LETTER

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Experimental niche evolution alters the strength of the diversity–productivity relationship

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The relationship between biodiversity and ecosystem functioning (BEF) has become a cornerstone of community and ecosystem ecology[1–3] and an essential criterion for making decisions in conservation biology and policy planning[4,5]. It has recently been proposed that evolutionary history should influence the BEF relationship because it determines species traits and, thus, species' ability to exploit resources[6,7]. Here we test this hypothesis by combining experimental evolution with a BEF experiment. We isolated 20 bacterial strains from a marine environment and evolved each to be generalists or specialists[8]. We then tested the effect of evolutionary history on the strength of the BEF relationship with assemblages of 1 to 20 species constructed from the specialists, generalists and ancestors[9]. Assemblages of generalists were more productive on average because of their superior ability to exploit the environmental heterogeneity[10]. The slope of the BEF relationship was, however, stronger for the specialist assemblages because of enhanced niche complementarity. These results show how the BEF relationship depends critically on the legacy of past evolutionary events.

Two fundamental ecological mechanisms can generate positive BEF relationships[11,12]. First, species may occupy complementary ecological niches, for example by feeding on different resources. In communities of complementary species, more of the total available niche space is filled in diverse communities, resulting in better community-wide resource use. Second, high-functioning and competitively dominant species are more likely to be found within species-rich communities (the sampling effect). Both mechanisms require a detailed understanding of species' phenotypic traits[13,14]. There has been, however, virtually no effort to understand how the evolution of species traits within ecological communities affects ecosystem functioning[6,7,15].

One important trait that determines complementarity is the degree of resource specialization, that is, the number of resources a species is able to exploit. Species niche width will tend to evolve to match the amount of available environmental variation[16,17]. In simple environments specialized types are expected to evolve, whereas generalists are more likely to appear in environments containing many resources[18,19]. The degree of specialization could alter the BEF relationship[10], so a full understanding the relationship must account for the evolutionary forces driving trait diversity.

In communities containing only specialist species that feed on different resources, the species do not compete with each other and their effects on ecosystem functioning (here productivity) are therefore additive (that is, the BEF relationship is linear; Fig. 1). However, the increased ability to exploit one resource might come with a lower ability to exploit any other, that is, there is a trade-off between resource usage ability[18] (Fig. 1a). For any type of trade-off, evolution towards generalization will affect the BEF relationship (Fig. 1b and Supplementary Information, section 1). First, ecosystem functioning at low diversity should be lower for specialists because they are inefficient at exploiting environmental heterogeneity. Second, generalization should reduce the contribution of additional species to ecosystem functioning and, thus, the slope (that is, strength) of the BEF relationship. Generalization also increases niche overlap and thus produces a nonlinear, saturating BEF relationship. We therefore predicted that the ecosystem functioning would be higher for generalists at low diversity and that the slope of the BEF relationship would be reduced in communities of generalists.

We tested these predictions by experimentally evolving niche specialization and conducting BEF experiments. Briefly, we promoted the evolution of generalist and specialist strategies from 20 ancestral bacterial strains that had been isolated from a marine environment. Each strain was grown either on a single resource (one different carbon substrate for each strain) or on a mixture of 31 resources (with a total resource availability equal to that in the single-resource treatment). Bacteria were serially transferred to fresh medium every 48 h for 32 transfers, allowing evolutionary adaptations[20] (Supplementary Information, section 2). Bacteria grown on the mixed medium (hereafter called generalists) tended to have higher performance on a wide array of substrates in comparison with the bacteria evolved on the simple medium consisting of a single resource (hereafter called specialists; see below).

We conducted the BEF experiment on the mixed medium of 31 resources for both evolutionary schemes (generalist and specialist) and with the ancestral strains (hereafter referred as species). We used the productivity (bacterial metabolic activity) after 48 and 72 h as our measure of ecosystem functioning[15]. The selection period resulted in a substantially increased productivity at all levels of species richness for both specialists and generalist assemblages (Fig. 2). Productivity significantly increased with the logarithm of species richness (48 h, $F_{1,1,506} = 291.2$, $P < 0.001$; 72 h, $F_{1,1,506} = 179.2$, $P < 0.001$), indicating that productivity was a saturating function of species richness. Productivity in monocultures differed significantly among treatments (48 h, $F_{2,1,506} = 1{,}751.9$, $P < 0.001$; 72 h, $F_{2,1,506} = 2{,}309.3$, $P < 0.001$), with ancestors performing the worst, followed in order by specialists and generalists. The slope of the BEF relationship also differed significantly among treatments (48 h, $F_{2,1,506} = 16.2$, $P < 0.001$; 72 h, $F_{1,1,506} = 8.8$, $P < 0.001$), being steeper for specialists. A model accounting for species composition also showed that the BEF relationship of ancestors was best described by a linear function of species richness and a nonlinear (saturating) function for the two evolutionary treatments (Supplementary Information, section 3). There was no relationship between the contribution of ancestors to the BEF and the contribution of their evolved counterparts (Supplementary Information, section 4). The difference between the slopes of the specialist and generalist BEF relationships was even stronger after 72 h, a result similar to those of experiments conducted with plant[21,22] and marine[23] communities.

We investigated whether the difference in the strength of the BEF relationship resulted from specialization, by growing each of the ancestral strains and specialist and generalist lineages on the 31 individual carbon substrates to estimate their final niche width. We recorded the number

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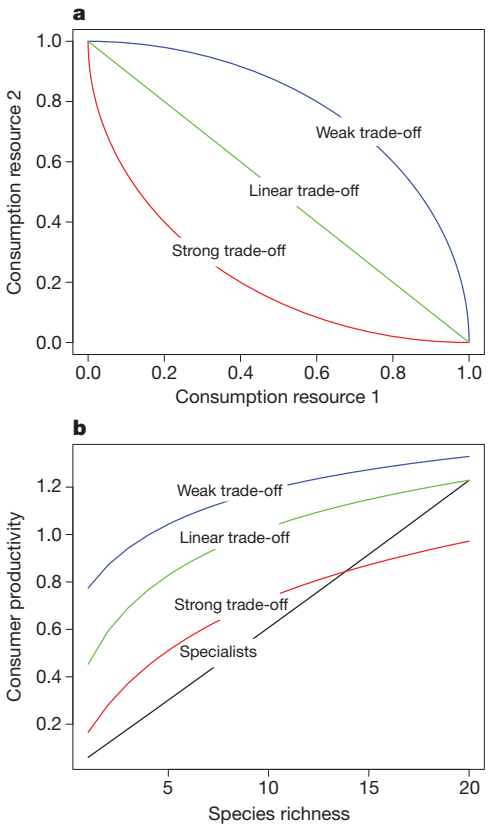

Figure 1 | Theoretical predictions of the effect of niche specialization on the strength of the BEF relationship. **a**, Hypothetical relationship between the consumption rates of a generalist on two resources. A perfect trade-off follows a straight line and any deviation to this reflects a relative cost (strong trade-off) or benefit (weak trade-off) of generalization. **b**, The BEF relationship will be affected by generalization and the type of trade-off. The figure represents a simulation experiment with $N = 20$ resources (R_i) and up to as many ($S \leq N$) consumer species (C_j). The dynamics of this system is given by the simple chemostat model $dR_i/dt = eR_0 - eR_i - \sum_{j=1}^{S} \alpha_{ij} R_i C_j$, $dC_j/dt = \sum_{i=1}^{N} \alpha_{ij} R_i C_j - mC_j - eC_j$, where e is the dilution rate, R_0 is the resource concentration in the inflow (for simplicity, we suppose that all resources have the same concentration in the inflow), α_{ij} is the per capita consumption rate of the resource i by consumer j, and m is the mortality rate. The ecosystem productivity at equilibrium is equivalent to the total resource consumption and is given by $\Phi = \sum_{i}^{N} \sum_{j}^{S} \alpha_{ij} R_i C_j$. Our simulation parameters are $R_0 = 1$, $e = 0.1$ and $m = 0.1$. We specified for the specialist that the consumption rate for its preferential resource is $\alpha_{ii} = 1$ and $\alpha_{i,j \neq i} = 0$ for the alternative resources. For the generalists, we specified the performance on the preferential resource to be $\alpha_{ii} = 0.6$ and an equal partitioning of the consumption rates between alternative resources that sums to 0.4 for the linear trade-off, 0.1 for the strong trade-off and 0.55 for the weak trade-off. We simulated communities of 1 to 20 species for specialists and generalists with linear, weak and strong trade-offs. Further details of the model and analytical results are given in Supplementary Information.

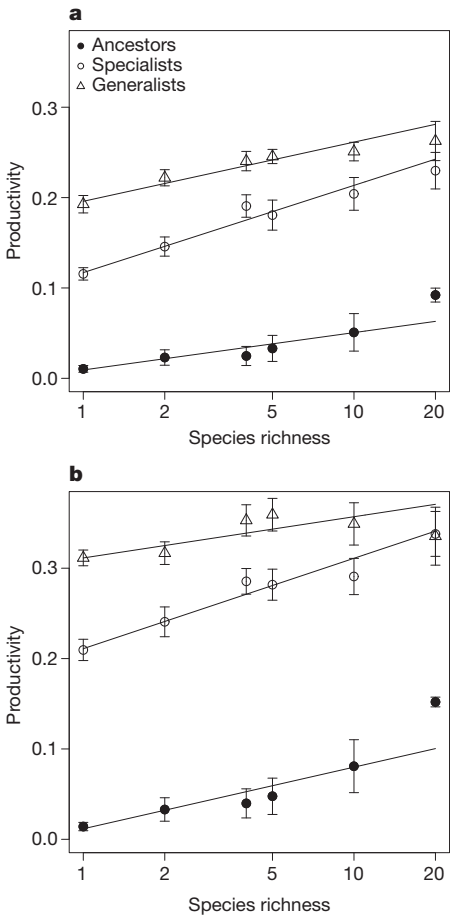

Figure 2 | Evolutionary treatments affect the strength of the biodiversity–productivity relationships. Productivity is measured as the absorbance at 590 nm after 48 h (corresponding to the conditions experienced during the selection experiment; **a**) and 72 h (**b**). Data show mean ± s.e. ($n = 504$ per evolutionary treatment). Lines depict the results of the analysis of covariance model.

of substrates each lineage was able to exploit. Bacteria cultured on single substrates adapted to fewer substrates than bacteria cultured on mixed medium (Table 1 and Supplementary Information, section 5). The generalists were able on average to exploit a larger number of substrates (10.75 ± 1.49 (s.e.) of the 31 substrates) than the specialists (4.80 ± 0.51 (s.e.); t-test for paired samples, $t_{19} = 3.45$, $P = 0.002$). The average number of shared substrates between all pairs of strains and lineages was also much higher for generalists than for ancestors and specialists (Table 1). This ability to exploit more substrates is reflected in the performance of the bacteria when grown on the mixed medium. The maximal performance recorded for each substrate for generalists was also significantly higher than the maximal performance for specialists (t-test for paired samples, $t_{30} = 2.95$, $P = 0.006$; Table 1), which suggests there was no trade-off in resource usage ability. It has been shown that concurrent adaptation to multiple resources does not always limit the capability to exploit each resource individually[8]. In fact, mutations increasing fitness on a given resource can even sometimes increase fitness on other resources (that is, synclinal selection[24]), preventing the occurrence of trade-offs. We also checked for within-lineage genotypic variability and found it to be low (Supplementary Information, section 6). Most generalist lineages were composed of generalist genotypes, but some consisted of mixtures of coexisting specialist genotypes. However, the qualitative results and conclusions (Fig. 2) were unaffected when the analysis was restricted to only those lineages that were composed of the most generalist genotypes (Supplementary Information, section 6).

Some lineages that evolved on the mixed medium responded weakly to the experimental evolution and remained specialists, and some lineages from the specialist treatment evolved towards generalization (Supplementary Information, section 5). Consequently, there is variance in ecosystem functioning that should be better predicted by functional diversity[7]. In addition to enhanced complementarity, better performance of the generalists on each carbon source might also have contributed to the difference in the BEF relationships between evolutionary treatments (Fig. 2). We calculated a niche diversity index (NDI) for every assemblage of the specialist and generalist treatments. The NDI is the number of different substrates a community is able to exploit, calculated on the basis of assays of individual species[25] (Supplementary Information, section 7). Given that generalists were more productive on each substrate, we expected that for a given NDI

Table 1 | The effect of experimental evolution on specialization

	Ancestral strains	Specialist lineages	Generalist lineages
No. of substrates used	4.00 ± 0.69	4.80 ± 0.51	10.75 ± 1.49
No. of substrates used by the 20 species assemblages	16	14	29
Average no. of shared substrates between strain–lineage pairs	2.25 ± 0.08	2.74 ± 0.11	5.80 ± 0.24
Average maximal productivity for each substrate	0.21 ± 0.07	0.23 ± 0.08	0.42 ± 0.10
Productivity of monocultures on the mixed medium	0.01 ± 0.01	0.12 ± 0.01	0.19 ± 0.02

Niche specialization was assessed from performance assays on the 31 carbon sources. A strain (or lineage) was considered able to exploit a substrate when its absorbance at 590 nm after 48 h was larger than the 95% distribution of the blanks. All data, mean ± s.e.

productivity would be larger for the generalist assemblages. We found that productivity significantly increases with community NDI ($F_{1,667} = 84.0$, $P < 0.001$; Fig. 3) (we note that the analysis is conducted on the NDI range from 3 to 14 to meet the requirements of analysis of covariance) and the logarithm of species richness ($F_{1,667} = 5.23$, $P = 0.022$). The intercepts and the slope of the NDI–productivity relationship differ between evolutionary treatments (48 h, $F_{1,667} = 72.6$, $P < 0.001$; 72 h, $F_{1,667} = 19.4$, $P < 0.001$). Overall, most of the variance is accounted for by the NDI and the effect of the evolutionary treatments on the intercept. The larger amount of explained variance by the NDI argues for complementarity as the dominant mechanism. Our experimental evolutionary treatments therefore affected both species complementarity and maximal productivity at equivalent complementarity.

The ancestral strains had not previously encountered the experimental conditions, so it is unsurprising that the intercept of the BEF relationship was greatly reduced as a result of their maladaptation (Fig. 2). Nonetheless, there is still a significant positive BEF relationship. Because each species was equally represented in the experiment, it is possible to estimate the degree to which they were associated with higher- or lower-than-average levels of functioning[26]. We found that the inferred species contributions were dominated by a single ancestral strain, whereas they were distributed more equitably in the specialist and the generalist treatments (Supplementary Information, sections 3 and 4). We note there is no significant correlation between the ancestors' contribution to the BEF and the contribution of their evolved counterparts. The data therefore provide evidence that evolutionary history could affect both mechanisms of the BEF relationship. First, the BEF relationship will be stronger for communities of specialists because of enhanced complementarity. Second, if most species are maladapted, few species are able to contribute to functioning and sampling effects dominate. Such a mechanism might be particularly important for ecosystem functioning in variable environments, where species are far from their optimal fitness peaks[27].

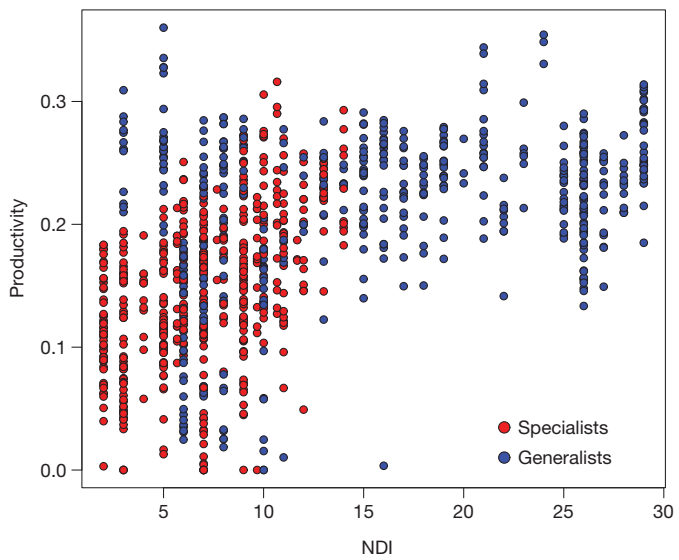

Figure 3 | The relationship between NDI and ecosystem functioning. NDI is the total number of carbon substrates a community is able to exploit, assessed from the individual ability of each lineage to exploit the carbon substrates and the community composition.

In this study, we have deliberately evolved independent lineages of specialists and generalists to compare assemblages of species that come from the same ancestral strain but have different evolutionary histories. It is likely that in nature species will diversify in complex assemblages of specialists and generalists depending on the environmental context[28]. We note that we cannot exclude the possibility that the generalists were an ensemble of specialist genotypes. Previous work has, however, shown that selection in a heterogeneous environment is most likely to result in the evolution of generalists[8]. In any case, the genotypic variability in a population, leading to increased generality, is still a species' trait that will influence the BEF relationship. Some of the changes observed between the evolved lineages might also have come through physiological adaptation. However, after at least a hundred generations we have found highly contrasting metabolic profiles (Table 1), no correlation between species contributions to the BEF relationship (Supplementary Information, section 3) and a strong response to selection (Supplementary Information, section 2). All of these observations are consistent with evolutionary changes.

A variety of BEF relationships have been observed and different ecological mechanisms have been inferred[22,29,30]. Our results provide strong support for the role of complementarity and evolutionary history in BEF. We found that specialists contribute more to the BEF. Monocultures of generalists were also found among the most productive assemblages. For conservation decisions, these results emphasize that, on average, the loss of specialists will have stronger effects on ecosystem functioning, but that losing a generalist species might have disproportionate effects when there is low redundancy. Our understanding of the mechanisms underlying the BEF relationship has now moved to a point where we cannot only distinguish among mechanisms, but can also manipulate these mechanisms experimentally. Investigations should now turn to understanding the evolutionary pressures that maintain niche diversification in natural communities, along with the trade-offs involved, and their effect on the BEF relationship.

METHODS SUMMARY

We isolated 31 phage-free bacterial strains from coastal sea water sampled off the Bay of Blanes, Spain, on the basis of their morphologies. We sequenced the 16S ribosomal DNA genes of the ancestral strains to confirm that different taxa were used in the experiment (Supplementary Information, section 8). Each strain individually underwent selection on a different, single-carbon substrate of an EcoPlate to obtain specialists, and underwent selection on a highly mixed medium made from a mixture of all 31 EcoPlate carbon substrates to obtain generalists. We transferred the bacteria to a fresh medium every 48 h during 64 d of incubation at 20 °C. After the selection period, we kept 20 lineages from among those that persisted and conducted a BEF experiment. We assembled microcosms with diversity levels, s, of 1, 2, 4, 5, 10 and 20 species for each of the three treatments (ancestral strains and specialist and generalist lineages). For each evolutionary treatment and diversity level, we created $20/s$ different assemblages by randomly selecting species from the species pool without replacement (for example, if $s = 5$ we randomly assigned the 20 species to four assemblages)[9], for a total of 42 assemblages. We carried out this process independently four times, so there were a total of 168 different assemblages for each evolutionary treatment. Each assemblage was replicated three times for a total of 1,512 microcosms (the product of three treatments, three replicates and 168 assemblages). We measured the light absorbance at 590 nm after 48 and 72 h to approximate productivity (reported

values were corrected by removing the average value of the blanks). We also conducted assays for each ancestral strain and the specialist and generalists lineages by incubating them on the 31 carbon substrates for 48 h at 20 °C. The assays were used to quantify generality and the niche diversity in the assemblages.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Bacterial isolation. Bacterial strains were isolated from coastal sea water sampled from the Bay of Blanes, Spain (40° 40′ N, 2° 50′ E) on 20–21 September 2007. Five replicate samples of 100 µl of sea water were spread on marine agar plates (BD Difco Marine Agar 2216; autoclaved for 20 min at 121 °C) and grown for 5 d at 12 °C, which was the *in situ* temperature at sampling time. Ninety-five colonies with distinct morphotypes (that is, size, shape and colour) were isolated over four weeks, clean-streaked three times and frozen in glycerol at −80 °C.

We then sequenced the 16S rDNA genes of the ancestral strains to confirm different taxa. A single colony of the 95 isolates was picked and dissolved in 10 µl TE (10 nM Tris, 1mM EDTA, pH 8.0) buffer, heated for 5 min at 95 °C and centrifuged briefly. The supernatant (1 µl) was used as the PCR template for 16S rDNA gene amplification. The PCR reaction buffer (total volume, 25 µl) contained 200 µM of each deoxynucleoside triphosphate in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM of primers 27F and 1492R[31] and ~2.5 units of puReTaq polymerase as included in the illustra puReTaq Ready-To-Go PCR beads kit (GE Healthcare). The PCR thermal cycling programme was as follows: 95 °C for 2 min; 30 cycles of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s; and 72 °C for 7 min. The PCR products were sent to AGOWA Genomics for unidirectional sequencing using primers 27F and 519R[31]. The quality of the sequences was controlled by removing traces of the sequencing primers by using PHRED[32] with a base-calling score of $n \geq 20$. Ambiguous base calls (that is, 'N's) at the ends of the sequences were also trimmed away. All sequences were analysed using the programs MALLARD[33] and CHECK_CHIMERA from the Ribosomal Database Project[34]. Neither program detected any chimaeras. Resulting sequences were then compared with the SILVA database[35] using the program BLAST[36]. A phylogenetic tree was built to infer the relationships among the ancestral strains and their closest known relatives. In combination with their morphological characteristics, we considered the 95 selected strains as different taxa (Supplementary Information, section 6).

All 95 bacterial strains were tested for production of prophage (that is, to see whether they contained an inducible viral genome) on treatment with and without (control) the inducing agent mitomycin C at a final concentration of 1 µg ml^{-1} (ref 31). Incubations were carried out in 96-well microplates for 24 h. The growth kinetics of each strain was obtained by inoculating 10 µl of overnight cultures in 200 µl marine broth culture medium (BD Difco Marine Broth 2216; autoclaved for 20 min at 121 °C). Cultures were allowed to grow in the dark at 20 °C for 48 h. Changes in cell density were measured by the amount of light absorbance at 590-nm wavelength (FLUOstar OPTIMA spectrophotometer, BMG) every 15 min. We calculated the difference in the absorbance at stationary phase between control and treated samples, and found a bimodal distribution (no effect and strong effect of mitomycin C) with a threshold corresponding to a 30% growth reduction by mitomycin C. We defined a strain containing cells with an inducible viral genome as a strain with a growth reduction larger than this threshold, and therefore consider strains under this threshold to be phage free. Of those strains that fell below the 30% threshold, we randomly selected 31 strains for the selection experiment.

Selection experiment. BIOLOG EcoPlates contain 31 different carbon substrates (plus one blank) belonging to different chemical families. In addition to the carbon substrates, each well contains a fluorogenic tetrazolium dye (5 cyano-2,3 ditolyl tetrazolium chloride), which is reduced to a violet-fluorescent formazan molecule when the carbon source is oxidized. Colour development was measured spectrophotometrically at 590 nm with a FLUOstar OPTIMA spectrophotometer and used as a proxy of metabolic activity[37]. Each of the 31 strains was used to establish three replicates of specialist and generalist selection lines. For the specialist treatment, each strain was assigned at random to one of the 31 carbon substrates. The EcoPlates used for the specialist treatment were prepared 2 h before each transfer with the addition of 140 µl of M9 minimal salts (0.1 g l^{-1} NH_4Cl, 6 g l^{-1} Na_2HPO_4, 3 g l^{-1} KH_2PO_4, 0.5 g l^{-1} NaCl) with salinity adjusted to 35.6 by the addition of NaCl, to match the salinity of the environment from which they were sampled. For the generalist treatment, we obtained a complex medium by mixing all of the 31 carbon EcoPlate substrates. The EcoPlates were prepared as for the specialists and after 30 min the contents of the EcoPlates (except the blanks) were transferred into a sterile flask, mixed with an orbital shaker and redistributed across a 96-well sterile microplate.

Each colony was initially grown for 24 h at 20 °C in 0.5 ml marine broth medium under constant orbital shaking. This solution (10 µl) was then used to inoculate each EcoPlate well from the two selection treatments. We intentionally did not wash the cells of marine broth medium to transfer a small quantity of this medium and assure the survival of the strains. Preliminary trials showed most of the strains did not initially survive in the EcoPlates without the marine broth. Consequently, for the first seven transfers we added a minimal quantity of marine broth, which we reduced from 7% (v/v) to 0% in steps of 1% at each transfer. The bacteria were incubated for 48 h in the dark at 20 °C in humid chambers. Bacteria were serially transferred to maintain maximal growth rate and to renew the substrate. A transfer consisted of inoculating every well from a new plate with 10 µl of the corresponding previous well. Before each transfer, new EcoPlates were prepared as described above. The mixed medium was prepared at every tenth transfer and stocked at 4 °C. The selection experiment ran for 32 transfers (several hundreds generations). At the thirty-second transfer, the contents of every well was amended with glycerol (50% v/v) and frozen at −80 °C.

We measured light absorbance after conducting the selection experiment. Several lineages or replicates went extinct during the experiment, mostly for the specialist treatment. Therefore, we selected the most productive replicate among the lineages that survived the specialist treatment, and the corresponding lineages of the generalist treatment and the ancestors. We used 20 strains/lineages out of the 31 that were subject to the selection experiment for the BEF experiment.

BEF experiment. We assembled random combinations of species at six levels of species richness for each of the three treatments (ancestors, evolved specialists and generalists). We used an experimental design that allowed separation of the effects of species richness and species composition[9,26]. The experimental design consisted of a set of 20/*s* microcosms, each with *s* species present. Within this set, the microcosm assemblages were constructed by sampling all of the 20 species without replacement. The construction of a system of microcosms was carried out independently four times. We chose values of *s* to be every factor of 20 ($s = 1, 2, 4, 5, 10, 20$), so for any given *s* the number of assemblages considered was $4 \times 20/s$. Each assemblage was replicated three times, so in the experiment as a whole there were $3 \times 3 \times 4 \times (1 + 2 + 4 + 5 + 10 + 20) = 1{,}512$ microcosms.

Bacterial communities were assembled in six sterile 96-well, 1-ml microplates. Bacteria were initially grown for 24 h at 20 °C in 6 ml marine broth medium under constant orbital shaking in humid chambers. The cultures were centrifuged (5 min at 3,500 r.p.m.) and washed by eliminating the marine broth and adding 6 ml M9 minimal salts with buffered salinity. Because the cultures had different productivities in the marine broth, we first measured cell density by flow cytometry[38]. We adjusted the cell density to a concentration of 5×10^{-5} cells ml^{-1} with buffered M9. The bacteria were left in starvation for 2 h before 20 µl (40 µl for the monocultures) was inoculated into the appropriate wells. Once the cultures were distributed in the appropriate wells of the six plates, 10 µl of each community was transferred into three replicated microplates containing 140 µl of the mixed medium (that is, the assemblages were initiated with 5×10^{-3} cells), for a total of 18 microplates. The cultures were incubated at 20 °C in humid chambers for 72 h. Light absorbance at 590 nm was measured at 48 and 72 h.

Assays. Assays were conducted to measure strain/lineage performances on each carbon substrate at the end of the experiment. Before the assays, frozen cultures from the end of the selection period were reconditioned in 6 ml marine broth medium for 24 h at 20 °C in humid chambers under constant orbital shaking. The cultures were centrifuged (5 min at 3,500 r.p.m.) and washed by removing the marine broth and adding M9 minimal salts with buffered salinity to adjust cell density to 3.3×10^{-6} cells ml^{-1} (a pilot study showed that this concentration was optimal to obtain a signal differentiating strains). The cultures were left in starvation for 2 h. The EcoPlates were prepared with 120 µl of the buffered M9 solution. The EcoPlates were incubated with 30 µl of culture. Each strain/lineage was incubated in triplicate at 20 °C in humid chambers. Light absorbance at 590 nm was measured after 48 h.

Statistical analyses. The selection treatment (three levels) and species richness (log-transformed) were entered into an analysis of covariance of the bacterial productivity. The dependent variable was the light absorbance at 590 nm and we analysed the effects of the experimental treatments after 48 and 72 h. The results of the assays were averaged over the three replicates. A strain/lineage was considered to be able to exploit a carbon substrate when the light absorbance was larger than the 95% of the distribution of the blanks. For each assemblage, we calculated the NDI, which is the total number of carbon substrates that a community is able to exploit, calculated on the basis of the community composition and the individual ability of each strain/lineage to exploit the carbon substrates. A second analysis of covariance was conducted (excluding the ancestors) considering the NDI instead of species richness. We also fitted a linear model that assessed the effect of species richness and species identity on ecosystem functioning without requiring knowledge of the contribution of individual species to ecosystem functioning in mixture (see ref. 26 for details of this methodology). The model returns species-specific coefficients that could be interpreted as the contribution of individual species to ecosystem functioning relative to the average species.

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