 is subsumed into the overlap between O1 and O2), with propagule sampling, we are left with 4% of high-selected and 38% of low-selected cases. With migrant pool sampling, we are left with no high-selected cases and 25% of low-selected cases.

For both high and low lines, the likelihood of H1 and H2 being satisfied varies inversely with the mutation rate \( P_{\text{mut}} \) (SI Table 4).

\( P_{\text{mut}} = 0 \), even the high-selected ecosystems satisfy H1 and H2 in 30% of cases; this is because without mutation, a single species that is alone capable of performing the selected function cannot evolve, so solutions based on interactions among multiple species are more likely. No obvious relation exists between satisfaction of H1 and H2 and the time between selection events.

**Discussion**

We have demonstrated a robust response to artificial selection in our model ecosystems. Our results suggest that individual-level selection pressure has a degrading effect on the response to artificial ecosystem selection, as expected from evolutionary theory. Between artificial ecosystem selection events, individual-level selec-
tion pressures on metabolic requirements and environmental preferences cause the species composition of the population to change. Although at the individual level this change is adaptive, at the higher level it amounts to drift, phenotypic variation without selection pressure. The genetic composition of different communities moves in different directions due to ecological interactions and the random individual-level mutations occurring within them. Artificial ecosystem selection prunes away those communities that move in the wrong direction and creates replicant variations of those that move in the right direction. Thus artificial ecosystem selection can be viewed as an external steering of the ongoing ecological and evolutionary processes within the microbial community that moves it along a different trajectory from that which it would have followed under the influence of individual-level selection pressure alone.

If a species from within a selected community is found that as a monoculture matches or exceeds the performance of the selected community, then it could be argued that ecosystem-level selection was implicitly selecting this species, and that the rest of the community is irrelevant to the observed response. Considering each species in the context of a wild-type community (supplied here by the control line community from the same selection run), we can identify cases where a single species may be solely responsible for the observed response to selection but requires the presence of a non-specific background community for the desired property to be expressed, as was observed in artificial selection experiments performed on beetle communities (6, 7). In this scenario, the background community acts as a non-evolving part of the environment that could have been incorporated into selection experiments at a lower level, hence community-level or ecosystem-level selection is not required. However, if the expression of the desired property requires a particular community, then selection above the level of the species or clonal group is required, because multiple species are concurrently selected. If multiple species are required and beneficial interactions are contributing significantly to community performance, then it suggests that selection has acted on those interactions, i.e., selection has acted on higher-level traits.

Our two sets of tests establish that a significant fraction of artificially selected ecosystem responses (especially on the low line) cannot be accounted for by a single type of organism within the community, and that interactions among different types of organisms contribute significantly to the response in the majority of cases. The tests performed may be combined in an attempt to falsify (or not) the proposition that the ecosystem selection process acts on traits above the level of individuals or species. What qualifies as a falsification depends on one’s view as to the reasonableness of the tests, but if we adopt the harsh criterion that any one of our observations O1–O4 amounts to a falsification, we are still left with a significant fraction of ecosystems on the low line where higher-level selection has acted on higher-level traits.

Almost all of the high-selected ecosystems (91% with propagule sampling, 100% with migrant pool sampling) appear to have a selected function that is based on the strong contribution of a single species. A further 5% with propagule sampling show no contribution of interactions, i.e., a response that could be due to the individual contributions of two or more species without interaction. Such solutions could be found by lower-level selection methods, leaving only 4% of cases with propagule sampling where higher-level traits were selected. However, with the low-selected ecosystems, a significant number of cases (38% with propagule sampling, 25% with migrant pool sampling) have a selected function based on beneficial interactions among multiple species. Higher-level selection is necessary more often in the low than in the high line, because selecting for low $\Phi$ (being close to the target environmental state) is a priori a harder task, requiring more than one type of microbe to be present in the community, whereas a good high-$\Phi$ solution may be found with a single microbe type. Single-species solutions are found more often in ecosystems selected with migrant pool sampling (which mixes individuals from several source communities and is thus analogous to sexual reproduction), because this method tends to break associations among species and thereby decrease the chance that particular species interactions will be stably transmitted.

The flask ecosystems used in the experiments reported here meet the three criteria for units of selection (18). Phenotypic variation among units occurs by sampling error when inoculating a new batch of flasks from the previous batch and by mutation during ecosystem development. Differential fitness based on this variation is externally imposed by the nature of the artificial selection process. Heritability can be inferred from the observed response to selection [although no direct measurements were made of the similarity of offspring ecosystems to their parent ecosystem(s)]. If there were no heritability, no sustained deviation from the control line would have been observed. The heritable information is encoded in the genotypes of the different microbe species and their relative frequencies in the flask communities, and transmitted by the inoculation method, leading to the formation of new ecosystems similar to the source ecosystem that provided their inoculum.

Although the simulated ecosystems used here meet the criteria for units of selection within the artificial ecosystem selection scenario, caution must be exercised in drawing any inferences concerning ecosystem-level selection in nature. The artificial ecosystem selection experiments described here (and elsewhere (refs. 1, 2, 11–13)) impose highly specialized conditions: the ecosystems are isolated by hosting each microbial population in a separate container; the transmission mechanism by which individuals are sampled and used to inoculate new flasks is externally provided; and differential fitness at the ecosystem level results from an arbitrary measure of the abiotic environment and does not take into account differences in viability or proliferation. Hence, although we note a strong response to artificial ecosystem selection using the more naturally plausible migrant pool sampling method, all we can say regarding the operation of higher-level selection in nature is that if the same experimental conditions (that were externally imposed here) occur in a natural setting, it is theoretically possible for selection at the level of the ecosystem to shape ecosystem properties and the underlying community. It has been argued elsewhere that spatial separation of semi-isolated populations may provide similar conditions and allow some forms of higher-level selection to operate (19). A spatial version of this model is currently in preparation that will be used in part to determine the scope (if any) for natural ecosystem selection in a more realistic setting.

**Methods**

Each run in an artificial ecosystem selection experiment involves a batch of 20 flasks, subjected to the same randomly generated set of flux parameters for nutrients and abiotic factors throughout, giving identical environmental conditions in the absence of microbial activity. At the start of each run, the liquid medium in each flask is allowed to reach equilibrium before being seeded with a microbial inoculum. An iteration of ecosystem selection is defined as the creation of a new batch of flask ecosystems (using microbes sampled from the fittest ecosystems of the previous iteration), the propagation of these ecosystems for a fixed period, and the subsequent assignation of a fitness score to each flask. The flask ecosystems used in the experiments reported here meet
Flask ecosystem fitness was based on the distance (Φ) of the abiotic environment from an arbitrary target state (see Eq. 1). To measure Φ, the target and actual state vectors of the abiotic environment are normalized, so that the vector of relative proportions of all abiotic factors is the ecosystem property on which artificial selection is based. Φ is measured on the final state of each flask after each iteration. This method should reduce noise, because the state of the abiotic environment is in part a cumulative function of the environment-altering activity of the population over time. The high line was selected to maximize Φ. The low line was selected to minimize Φ. For the control line, source ecosystems were selected at random from the previous batch. The randomly selected control line was used, because an abiotic line would not control for any inherent tendencies of the flask ecology to alter their environment in a particular way irrespective of higher-level selection. All lines also had to meet a criterion of viability, that is, only flask ecosystems with a living microbial population could be selected to provide inocula for the next iteration.

The first set of experiments was designed to look at the effects on the response to artificial ecosystem selection of different sampling methods, varying mutation rate, and varying the propagation time (the time period for which each ecosystem was allowed to develop before fitness testing). The default settings are a target vector \( \bar{a} = (0.2, 0.3, 0.5) \), propagation time \( T_{prop} = 5,000 \) time steps, and individual mutation rate \( P_{mut} = 0.01 \), i.e., a 1% chance of a new allele value at each offspring locus during each reproduction event. First, the effect of different sampling methods is considered for the default settings. Second, the effect of varying the mutation rate \( P_{mut} \) during microbe reproduction is examined for the same target vector and propagation time, with the propagule sampling method. Values for \( P_{mut} \) are taken from the set \( \{0.001, 0.01, 0.03, 0.05, 0.1\} \). Third, the effect of varying the propagation time \( T_{prop} \) is examined for the default target vector and individual-level mutation rate, again with the propagule method. Values for \( T_{prop} \) (measured in simulation time steps) are taken from the set \( \{2,000, 5,000, 10,000, 20,000\} \). Each run has a population of 20 flask ecosystems, undergoing 60 ecosystem selection iterations (e.g., 300,000 time steps when \( T_{prop} = 5,000 \)), with 30 iterations of directed selection followed by a further 30 iterations of random selection. In each run, the initial seed population and flux parameters were held fixed for all flask ecosystems and treatments (high, low, and random), thus giving identical initial conditions. However, in each experiment, a number of runs are undertaken with different initial seed populations and flux parameters; this repetition allows reliable results to be generated, despite the high level of stochasticity in the system. Φ values are recorded for each ecosystem at the end of each iteration. For each run, Φ is then averaged over all flask’s in the population. Finally, Φ is averaged over all runs in each experiment to give the values used in the results.

The evolved ecosystems were tested to determine their performance in perturbed conditions and to provide data for the second group of experiments. For these tests, a baseline value for Φ is required for each artificially selected ecosystem. This is obtained by running a mock iteration of ecosystem selection: 20 identical propagules of 100 individuals sampled from the fittest ecosystem in the final (30th) iteration of directed ecosystem selection, were run forward with all parameters duplicated from the selection experiments but with mutation disabled (\( P_{mut} = 0 \)) to prevent it changing the species composition of the community. Short runs were used (\( T_{prop} = 2,000 \)), because in the absence of mutation, this is sufficient for the ecosystem to reach a reasonably steady state. The mean final value of Φ measured across all 20 replicated ecosystems was taken to be the baseline score for comparison. The effect of perturbations to the material fluxes through the flask environment was found by running a similar test on the selected community with randomly chosen flux parameters that were different from its normal conditions. For each selected ecosystem, a propagule of 100 individuals was used to inoculate 20 flasks, in each of which the flux parameters were randomly generated (all other parameters were kept the same). After a mock iteration, Φ was measured for each of the 20 variant sets of flux parameters.

The second group of experiments was designed to establish the basis of the response to artificial ecosystem selection and whether it was created by selection acting above the level of the clonal group. This was done by looking for species in each selected community that were capable of inducing the observed functionality on their own. For every species in each selected ecosystem, a propagule of 100 individuals was allowed to develop as a monoculture population. After a mock iteration, Φ was measured for each clonal variant. In another test, for every species from each selected ecosystem, 25 individuals were combined with 75 randomly sampled individuals from the associated control line community (because these control line communities are adapted to the same environmental conditions as the artificially selected community, the control line community acts as a non-selected wild-type community). After a mock iteration, Φ was measured for each composite community. Twenty repetitions were performed and mean Φ scores taken to account for variation between runs.

To verify O4, the expected score (\( \Phi_E \)) for each intact community was compared with the observed community score (\( \Phi_O \)). \( \Phi_E \) was found as the weighted sum of the monoculture Φ values for each species in the selected community:

\[
\Phi_E = \sum_s p_i \Phi_i
\]

where \( S \) is the number of species, \( p_i \) is the fraction of the total community made up of species \( i \), and \( \Phi_i \) is the monoculture score of species \( i \). In the comparisons of \( \Phi_O \) and \( \Phi_E \) used in verifying O4, mean values from all 20 repetitions of the baseline test of the selected community were used and a significant difference among them was said to exist only if the absolute difference between the mean values for \( \Phi_O \) and \( \Phi_E \) was greater than the sum of their standard deviations.

Further details of the model are in SI Text. Model parameter values are given in SI Table 2, and any deviations from these values are noted in the text.

This work was supported by the Leverhulme Trust.

Supporting Information to “Artificial selection of simulated microbial ecosystems”

Hywel T.P. Williams  Timothy M. Lenton

March 4, 2007

1 Model Description

Conceptually, the Flask model simulates the ecology and evolution of microbial communities. References to other work describing the Flask model and the typical dynamics of the flask ecosystems can be found in the main paper. Each community is suspended in a liquid medium held in a flask subjected to a continuous fixed-rate chemical flux, giving growing conditions similar to those found in a chemostat. Individual microbes grow and reproduce dependent on food supply and environmental conditions within the flask, and nutrient cycling loops and stable ecologies emerge from the indirect interaction of individuals via the flask environment. Mutation may occur during microbe reproduction, allowing the genesis of new microbial strains.

The composition of the liquid medium in each flask determines the environment of the microbes. Some of the chemicals present may be consumed as food by the microbial population and converted to biomass, while others are non-consumable and form part of the abiotic environment. In addition it is assumed that the liquid medium has properties such as temperature, pH, salinity, etc., and that these both affect, and can be affected by, microbial activity. We will refer to these non-consumable chemicals and physical properties of the flask environment collectively as ‘abiotic factors’ for ease of discussion; while chemical nutrients are also abiotic we feel that their role as the subjects of metabolism justifies this notational convenience. The effect of the microbes on abiotic factors is modelled here as a side-effect of metabolism, with a genetically specified effect caused by each microbe for every unit of biomass created.

The composition of the abiotic environment resulting from the interaction of the input and output fluxes with the collective actions of the microbial population forms a ‘phenotypic’ ecosystem trait that is used as the basis for selection. Offspring ecosystems are formed by innoculating sterile flasks with seed populations sampled from the most successful ecosystems in the previous iteration. The response to selection is measured as the change over time in the distance of the environmental state variables from some pre-specified target vector. The artificial ecosystem selection method is described fully in the main paper.

1.1 The flask environment

Each flask contains a neutral liquid matrix in which is suspended a microbial population. There is a flow of liquid medium through the flask which occurs continuously at a fixed rate.
The inflow brings with it influxes of nutrients at fixed concentrations and steady inputs to abiotic factors, while the outflow removes a fixed proportion of stored nutrients and abiotic factors. The liquid medium in each flask is assumed to be well-mixed, so that in the absence of perturbation the composition of the medium in each flask will reach a homogeneous steady state equilibrium. Each microbe both consumes and excretes chemical nutrients, and also has an effect on the levels of the abiotic factors as a side-effect of metabolism (explained in Section 1.3.4 below).

The state of the flask environment is given by a vector $V$:

$$V = (n_1, ..., n_N, a_1, ..., a_A) = (v_1, ..., v_{N+A})$$

where $n_i$ is the concentration of nutrient $i$, $a_i$ is the level of abiotic factor $i$, or equivalently, $v_i$ is the level of the $i^{th}$ environmental state variable. $N$ is the number of chemical nutrients, and $A$ is the number of abiotic factors. The change in $V$ over time is given by Equation 3, which relates the rate of change of each $v_i$ over time to the rates of influx and outflux of that variable and the net effect of microbial activity. $I_i$ is the rate of influx of $v_i$, $O_i$ is the rate of outflux, and $E_i$ is the effect on $v_i$ of current microbial activity. The form of Equation 3 is general to nutrients and abiotic factors, although $E_i$ is calculated differently for nutrients and abiotic factors (see Sections 1.3.1 and 1.3.4).

$$\frac{dv_i}{dt} = I_i - O_i v_i + E_i \quad (3)$$

1.2 Microbes

Microbes are modelled as simple organisms that consume and excrete nutrients in fixed proportions and affect the levels of abiotic factors in their environment as a side-effect of metabolism. The precise ratios in which nutrients are consumed and excreted, and the nature of the by-product effect on abiotic factors, are genetically encoded for each individual, as are its preferred abiotic conditions (i.e., the state of the abiotic environment in which its growth rate will be maximised). Microbes grow by converting consumed nutrients to biomass and reproduce by splitting when their biomass reaches a fixed threshold. Biomass is reduced at a fixed rate to represent the inevitable thermodynamic inefficiency of metabolism and the cost of maintaining cellular machinery. Microbes die if their biomass drops below a fixed threshold, which can happen in sustained periods of nutrient limitation.

A microbe can be represented by a vector $M$:

$$M = (B, \lambda, \mu, \alpha, \beta)$$

where $B$ is the current biomass of the microbe, $\lambda = (\lambda_1, ..., \lambda_N)$ represents the ratio in which nutrients are consumed, $\mu = (\mu_1, ..., \mu_N)$ the ratio in which excreta is returned to the environment as nutrients, $\alpha = (\alpha_1, ..., \alpha_A)$ the microbe’s effect on abiotic factors as a side-effect of metabolism, and $\beta = (\beta_1, ..., \beta_A)$ the relative proportions of abiotic factors in the environment at which growth rate is maximised. Clearly $\sum_{i=1}^{N} \lambda_i = 1$ and $\sum_{i=1}^{N} \mu_i = 1$ hold since all materials consumed and excreted must be accounted for; there is no such constraint on $\alpha$ since the effect of the microbe on the abiotic environmental factors does not necessarily involve mass transfer and is thus treated generally. $\sum_{i=1}^{A} \beta_i = 1$ also holds, since environmental
preference is represented here as a vector specifying the optimal proportion of each abiotic factor relative to the other abiotic factors in the flask environment. Of the quantities in the microbe state vector $M$, only $B$ is a variable during the lifetime of an individual, since $\lambda$, $\mu$, $\alpha$ and $\beta$ are genetically encoded and thus fixed.

### 1.2.1 Genotype

The genotype for a microbe is an array with $2N + 2A$ loci taking values in the range $[-1.00, 1.00]$. The genotype is subdivided into two sets of $N$ loci for consumption and excretion and two sets of $A$ loci for influence on abiotic factors and preferred environmental conditions. The microbe phenotype is formed by mapping and transforming the values in its genotype according to fixed rules.

\[
\begin{array}{cccc}
\{N \text{ consumption loci}\} & \{N \text{ excretion loci}\} & \{A \text{ effect loci}\} & \{A \text{ preference loci}\} \\
\downarrow & \downarrow & \downarrow & \downarrow \\
\lambda & \mu & \alpha & \beta
\end{array}
\]

#### Phenotype

The consumption ratio $\lambda$ (specifying the fixed microbe-specific proportions in which nutrients are consumed) is found by linearly mapping the $N$ alleles for consumption to the range $[0.00, 1.00]$ and normalising to give the fraction of total consumption that is made up of each nutrient. The excretion ratio $\mu$ (specifying the fixed microbe-specific proportions in which excreta is returned to the environment as nutrients) is found similarly. For example, if $N = 3$ and the consumption loci of the genotype are $(-0.4, 0.7, 0.1)$, this would map linearly to $(0.3, 0.85, 0.55)$ and give a normalised consumption ratio of $\lambda = (0.18, 0.5, 0.32)$.

The vector $\alpha$ determining a microbe’s effect on the abiotic factors in the environment is found by directly mapping the $A$ alleles from the relevant part of the genotype to the values for the phenotypic trait without scaling or transformation, i.e., values remain in the range $[-1.00, 1.00]$. These values give the alteration caused in the level of each abiotic factor by the creation of a single unit of biomass during microbe metabolism (see Section 1.3.4 for more details). A microbe’s preferred abiotic environment $\beta$ is determined by linear mapping and normalisation of the relevant $A$ alleles of the genotype, using the same scheme as that for finding the consumption and excretion ratios $\lambda$ and $\mu$. The microbe’s preferred environment is thus expressed as a vector of the relative proportions of each abiotic factor; the microbe’s growth rate will be maximised when the state of the environment matches this preference (see Section 1.3.3).

Note that ‘genotypes’ as defined here are highly abstracted analogues of their biological inspiration; the developmental stage used here is direct and deterministic, and there is no possibility of significant epistatic interactions or pleiotropy. However, the use of the term ‘genotype’ here is justified since the genotype is the mechanism of microbe heredity, the determinant of microbe phenotype, and the subject of mutations leading to phenotypic variation.
1.2.2 Reproduction and mutation

If the genetic specification of the microbe causes it to be successful in its environment (i.e., if its nutrient demands and preferred abiotic conditions are suited to the current state of the liquid medium held in the flask), the microbe will consume nutrients and grow by increasing its biomass. If a microbe’s biomass reaches the reproduction threshold $T_R$, it reproduces asexually by splitting. The parent microbe donates half of its biomass to the offspring microbe, which also receives a copy of the parent’s genotype. Mutation of the offspring genotype may occur during reproduction, implemented as a potential for copying error at each locus of the genotype which causes the allele value for that locus to be randomly reassigned from the range $[-1.00, 1.00]$. Mutations occur at each locus with low probability $P_{mut}$. No mechanism for genetic recombination is implemented.

1.2.3 Maintenance cost and death

Unsuccessful microbes will not consume enough nutrients to grow and may reduce in biomass due to a fixed rate of biomass decrement which is incorporated in the model as a proxy for the combined energy costs of maintaining cellular machinery and metabolic inefficiency. This ‘cost of living’ reduces biomass at a fixed rate $\gamma$ per simulation timestep, with the decrement assumed to be lost from the flask environment as unrecoverable heat radiation. The inclusion of this cost ensures that nutrients cannot be infinitely recycled and thus preserves the thermodynamic integrity of the model.

If the biomass of a microbe falls below a threshold $T_D$ the microbe is assumed to die from starvation. In addition to this, each living microbe may die ‘from natural causes’ with a low probability $P_D$ at each timestep. This mechanism is intended to be a catch-all for death by predation, senescence, etc., and serves to thin out the microbial population in an unbiased way and thus promote continuing selection and competition between microbes. Note that the value of $P_D$ is related to the washout rate of living microbes in chemostat models. When a microbe dies it is assumed that its remaining biomass is washed out and lost from the system.

1.3 Microbe metabolism

1.3.1 Nutrient consumption / excretion

At each timestep of the simulation, each living microbe $j$ will attempt to consume a total of $C_{j}^{max}$ units of nutrient, with the contributions to this total made up of each different nutrient type fixed in the relative proportions defined by the microbe’s genetically specified consumption ratio. The size of $C_{j}^{max}$ is limited by a global maximum level $C^{max}$ and is calculated on an individual basis for each microbe $j$. This calculation takes into account the match between the current state of the abiotic environment and the genetically specified preferences of microbe $j$ and will be covered in Section 1.3.3 below. The actual amount consumed $C_{j}^{act}$ is less than or equal to $C_{j}^{max}$ and depends on nutrient availability.

In order to ensure that the microbe population doesn’t consume more nutrients than currently exist in the flask environment, individual demands may need to be scaled, and to ensure that no artefacts are introduced into the model by this scaling, it must not favour any particular individual. At each timestep the total nutrient demand for the entire microbe population is calculated and compared to the amounts of nutrients available. It is assumed
that all microbes are continuously and simultaneously feeding, so in the case that there is an insufficient amount of a nutrient available to meet the entire population demand, the demand of every individual microbe that requires that nutrient is scaled down equally, so that the total amount consumed by the population matches what is available.

Mathematically, we have constraints $C_{j}^{\text{max}} \leq C_{j}^{\text{act}} \leq C_{j}^{\text{max}}$, and then:

$$C_{j}^{\text{act}} = C_{j}^{\text{max}} N \prod_{i=1}^{N} w_{ij}$$

where $C_{j}^{\text{act}}$ is the actual total quantity of nutrients consumed by microbe $j$ after scaling for nutrient limitation has been applied and $w_{ij}$ is the scaling factor for nutrient $i$ for microbe $j$. Values for $w_{ij}$ are calculated sequentially by noting that the population demand $D_{i}$ for nutrient $i$ (after all individual demands for nutrient $i - 1$ have been scaled appropriately) and $w_{ij}$ are related. Recalling that $\lambda_{ij}$ is the proportion of consumption taken as nutrient $i$ by microbe $j$ and $n_{i}$ is the total amount of nutrient $i$ currently available in the flask environment, we can derive the full set of all $w_{ij}$ and $D_{i}$ for every nutrient $i$ and living microbe $j$ by solving iteratively for each value of $D$ and $w$, starting with an assumed value of $w_{0j} = 1$ (valid since nutrient 0 does not exist).

$$w_{0j} = 1 \quad \forall j$$

$$w_{ij} = \begin{cases} \min(1, \frac{n_{i}}{D_{i}}) & \lambda_{ij} > 0 \\ 1 & \lambda_{ij} = 0 \end{cases}$$

$$D_{i} = \sum_{j}^{\text{living}} \left( \lambda_{ij} C_{j}^{\text{max}} \prod_{k=0}^{i-1} w_{kj} \right)$$

Then having established the value of $C_{j}^{\text{act}}$ we can go on to derive:

$$C_{j} = C_{j}^{\text{act}} (\lambda_{1j}, ..., \lambda_{Nj})$$

$$C_{i}^{\text{pop}} = \sum_{j}^{\text{living}} \lambda_{ij} C_{j}^{\text{act}}$$

where $C_{j}$ is the actual consumption vector for microbe $j$ and details how much of each nutrient is consumed by microbe $j$ at a particular timestep. $C_{i}^{\text{pop}}$ is the total amount of nutrient $i$ consumed by all living microbes.

This scheme means that each microbe always consumes nutrients in the relative proportions specified by its genetically determined consumption ratio. If nutrient limitation means that the amount of a particular nutrient consumed by a microbe is scaled down, the amounts of the other nutrients its consumes are also scaled down by an equivalent factor to maintain the fixed relative proportions of consumption.

Consumed nutrients are converted to biomass with a standard efficiency of $\theta$, with the waste being excreted as nutrients (i.e., $C_{j}^{\text{act}} = 10$ units of food consumed with efficiency of $\theta = 0.6$ makes 6 units of biomass and 4 units of excreta). Excreta is returned to the environment as
nutrients in the fixed proportions specified by the microbe’s genetically determined excretion ratio. We can thus define the excretion vector $X_j$ for every microbe $j$, and an expression for the total amount $X^{pop}_i$ of nutrient $i$ collectively excreted and returned to the environment by the population:

$$X_j = (1 - \theta)C^\text{act}_j(\mu_{1j}, \ldots, \mu_{Nj})$$

(7)

$$X^{pop}_i = \sum_j \text{living} (1 - \theta)C^\text{act}_j \mu_{ij}$$

(8)

1.3.2 Growth

At each timestep of the simulation a microbe $j$ will consume $C^\text{act}_j$ units of nutrient, which are converted to biomass with a fixed efficiency of $\theta$. Taking into account the previously defined maintenance cost $\gamma$, we can now state the growth rate (rate of change of biomass) of a microbe $j$ as:

$$\frac{dB_j}{dt} = \theta C^\text{act}_j - \gamma$$

(9)

Note that Equation 9 specifies the growth of an individual. Growth of a population occurs only as a result of individual growth and reproduction, and is not specified a priori as in more traditional population ecology models such as Lotka-Volterra systems.

1.3.3 Effect of abiotic factors on metabolic rate

The model is designed so that the state of the abiotic environment affects the growth rate of microbes, and this is implemented as a feedback from the environmental state variables for abiotic factors onto the consumption demands of all microbes. As mentioned above, each microbe will attempt to consume a maximum amount $C^{\text{max}}_j$ of nutrients at each timestep, with this demand being met depending on nutrient availability. The attempted consumption amount $C^{\text{max}}_j$ is calculated for each microbe $j$ as a function of the match between the current state of the abiotic environment and the microbe’s genetically specified preferences. This function has Gaussian form and falls away smoothly from its maximum value as the distance between the current environment and the optimum increases. Mathematically, we can capture this as below:

$$C^{\text{max}}_j = \psi_j C^{\text{max}}$$

(10)

$$\psi_j = e^{-\tau (\rho_j)^2}$$

(11)

$$\rho_j = \sqrt{\sum_{i=1}^{A} (\hat{\alpha}_i - \beta_{ij})^2}$$

(12)

$$\hat{\alpha}_i = \frac{a_i}{\sum_{i=1}^{A} a_i}$$

(13)
where $C^{\text{max}}$ is a universal constant defining the maximum rate of consumption for any microbe, $\psi_j$ is a microbe-specific measure of the microbe’s satisfaction with the current abiotic environment given its preferences, $\tau$ is a universal constant parameter that sets the level of influence of the abiotic environment on growth rate (high $\tau$ means a stronger influence, $\tau = 0$ means no influence), $\rho_j$ is a measure of the distance between the current abiotic environment and the microbe’s preferred environment, $\hat{a}_i$ is the normalised level of abiotic factor $a_i$, and $\beta_{ij}$ is microbe $j$’s preferred normalised level for factor $a_i$.

### 1.3.4 Effect of microbial activity on environment

Microbes can affect the amount of nutrients in the flask environment as well as the levels of the abiotic factors. During metabolism microbes remove nutrients from the environment by consumption and add nutrients to the environment by excretion. Also, during metabolism, microbes affect the levels of abiotic environmental factors as a side-effect of their metabolic action.

The metabolic effect on abiotic factors is implemented in the model as a mechanism by which microbes can affect their abiotic environment. In the real world, all organisms have an effect on their abiotic surroundings, and while metabolism is one way in which organisms do this (particularly in the microbial world), other kinds of behaviour are also significant. However, to keep the model as simple as possible we have only implemented the interaction of microbes with their abiotic environment as a side-effect of metabolism. This method has the advantage of making the size of a microbe’s effect on the environment proportional to its growth rate, so that fast-growing and more fecund microbial strains have a greater influence than dormant or slow-growing strains, as seems intuitively correct.

Equation 3 relates the rate of change of each environmental variable $v_i$ to the combined effect $E_i$ of the microbial population on that variable. The calculation of $E_i$ is treated differently for nutrients and abiotic factors. First of all, let the vector $E$ be the vector of effects of the population on all environmental variables, and note that this vector can be sub-divided into nutrient and abiotic components.

$$E = (E_n, E_a) = (E_{n1}, ..., E_{nN}, E_{a1}, ..., E_{aA})$$

We can use our previous definitions of nutrient consumption/excretion to work out the population effect on nutrient state variables. This gives us an expression for the effect $E_{ni}$ of the population on nutrient $i$:

$$E_{ni} = -C_i^{\text{pop}} + X_i^{\text{pop}}$$

The effect of each microbe on each abiotic factor is determined by its genetically specified effect vector $\alpha$ and is applied for every unit of biomass created. The expressions for microbe growth combined with this vector can be used to work out the population effect $E_{ai}$ on each abiotic state variable $i$:

$$E_{ai} = \sum \frac{dB_i}{dt} \alpha_{ij}$$
1.4 Simulation method

At the start of each simulated timestep, nutrient/abiotic influx is added to the environment. This is followed by simultaneous update of all microbes in the population for metabolism, death (by starvation or random selection), and reproduction, in that order. When the biota are updated, nutrient/abiotic outflux is removed from the environment and the system update is complete for that timestep.

The update equation for the flask environment (Equation 3) and the microbial growth equation (Equation 9) are continuous differential equations. At each timestep these differential equations are discretised by calculating their instantaneous value and adding it to the existing values of the quantities concerned. For environmental update we have $\Delta V = \frac{dV}{dt}$ and then $V_t = V_{t-1} + \Delta V$. For microbe growth we have $\Delta B = \frac{dB}{dt}$ and $B_t = B_{t-1} + \Delta B$. In effect this is equivalent to numerical integration using Euler’s forward method with an integration timestep equal to one simulated timestep in the flask ecosystem.
2 Additional Results

The results given in this section support those given in the main body of the paper. Some interpretation is given in figure/table captions here, but these results are best understood by reference to the main paper.
Table 2: Parameter values used in the simulation, deviations from these values are reported in the text.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>Number of nutrients</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>Number of abiotic factors</td>
</tr>
<tr>
<td>$T_R$</td>
<td>120</td>
<td>Reproduction threshold (biomass units)</td>
</tr>
<tr>
<td>$T_D$</td>
<td>50</td>
<td>Starvation threshold (biomass units)</td>
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<tr>
<td>$P_{mut}$</td>
<td>Varied</td>
<td>Probability of mutation at each genotype locus during reproduction</td>
</tr>
<tr>
<td>$P_D$</td>
<td>0.002</td>
<td>Probability of death by natural causes at each timestep</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>Maintenance cost (biomass units/timestep)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>0.6</td>
<td>Nutrient conversion efficiency</td>
</tr>
<tr>
<td>$C^{max}$</td>
<td>10</td>
<td>Maximum nutrient consumption rate (units/timestep)</td>
</tr>
<tr>
<td>$\tau$</td>
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<td>Level of influence of abiotic environment on metabolism</td>
</tr>
<tr>
<td>$I_{N}^{\text{min}}$</td>
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<td>Minimum rate of nutrient influx (units/timestep)</td>
</tr>
<tr>
<td>$I_{N}^{\text{max}}$</td>
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<td>Maximum rate of nutrient influx (units/timestep)</td>
</tr>
<tr>
<td>$O_{N}^{\text{min}}$</td>
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<td>Minimum rate of nutrient outflux (fraction/timestep)</td>
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<td>$O_{N}^{\text{max}}$</td>
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<td>Maximum rate of nutrient outflux (fraction/timestep)</td>
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<tr>
<td>$I_{A}^{\text{min}}$</td>
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<td>Minimum rate of abiotic factor influx (units/timestep)</td>
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<td>Maximum rate of abiotic factor influx (units/timestep)</td>
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<tr>
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<td>Minimum rate of abiotic factor outflux (fraction/timestep)</td>
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<td>$O_{A}^{\text{max}}$</td>
<td>0.25</td>
<td>Maximum rate of abiotic factor outflux (fraction/timestep)</td>
</tr>
<tr>
<td>$K_f$</td>
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<td>Number of flask ecosystems in each batch</td>
</tr>
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<td>$K_m$</td>
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<td>Number of individuals in flask innoculum</td>
</tr>
<tr>
<td>$T_{\text{prep}}$</td>
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<td>Flask equilibriation time prior to seeding (timesteps)</td>
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<tr>
<td>$T_{\text{prop}}$</td>
<td>Varied</td>
<td>Propagation time for flask ecosystems</td>
</tr>
</tbody>
</table>
Artificial selection of simulated microbial ecosystems

Williams and Lenton. 10.1073/pnas.0610038104.
Supporting Information

Files in this Data Supplement:

SI Text
SI Table 2
SI Figure 3
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*Fig. 3*. Migrant pool sampling gives a strong response to artificial ecosystem selection. This response is similar to that achieved with propagule sampling (Fig. 1). Mean F± 1 SE plotted. Here 49 runs were performed using migrant pool sampling with T_prop = 5,000 and P_mut = 0.01 for all runs, with arbitrary target vector (fA_1 , fA_2 , fA_3 ) = (0.2, 0.3, 0.5). Data are plotted for directed selection for either increase (high line) or decrease (low line) in distance of the abiotic environment from target state, F, as well as for a random selection control line that shows behavior in the absence of artificial ecosystem selection. Directed selection is stopped after iteration 30, at which point all selection is random.

*Fig. 4*. Response to artificial ecosystem selection (using propagule sampling method) is achieved with different target vectors for normalized abiotic environmental state. Mean F± 1 S.E. plotted for all runs. Here T_prop = 5,000 and P_mut = 0.01 for all runs, but different target vectors for abiotic environmental states are used. Sub-figure captions give target vectors and number of runs performed for each. Two
data sets with different target vectors are shown, supplementing the example shown in the main body of the paper (Fig. 1). Directed selection is stopped after iteration 30, at which point all selection is random.

SI Figure 5 <content/suppl/2007/05/11/0610038104.DC1/10038Fig5.pdf>

*Fig. 5*. Response to artificial ecosystem selection (using propagule sampling) with different mutation rates for individual reproduction. Here the target vector is \((\bar{\alpha}_1, \bar{\alpha}_2, \bar{\alpha}_3) = (0.2, 0.3, 0.5)\) and \(T_{\text{prop}} = 5,000\) for all runs. Mean \(F \pm 1\) SE plotted for all runs. \(P_{\text{mut}}\) takes values from the set \{0, 0.01, 0.03, 0.05, 0.1\} (number of runs for each value given in sub-figure captions). Fig. 5/a/: When the chance of mutation during individual reproduction is reduced to zero a strong response to selection is observed, and the adapted response is sustained even after directed selection pressure is removed at iteration 30. Fig. 5 /b-e/: As the probability \(P_{\text{mut}}\) of mutation at each genetic locus during individual reproduction is increased, the response to selection is weakened and relaxation to the nonselected state after iteration 30 is accelerated.

SI Figure 6 <content/suppl/2007/05/11/0610038104.DC1/10038Fig6.pdf>

*Fig. 6*. Response to artificial ecosystem selection (using propagule sampling) when propagation time is varied. Here the target vector is \((\bar{\alpha}_1, \bar{\alpha}_2, \bar{\alpha}_3) = (0.2, 0.3, 0.5)\) and \(P_{\text{mut}} = 0.01\) for all runs. Mean \(F \pm 1\) SE plotted for all runs. \(T_{\text{prop}}\) takes values from the set \{2,000, 5,000, 10,000, 20,000\} (number of runs for each given in sub-figure captions). As \(T_{\text{prop}}\) increases, the response to artificial ecosystem selection decreases, showing an inverse relationship between the time between selection events and the elicited response.

SI Figure 7 <content/suppl/2007/05/11/0610038104.DC1/10038Fig7.pdf>

*Fig. 7*. Response to artificial ecosystem selection (using propagule sampling) varies inversely with mutation rate and propagation time. Plots show mean absolute difference in \(F\) between high and low lines (\(\pm 1\) SE) after 30 iterations of ecosystem selection, measured on multiple
runs with varying mutation rate $P_{mut}$ and propagation time $T_{prop}$. The target vector is $(\hat{a}_1, \hat{a}_2, \hat{a}_3) = (0.2, 0.3, 0.5)$ for all runs.

*Fig. 8*. Ecosystems relax toward the non-selected condition when directed selection is removed. Relaxation depends on mutation rate, but not on propagation time. Difference in mean $F$ between high and low selected lines ($\pm 1$ SE) plotted against time for different mutation rates and propagation time. The target vector is $(\hat{a}_1, \hat{a}_2, \hat{a}_3) = (0.2, 0.3, 0.5)$ for all runs.
Selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Supplementing the example shown in the main body of the paper (Paper Figure 1), Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Selected vectors for abiotic environmental state are real. Sub-figure captions give target vectors and selected vectors for abiotic environmental state. Mean ± 1 s.e. plotted for all runs. Here \( T_{\text{prop}} = 5000 \) and \( P_{\text{mut}} = 0.01 \) for all runs. Here, \( \tau = 5000 \) and \( P_{\text{mut}} = 0.01 \) for all runs, but different target vectors for abiotic environmental state are used. Directed selection is stopped after iteration 30, at which point all selection is random.

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Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.

Directed selection is stopped after iteration 30, at which point all selection is random.
Selection is stopped after iteration 30, at which point all selection is random.

Supplementing the example shown in the main body of the paper (Paper Figure 1), Directed selection is stopped after iteration 30, at which point all selection is random. Directed selection is stopped after iteration 30, at which point all selection is random. Directed selection is stopped after iteration 30, at which point all selection is random.

Figure 3: Migrant pool sampling gives a strong response to artificial ecosystem selection. This response is similar to that achieved with propagule sampling (Paper Figure 1). Mean $\Phi \pm 1\text{s.e.}$ plotted. Here 49 runs were performed using migrant pool sampling with $T_{\text{prop}} = 5000$ and $P_{\text{mut}} = 0.01$ for all runs, but different target vectors for each run. Directed selection is stopped after iteration 30, at which point all selection is random.

Figure 4: Response to artificial ecosystem selection (using propagule sampling method) is achieved with different target vectors for normalised abiotic environmental state. Mean $\Phi \pm 1\text{s.e.}$ plotted. Here 90 runs were performed using migrant pool sampling with $T_{\text{prop}} = 5000$ and $P_{\text{mut}} = 0.01$ for all runs, but different target vectors for abiotic environmental state are used. Directed selection is stopped after iteration 30, at which point all selection is random.
Figure 5: Response to artificial ecosystem selection (using propagule sampling) with different mutation rates for individual reproduction. Here the target vector is $(\bar{a}_1, \bar{a}_2, \bar{a}_3) = (0.2, 0.3, 0.5)$ and $T_{prop} = 5000$ for all runs. Mean ±1 s.e. plotted for all runs. $P_{mut}$ takes values from the set {0, 0.01, 0.03, 0.05, 0.1} (number of runs for each value given in sub-figure captions). Figure 5(a): When the chance of mutation during individual reproduction is reduced to zero a strong response to selection is observed, and the adapted response is sustained even after directed selection pressure is removed at iteration 30. Figures 5(b)-5(e): As the probability $P_{mut}$ of mutation at each genetic locus during individual reproduction is increased, the response to selection is weakened and relaxation to the non-selected state after iteration 30 is accelerated.
Figure 6: Response to artificial ecosystem selection (using propagule sampling) when propagation time is varied. Here the target vector is $(\bar{a}_1, \bar{a}_2, \bar{a}_3) = (0.2, 0.3, 0.5)$ and $P_{\text{mut}} = 0.01$ for all runs. Mean ± 1s.e. plotted for all runs. As $T_{\text{prop}}$ increases, the response to artificial ecosystem selection decreases, showing an inverse relation. $T_{\text{prop}}$ takes values from the set $\{2000, 5000, 10000, 20000\}$ (number of runs for each given in sub-figure captions).
Figure 7: Response to artificial ecosystem selection (using propagule sampling) varies inversely with mutation rate and propagation time. Plots show mean absolute difference in $\Phi$ between high and low lines ($\pm$1 s.e.) after 30 iterations of ecosystem selection, measured on multiple runs with varying mutation rate $P_{mut}$ and propagation time $T_{prop}$. The target vector is $(\bar{a}_1, \bar{a}_2, \bar{a}_3) = (0.2, 0.3, 0.5)$ for all runs.
(a) \( T_{prop} = 5000 \) for all runs, \( P_{mut} \in \{0, 0.01, 0.03, 0.05, 0.1\} \) for \{43, 57, 87, 42, 73\} runs \{2000, 5000, 10000, 20000\} for \{46, 57, 60, 75\} runs respectively with each value. Number of iterations are a good measure of elapsed time since all iterations are of same duration.

(b) \( P_{mut} = 0.01 \) for all runs, \( T_{prop} \in \{2000, 5000, 10000, 20000\} \) for \{46, 57, 60, 75\} runs respectively with each value. Data values plotted is a good measure of elapsed time since all iterations against elapsed time (in model time steps), rather than against the number of selection events (in ecosystem selection iterations), to allow comparison. Vertical dashed lines mark the point when directed selection was replaced by random selection (i.e., end of iteration 30).

(c) Real-time comparison of response to artificial ecosystem selection when time between selection events is varied. Figure shows relaxation of selected function after directed selection pressure is removed. Here the difference between the high and low selected lines is normalised by dividing by the mean distance from the target abiotic state vector over the last 10 iterations of directed selection. This analysis highlights the similarity in relaxation rate measured over elapsed time, as opposed to the difference when measured over the number of elapsed iterations, when mutation rate is kept the same but propagation time is varied.

Figure 8: Ecosystems relax towards the non-selected condition when directed selection is removed. Relaxation depends on mutation rate, but not on propagation time. Difference in mean \( \Phi \) between high and low selected lines (±1 s.e.) plotted against time for different mutation rates and propagation time. The target vector is \((\bar{a}_1, \bar{a}_2, \bar{a}_3) = (0.2, 0.3, 0.5)\) for all runs.
Table 3: Mean performance ($\Phi$) scores from tests of artificially selected ecosystems with perturbed environmental fluxes.

<table>
<thead>
<tr>
<th>Sampling</th>
<th>$T_{prop}$</th>
<th>$P_{mut}$</th>
<th>Runs</th>
<th>Control</th>
<th>Low Baseline</th>
<th>Perturbed</th>
<th>High Baseline</th>
<th>Perturbed</th>
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<td>0.43</td>
<td>0.94</td>
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<tr>
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<td>57</td>
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<td>0.19</td>
<td>0.36</td>
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Data shown for control line communities, baseline performance of selected communities, and flux-perturbed performance of selected communities. Results are given for propagule sampling (with varied $T_{prop}$ and $P_{mut}$) and for migrant pool sampling (with default settings), for both high and low selected lines. Perturbing the environmental fluxes has a significant effect on community performance, increasing $\Phi$ for low-selected communities and decreasing $\Phi$ for high-selected communities.
Table 4: Proportion of ecosystems selected that satisfy observations O1, O2, O3 and O4. Also shown is the proportion which do not satisfy different combinations of the observations, i.e., the proportion which may be argued to satisfy hypotheses H1 and H2, depending on what falsification of H1 is accepted.

<table>
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<tr>
<th>Sampling</th>
<th>Line</th>
<th>$T_{\text{prop}}$</th>
<th>$P_{\text{mut}}$</th>
<th>Runs</th>
<th>O1</th>
<th>O2</th>
<th>O3</th>
<th>O4</th>
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<th>$(O2 \cup O4)^c$</th>
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<th>$(O1 \cup O2 \cup O4)^c$</th>
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</table>

The possible combinations of observations that falsify H1 are: (1) O1 alone, (2) O2 alone, (3) O3 (implies O1 and O2), (4) either O1 or O2 or both. These give sets where H1 and H2 are both satisfied: (1) $(O1 \cup O4)^c$, (2) $(O2 \cup O4)^c$, (3) $(O3 \cup O4)^c$, (4) $(O1 \cup O2 \cup O4)^c$, as shown in the table. For ecosystems selected using propagule sampling data is shown for variations in $T_{\text{prop}}$ and $P_{\text{mut}}$. There is an inverse relation between mutation rate and the likelihood of satisfying H1 and H2 by combination (4) (final column).
Table 5: Proportional subsets of artificially selected ecosystems representing intersections between the sets for O1, O2, O3 and O4.

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Line</th>
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<th>$P_{mut}$</th>
<th>Runs</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
<th>(6)</th>
<th>(7)</th>
<th>(8)</th>
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Data shown for high and low lines, for both propagule and migrant pool sampling methods. Variation shown along axes for $P_{mut}$ and $T_{prop}$. Target vector $(\bar{a}_1, \bar{a}_2, \bar{a}_3) = (0.2, 0.3, 0.5)$. 