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## Levels and limits in artificial selection of communities

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1 **TITLE**

2 Levels and limits in artificial selection of communities.

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19

20 **AUTHOR CONTRIBUTIONS**

21 M.B. conceived the study, directed research, performed some statistical analyses and wrote the article,  
22 and all authors contributed substantially to revisions. B.K. performed the selection experiment under

23 the supervision of T.Z.L. and some statistical analyses under the supervision of J.M. and M.B.; T.Z.L.  
24 performed the T-RFLP analysis.

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45 **Abstract**

46 Artificial selection of individuals has been determinant in the elaboration of the Darwinian theory of  
47 natural selection. Nowadays, artificial selection of ecosystems has proven its efficiency and could  
48 contribute to a theory of natural selection at several organization levels. Here, we were not interested  
49 in identifying mechanisms of adaptation to selection, but in establishing the proof of principle that a  
50 specific structure of interaction network emerges under ecosystem artificial selection. We also  
51 investigated the limits in ecosystem artificial selection to evaluate its potential in terms of managing  
52 ecosystem function. By artificially selecting microbial communities for low CO<sub>2</sub> emissions over 21  
53 generations (n = 7,560), we found a very high heritability of community phenotype (52%). Artificial  
54 selection was responsible for simpler interaction networks with lower interaction richness. Phenotype  
55 variance and heritability both decreased across generations, suggesting that selection was more likely  
56 limited by sampling effects than by stochastic ecosystem dynamics.

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## 63 INTRODUCTION

64 Because of the time required for natural selection to occur in nature, artificial selection has been a  
65 major argument in the development of the theory of heredity with modification by Darwin, with the  
66 deep analysis of pigeon breeding genealogy (Darwin 1859). In the line of Darwin, several  
67 experimental studies have investigated the effects of artificial selection at the group level, to feed the  
68 debate on the level of natural selection (Williams 1966; Lewontin 1970; Wilson 1997). Experiments  
69 testing group artificial selection involved beetle populations (Wade 1976, 1977; Craig 1982), plant  
70 populations (Goodnight 1985), chicken populations (Craig & Muir 1996), but also two-species beetle  
71 communities (Goodnight 1990a, 1990b) or multiple-species microbial ecosystems (Swenson *et al.*  
72 2000a, 2000b). Recent research also focusses on the ecological consequences of selection of plant  
73 trait-associated microbiomes (Lau & Lennon 2012; Panke-Buisse *et al.* 2015). Consequences of these  
74 results for natural selection in nature have already been discussed (Goodnight & Stevens 1997).

75 In parallel, much of the work done in modern molecular genetics is focused on the genetic basis of  
76 organism phenotypes and changes in alleles frequencies associated with selected phenotypes.  
77 However, this cannot be the unique focus when dealing with community or ecosystem artificial  
78 selection: in addition to variations in gene frequencies, changes in ecosystem or community phenotype  
79 could be due to changes in intraspecies interactions among individuals and species composition  
80 (Goodnight 2000). A simulation model even demonstrate that ecosystem artificial selection can occur  
81 “without genetic changes”, i.e. only because of changes in species composition (Penn & Harvey  
82 2004). Before asking the question of genetic mechanisms involved in the modification of the  
83 ecosystem phenotype, it is important identifying the level at which phenotype variance occurs:  
84 community, population or individual genes? A first objective of this study was to bring an  
85 experimental proof of principle that community structure, especially the structure of interaction  
86 networks of communities, are significantly affected during the artificial selection procedure.

87 A second objective was to document how far we can go in changing ecosystem phenotype by artificial  
88 selection. Whereas limits in artificial selection have been well described and formalized at the

89 individual level (Robertson 1960; Hill 1982), the degree to which ecosystem properties may be  
90 improved by artificial selection remains unclear (Goodnight 2000). Two different interpretations of the  
91 nature of variation in ecosystem artificial selection are leading to opposite predictions on the link  
92 between variance and heritability and their consequences on the limits in ecosystem artificial selection.

93 Firstly, it has been well established that, during artificial selection of individual organisms, directional  
94 selection by truncation leads to a reduction in phenotypic variance, depending on the intensity of  
95 selection  $i$ .  $i = z/p$ , where  $z$  is the ordinate of the normal curve at the truncation point and  $p$  is the  
96 percentage of selected individuals (or the selection rate). In directional selection, the variance of the  
97 individuals selected in the parental generation decreases by a factor of  $1-i(i-x)$ , where  $x$  is the abscissa  
98 of the truncation point of the normal curve (Cochran 1951). Genetic variance can be “used up” by  
99 selection in a manner that is proportional to the relative reduction in parental phenotypic variance via  
100 the fixation of favorable alleles and the elimination of unfavorable alleles as well as rare alleles by  
101 drift (i.e. sampling effect). Exceptions occur when a selected trait involves a very large number of loci  
102 (Bulmer 1976). To summarize, a decreased genetic variance should lead to decreased phenotypic  
103 variance and consequently to a decrease in heritability and selection efficiency. This could be true for  
104 other level of organization such as the ecosystem, in which genetic variance could be “used up”  
105 through the successive loss of rare alleles, individuals or species. In this case, an observed decrease in  
106 the variance of ecosystem phenotype should be interpreted as a loss of genetic diversity by sampling  
107 effect; ecosystem phenotype variance and heritability should thus decrease along generations and be  
108 positively correlated. The limits in ecosystem artificial selection would thus be determined by the  
109 initial genetic diversity, size of the population and intensity of the sampling effect.

110 Another argument leads to an opposite prediction on nature of the limits in ecosystem selection and  
111 the sign of the correlation between variance and heritability. According to Lewontin, there are three  
112 conditions needed for selection to occur (Lewontin 1970): (i) there must be phenotypic variance  
113 among the different individuals experiencing selection; (ii) this phenotypic variance must be heritable;  
114 and (iii) phenotypic differences must be linked with different fitness values. In artificial selection  
115 experiments, this third condition is always true, as the breeder/experimenter selects individuals based

116 on phenotypic differences. But Penn *et al.* pointed out that Lewontin's first and second conditions  
117 (variance and heritability) could be at odds as far as ecosystem artificial selection is concerned (Penn  
118 2003; Penn & Harvey 2004). Indeed, Swenson *et al.* did not observe any effect of the size of the  
119 sample used to create the offspring generation (6.0 vs. 0.06 g of soil) on ecosystem phenotype  
120 variance; they interpreted this result as a proof that the intensity of the sampling effect was not  
121 determinant in the variance of the ecosystem phenotype, because its importance should have been  
122 lower with large samples than with small ones. Consequently, they proposed that ecosystem variance  
123 was determined by the stochastic dynamics of ecosystem or *butterfly effect* (Lorenz 1993), which  
124 occurs whatever the importance of initial differences due to the sampling effect (Swenson *et al.*  
125 2000b). For Penn *et al.* (2003; 2004), the stochastic ecosystem dynamics could potentially reduce the  
126 heritability of ecosystem phenotypes because it leads to differences between parental and offspring  
127 communities. As a consequence, a high variance in ecosystem phenotype due to the stochastic  
128 dynamics of ecosystem would be associated with a low heritability and vice versa. If true, a negative  
129 correlation should be observed between ecosystem phenotypic variance and heritability. In that case,  
130 artificial selection might involve more than a search for ecosystems with desired phenotypic traits; it  
131 might also be a selection of ecosystems quickly arriving at stable local equilibria, such that their  
132 properties can be reliably transmitted to the next generation. In ecosystem artificial selection, the  
133 limits to transmission of selected variations would thus rely on the ability for ecosystem dynamics to  
134 reach quickly a stable equilibrium, not on sampling effect and consequences on initial genetic  
135 diversity.

136 To test our hypotheses, we repeatedly selected for ecosystems with low CO<sub>2</sub> emissions, over 21  
137 selection events hereafter referred to as "generations". The control (random selection) and selection  
138 treatment (selection for low CO<sub>2</sub> emissions) each contained 6 independent lines of 30 communities  
139 apiece, which allowed us to test the effects of ecosystem selection in a statistically sound way (Fig. 1).  
140 For each generation, we also determined community biomass and carbon assimilation yield. To  
141 determine if a part of the community phenotype variance could be due to changes in community  
142 structure, we looked for non-random changes in community composition and ecological network

143 structure in lines of the control and selection treatment. In this aim, microbial community composition  
144 was determined by characterizing the T-RFLP-defined genetic units present in the last generation. To  
145 reveal ecological interaction network structure, we used co-occurrence patterns (see Faust & Raes  
146 (2012) for a detailed discussion); we built co-occurrence networks using genetic-unit-based correlation  
147 matrices and were thus able to explore how the structure of ecological interactions responded to  
148 ecosystem selection. To identify if the limits in ecosystem artificial selection were due to sampling  
149 effect or ecosystem dynamics, we confronted the two opposite predictions on the sign of the  
150 correlation between variance and heritability. In this aim, we calculated the heritability of CO<sub>2</sub>  
151 emissions at the ecosystem level by (i) regressing emissions by offspring communities against mean  
152 emissions by artificially selected parental communities and (ii) using the breeder's equation  
153 (Goodnight 2000).

154



## 155 MATERIALS AND METHODS

156

### 157 Artificial selection experiment

158 An initial source ecosystem was obtained from the outlet of the Valenton water treatment plant  
159 (France) in April 2012. It was stored at 4 °C for 3 weeks and then, before the experiment, a 250-ml  
160 sample was incubated at 25 °C for 12 h to allow for acclimation to experimental conditions. Terminal  
161 restriction fragment length polymorphism (T-RFLP) analysis (see below) revealed that this sample  
162 contained an initial microbial community composed of 55 genetic units (Shannon index = 1.61). The  
163 experiment involved a control and a selection treatment, which were each made up of 6 independent  
164 lines of 30 communities apiece (Fig. 1). In the control, three communities were randomly chosen from  
165 each generation of each line; these parental communities were then pooled to produce the offspring  
166 communities of the next generation. In the selection treatment, the three communities with the lowest  
167 CO<sub>2</sub> emissions were selected, and the same procedure was followed. The experiment spanned 21  
168 generations, producing a total of 7,560 communities. Our micro-ecosystems consisted of 50 µl inocula  
169 taken from the initial source ecosystem (about 10<sup>5</sup> CFU ml<sup>-1</sup>) to which 750 µl of sterile liquid medium  
170 (1/20 diluted LB: 10 g l<sup>-1</sup> Trypton, 15g l<sup>-1</sup> TSB, 500 mg l<sup>-1</sup> of yeast extract, pH=7.3) was added; they  
171 were cultivated in 96-deep-well microplates. In each plate, six control wells were filled with 800 µl of  
172 sterile liquid medium to detect any contamination. The microplates were incubated at 25 °C in the  
173 dark for 24 h; this time period was defined as the generation time. CO<sub>2</sub> emissions and microbial  
174 biomass were measured at the end of each 24-hour period. The new generation was created by taking  
175 50 µl from each pool of control or artificially selected communities.

176

### 177 Quantification of ecosystem properties

178 As stated above, communities were selected based on their CO<sub>2</sub> emissions. CO<sub>2</sub> measurements were  
179 performed using the MicroResp<sup>TM</sup> system (Campbell *et al.* 2003), adapted for use with aquatic

180 communities (Tlili *et al.* 2011). The CO<sub>2</sub> emissions of each community were quantified by examining  
181 the relative change in the cresol red indicator dye suspended above each well of the 96-well plates.  
182 After a 24-hour incubation period, the absorbance of the indicator dye was measured at 570 nm using  
183 a microplate reader (Synergy HT, BioTek, USA); the values obtained were converted into estimates of  
184 CO<sub>2</sub> emissions using a calibration curve previously established at 25 °C (Lerch *et al.* 2013). Each  
185 community's microbial biomass was estimated using a 200-μl sample. Samples were placed in the  
186 wells of a 300-μL microplate and their absorbance at 600 nm (Synergy HT, BioTek, USA) was  
187 measured to assess the density of bacterial cells (cells ml<sup>-1</sup>). Cell density was converted to C biomass  
188 (referred to as C<sub>Bio</sub> below, μg C ml<sup>-1</sup>) assuming 10<sup>-12</sup> g wet weight per cell, a water content of 70%,  
189 and a C content as 40% of dry weight (Bratbak & Dundas 1984). The metabolic efficiency of the  
190 microbial communities was estimated using carbon assimilation yield (*Y*), which was calculated as  
191 follows:

$$192 \quad Y = \frac{C_{Bio}}{C_{Bio} + C_{Min}}$$

193 where *C<sub>Bio</sub>* and *C<sub>Min</sub>* are the amounts of assimilated and mineralized carbon, respectively (Lerch *et al.*  
194 2007a).

195

## 196 **Community composition**

197 The composition of the bacterial communities was determined on the last generation (21<sup>th</sup>) using T-  
198 RFLP analysis. The details of the T-RFLP analysis are provided in Supplementary Information and the  
199 length of the restriction fragments is provided in **SI Tab. 1**. The richness of the T-RFLP profiles was  
200 expressed as the total number of T-RFs, and the evenness of the profiles was estimated using the  
201 Shannon index (*H'*) (Shannon 1948), which was calculated as follows:

$$202 \quad H' = \sum_{i=1}^s p_i \ln(p_i)$$

203 where  $p_i$  represents the relative abundance of a given T-RF  $i$ . Past work has found that these indices  
204 are significantly correlated with true soil bacterial community richness and diversity ( $R^2 = 0.71$ ,  $P =$   
205  $0.05$ ) only when communities contain less than 1,200 species (Blackwood *et al.* 2007), as was the case  
206 for our experimental communities.

207

## 208 **Statistical Analysis**

209 Statistical analyses were performed using the following variables quantified for each generation: (i)  
210 absolute CO<sub>2</sub> emissions of the control and treatment lines; (ii) relative CO<sub>2</sub> emissions of the six  
211 selection treatment lines (mean of each treatment line minus the overall control mean); (iii) mean  
212 relative CO<sub>2</sub> emissions of the selection treatment (overall treatment mean minus the overall control  
213 mean). Biomass and carbon assimilation yield were similarly quantified, transformed, and analyzed.

214 Data normality was tested using the *nortest* package in R (Gross & Ligges 2012). We found that the  
215 residuals of relative CO<sub>2</sub> emissions, biomass, and carbon assimilation yield were normally distributed,  
216 but those of the absolute values of these variables were not. The former were consequently analyzed  
217 using linear models (*stat* package in R), while the latter were examined using generalized linear mixed  
218 models employing restricted maximum likelihood (REML, *nlme* package in R (Pinheiro *et al.* 2013)  
219 Time (generation), treatment, and their interaction were the fixed effects; line and microplate position  
220 in the incubator were the random effects.

221 The T-RFLP profile data were analyzed using correspondence analysis (*ade4* package in R (Chessel *et*  
222 *al.* 2004) An intergroup constrained analysis coupled with a Monte Carlo permutation test was  
223 performed to examine differences in community composition between the control and the selection  
224 treatment. The data were also used to build co-occurrence networks (i.e., comprising direct and  
225 indirect interactions). Using the abundance of the different genetic units, we calculated Spearman  
226 correlation coefficients for different genetic unit pairs and constructed a correlation matrix. Only  
227 significantly correlated pairs were included in the networks ( $P \leq 0.05$ ) (Barberan *et al.* 2012). Distinct  
228 positive and negative co-occurrence networks were built for the control and selection treatment by

229 converting the correlation matrix into an adjacency matrix with either positive or negative correlation  
230 coefficients. A global network that combined positive and negative co-occurrences was also  
231 constructed to provide an overview of ecological network structure. In this network, modularity, also  
232 called compartmentalization, equals the number of isolated sub-networks. The adjacency matrix was  
233 resampled via bootstrapping (*boot* package (Ripley 1999) in R) with a view to quantifying the  
234 variance associated with the estimation of the four following interaction network indices (*statnet*  
235 package (Handcock *et al.* 2008) in R): (1) average degree (D) is the average number of interactions  
236 engaged in by one genetic unit (equal to 0 for an unconnected unit)—it is a good estimate of network  
237 complexity; (2) average betweenness (B) is the average number of shorter chains going through one  
238 node—it can signal the presence of keystone species in the network (from a topological standpoint,  
239 i.e., a node with many links); (3) connectance (C) is the proportion of possible links between species  
240 that are actually realized—this index links the ecological network’s overall structure to the behavior of  
241 the genetic units; (4) connectedness (Cd) is the probability that at least one chain exists between any  
242 pair of units—it quantifies all the direct and indirect interactions within the network. Index values for  
243 the control and selection treatment were compared using a Wilcoxon rank sum test with continuity  
244 correction for non-parametric data.

245

## 246 **RESULTS**

247

### 248 **Artificial selection**

249 Artificial selection was successful: ecosystems from the selection treatment emitted significantly less  
250 CO<sub>2</sub> than those of the control. Over the experiment's 21 generations, CO<sub>2</sub> emitted in selection  
251 treatment decreased by 0.253 μg C ml<sup>-1</sup> (-60%) and only by 0.142 μg C ml<sup>-1</sup> (-38%) in the control (SI  
252 Fig. 1). Three of the six treatment lines emitted significantly less CO<sub>2</sub> over time relative to the control  
253 (SI Fig. 2). Mean overall CO<sub>2</sub> emitted by the selection treatment decreased significantly more than that  
254 emitted by the control ( $R^2 = 0.23$ ,  $P = 0.015$ ) (Fig. 2a).

255

### 256 **Ecosystem function**

257 Relative carbon assimilation yield did not change over time in any of the treatment lines (SI Fig. 3) or  
258 in the group as a whole ( $R^2 = 0.009$ ,  $P = 0.29$ ) (Fig. 2b). In contrast, relative biomass production  
259 declined over time in each of the selection treatment lines (SI Fig. 4) and in the selection treatment as  
260 a whole ( $R^2 = 0.91$ ,  $P < 0.001$ ) (Fig. 2c). The lower CO<sub>2</sub> emissions of the treatment communities were  
261 thus due to lower biomass production. The greater  $R^2$  value for biomass (0.91) as compared with the  
262 selected CO<sub>2</sub> emissions (0.23) can be explained in two ways: (i) biomass measurements (made using  
263 optical density) were more accurate than the CO<sub>2</sub> measurements (made using the MicroResp<sup>TM</sup> system)  
264 (Campbell *et al.* 2003) or (ii) biomass values tend to integrate temporal variation, unlike CO<sub>2</sub>  
265 emissions.

266

### 267 **Community structure**

268 The analysis of the genetic composition of the last generation of microbial communities showed that  
269 neither the erosion of diversity nor the presence of a single, specific genetic unit could explain the

270 stronger decrease in CO<sub>2</sub> emissions in the selection treatment. Indeed, genetic diversity was similar in  
271 the control and selected communities (specific richness of 20 and 18 genetic units and Shannon index  
272 of 0.87 and 0.83 for the control and selection treatment respectively). The treatment explained 23% of  
273 the variance in community composition (constrained correspondence analysis; axis 1: 27%, axis 2:  
274 20%, Monte Carlo test:  $P = 0.002$ ) (Fig. 3). Despite an important variation in the different lines within  
275 the control and selection treatment, species composition differed significantly between the control and  
276 the treatment.

277

## 278 **Ecological network structure**

279 Microbial interaction networks can only be analyzed in terms of co-occurrence networks (see Faust &  
280 Raes (2012) for a detailed discussion of the difference between interaction and co-occurrence  
281 networks). . To build the co-occurrence networks in the control and selection treatment, we calculated  
282 the correlation coefficients for pairs of genetic units and selected those that were significantly  
283 positively or negatively correlated ( $P < 0.05$ ). The total number of such pairs, often referred to as  
284 “interaction richness” (Tylianakis *et al.* 2010), is a measure of interaction diversity, which is positively  
285 correlated with the rate of ecosystem processes (Snyder *et al.* 2006; Hoehn *et al.* 2008). Interaction  
286 richness was equal to 54 and 28 for the control and selection treatment, respectively. Connectance, i.e.  
287 realized interaction richness reported to potential interaction richness, was also lower in the selection  
288 treatment network than in the control for the overall network (-23%,  $P < 0.0001$ , Fig. 4e) as for the  
289 positive and negative co-occurrence networks (SI Fig. 5e, 6e). Interaction diversity can additionally  
290 be expressed by the average degree which reveals the average number of interactions involving each  
291 node (i.e., an individual genetic unit with at least one significant co-occurrence). Genetic units were  
292 involved in 3.2 interactions in the overall control network versus 2.0 in the overall selection treatment  
293 network (-35%,  $P = 0.038$ ) (Fig. 4c); this difference was not significant for the positive and negative  
294 networks ( $P = 0.10$  and  $0.11$ , respectively, SI Fig. 5c, 6c). Approximately 48 and 46% of co-

295 occurrences were negative in the control and treatment networks, respectively. Thus, artificial  
296 selection decreased interaction diversity but did not affect co-occurrence direction.

297 Another useful information can be obtained from the number of clusters or sub-networks, a network  
298 indicia called compartmentalization or modularity. Modularity was similar for the positive and  
299 negative co-occurrence networks (SI Fig. 5a,b, 6a,b), but the overall control network contained a  
300 single cluster and the overall treatment network contained four clusters (Fig. 4a,b), which indicates the  
301 presence of isolated microbial groups. Average betweenness specifies the average proportion of  
302 centrally located nodes, which are viewed as “hubs” or “keystone species” from a network  
303 perspective. The overall selection treatment network contained far fewer hubs than the overall control  
304 network (-87%,  $P = 0.003$ , Fig. 4d); this also hold for the positive and negative networks (SI Fig. 5d,  
305 6d). Finally, connectedness is the probability that at least one chain exists between any pair of units,  
306 i.e. it quantifies all direct and indirect interactions within the network. Once again, the overall  
307 selection treatment network had lower connectedness than the overall control network (-71%,  $P <$   
308  $0.0001$ , Fig. 4f); positive and negative networks showed similar patterns (SI Fig. 5f, 6f). Taken  
309 together, the results for these three indices show that the treatment network was formed by the  
310 juxtaposition of several small networks, characterized by many isolated compartments and contained  
311 fewer hubs.

312

### 313 **Ecosystem-level heritability**

314 Heritability has been defined as the covariance between average effects and average excess (Fisher  
315 1930), or as the proportion of total variance that can contribute to a response to selection (Goodnight  
316 2000). Heritability at the ecosystem level can be calculated using the slope of the regression between  
317 the mean trait values of the selected parental ecosystems and the trait values of the offspring  
318 ecosystems (Goodnight 2000). Accounting for the decrease in CO<sub>2</sub> emissions observed in the control,  
319 we found a very high overall heritability ( $h^2$ ) of  $51.9 \pm 1.4\%$  (mean  $\pm$  s.e.) ( $P < 2 \times 10^{-16}$ ,  $N = 3600$ ) (Fig.  
320 5). For lines 1 to 6 ( $N = 600$ ),  $h^2$  equaled  $61.7 \pm 3.9\%$  ( $P < 2 \times 10^{-16}$ ),  $70.4 \pm 4.3\%$  ( $P < 2 \times 10^{-16}$ ),

321 81.6±3.8% ( $P < 2 \times 10^{-16}$ ), 41.3±3.8% ( $P < 2 \times 10^{-16}$ ), 32.0±3.3% ( $P < 2 \times 10^{-16}$ ) and 32.5±3.2% ( $P < 2$   
322  $\times 10^{-16}$ ), respectively (SI Fig. 7). The dramatic differences in  $h^2$  among lines (32 to 82%) suggested  
323 that independent trajectories of the lines resulted in large differences in their selection potential.

324 To determine the sign of the correlation between heritability and ecosystem phenotype variance, we  
325 estimated  $h^2$  at each generation using the standard breeder's equation:  $h^2 = R/S$ , where R is the  
326 response to selection and S is the selection differential (Goodnight 2000). This calculation can yield  
327 results greater than 1 when population sizes are small. We found a significant positive correlation  
328 between heritability and variance for all the parental ecosystems within a given generation ( $P = 0.006$ ,  
329 Fig. 5), even if the relationship was weak ( $R^2 = 0.07$ ). This positive correlation may be due to a time  
330 effect because both the variance and heritability of the parental generation decreased over time ( $R^2 =$   
331  $0.10$ ,  $P = 0.0007$  and  $R^2 = 0.04$ ,  $P = 0.0316$ , respectively;  $N = 115$ ).

332

333



## 334 **DISCUSSION**

335

### 336 **Statistically sound evidence that ecosystems can be artificially selected**

337 Artificial selection of ecosystems was successful. Decreased CO<sub>2</sub> emissions were observed in both the  
338 selection treatment and the control because biomass declined over time, probably due to a dilution  
339 effect. However, the decrease in emissions was significantly greater in the selection treatment than in  
340 the control. Previous studies demonstrating artificial selection of complex ecosystem properties were  
341 lacking statistical robustness because of an absence of line replication (Swenson *et al.* 2000b) or  
342 synthetic regression analysis (Swenson *et al.* 2000a). Here, with a large data set obtained using 7,560  
343 ecosystems (12 independent lines of 30 ecosystems each allowed to run for 21 generations), we have  
344 clearly demonstrated that artificial selection can lead to a statistically significant difference in  
345 ecosystem properties.

346

### 347 **Levels of selection**

348 Evolution at the ecosystem level has mainly been discussed in terms of sources of variation related to  
349 target ecosystem phenotypes. Changes in ecosystem phenotype could be due to modifications in intra-  
350 or inter-species interactions or species composition (Goodnight 2000; Penn & Harvey 2004). An  
351 artificial selection experiment shows that, in two-species communities, artificial selection can lead to  
352 the emergence of correlated interspecific responses, which suggests that genetically based interactions  
353 among individuals are involved (Goodnight 1990a, 1990b). An individual-based evolutionary  
354 simulation model has also confirmed that artificial selection can act on complex communities by the  
355 way of ecological interactions (Williams & Lenton 2007). By showing that the group's response was  
356 not simply the sum of the responses of its individual components, the model revealed that many  
357 communities are selected because of selective pressures acting on ecological interactions, not on  
358 individual species.

359 In our study, we were not able to analyze all possible sources of variance, but our results provide  
360 several new pieces of knowledge regarding the nature of variance of ecosystem phenotype. Firstly,  
361 microbial ecosystem selection does not select for a single genetic species; indeed, at the end of the  
362 experiment, selected communities were not comprised of one or a few species. Instead, species  
363 richness was equally high in the selection treatment and the control. Mean numbers of genetic units  
364 and Shannon index values were similar for the control and selection treatment. However, rare species  
365 that could not be detected with our molecular methods might have been impacted. Secondly, changes  
366 in community composition are an ecosystem response to selection. Although a large amount of  
367 variance resulted from sampling effects and the lines' different evolutionary trajectories, the control  
368 and treatment groups nonetheless differed dramatically in community composition and structure (Fig.  
369 3). A significant effect of artificial selection on microbial community composition has also been  
370 observed for plant trait-associated microbiomes obtained in multigenerational selection experiments  
371 (Lau & Lennon 2012; Panke-Buisse *et al.* 2015). Thirdly, specific ecological network patterns,  
372 especially interaction richness, can be selected for: co-occurrence network analysis showed that  
373 ecological network structure was very different in the control versus the selection treatment (Fig. 4),  
374 especially with regards to interaction richness. Since lower interaction richness may be associated with  
375 lower rates of ecosystem function (Snyder *et al.* 2006; Hoehn *et al.* 2008; Tylianakis *et al.* 2010), it is  
376 likely that selection resulted in reduced CO<sub>2</sub> emissions by reducing interaction richness. It is thus  
377 unlikely that individual organism-level selection could have acted to shape ecological co-occurrence  
378 patterns, which emerge at the community level.

379 Our results call for epistemological considerations. First, in ecosystem artificial selection experiments,  
380 the nature of the selected entities (either community or ecosystem) depends on the amount of abiotic  
381 (even if biogenic) material transmitted with the inoculum, and its functional consequences. However,  
382 it was not possible to assess the role played by this abiotic material in our experiment. Secondly, the  
383 level of the selected unit was methodologically defined by the generation time and the volume of  
384 ecosystems. Reproduction is a step of the experimental protocol. This is responsible for a disjunction  
385 between the generation time and size of the selected unit as compared with the range of generation

386 times of microorganisms belonging to different species (Fig. 3) and the size of their populations.  
387 Nevertheless, artificial selection operates. This argues in favor of an enlarged vision of evolution  
388 based on operationally defined functional entities (CO<sub>2</sub> emitting ecosystems in our case), rather than  
389 on organism reproduction and their populations (see Bouchard 2014 and Doolittle 2014 for a detailed  
390 analysis).

391

### 392 **Limits on the artificial selection of ecosystems**

393 The results of our experiment indicate that variance in the parental generation decreased over time.  
394 This finding illustrates that genetic diversity is progressively lost through the fixation of favorable  
395 alleles (see Introduction). Variance in ecosystem phenotype could be due to the sampling effect of the  
396 community or to the stochastic ecosystem dynamics (see Introduction). Because a decreased variance  
397 is expected in the case of an important sampling effect and not in the case of an important stochastic  
398 dynamics, the decrease in variance over time suggests that sampling effect is at the origin of  
399 ecosystem phenotype variance.

400

401 Heritability calculated as the slope of the regression of CO<sub>2</sub> emissions by offspring communities  
402 against mean emissions by artificially selected parental communities (Fig. 5) provided a very high  
403 heritability value of 52% in the selection treatment, far greater than the 15% found by Goodnight  
404 (2000), the only other estimate, to our knowledge. This indicated a strong potential for changing an  
405 ecosystem phenotype in the desired direction by ecosystem artificial selection (see also the review by  
406 Goodnight & Stevens, 1997). In addition, heritability calculated using the breeder's equation declined  
407 over time, together with artificial selection efficiency. The reduced probability of improving  
408 ecosystem properties over time can be understood in the context of Fisher's geometric model (Fisher  
409 1930). At the ecosystem level, changes in species ecological interaction or community composition  
410 responsible for a given amount of change in ecosystem phenotype can, in fact, be selected for when  
411 the level of adaptation is low (i.e., during the first generations of an ecosystem selection experiment).

412 However, as fitness increases, such changes have a reduced ability to improve future fitness. Only  
413 changes causing smaller changes can increase fitness in the later generations.

414 Both variance and heritability were positively correlated. As explained in the Introduction, if the  
415 stochastic dynamics of ecosystem were responsible for ecosystem phenotype variance (Swenson *et al.*  
416 2000a, 2000b; Penn 2003; Penn & Harvey 2004), we should observe a negative correlation between  
417 variance and heritability. Conversely, by extrapolating Cochran hypothesis (1951) from the individual  
418 organism level to the ecosystem level, if the sampling effect was dominant, we should observe a  
419 positive correlation between variance and heritability. Our results show a positive correlation, which  
420 suggests that stochastic ecosystem dynamics did not have a large effect on heritability and selection  
421 efficiency, whereas sampling effect seems to be mainly at the origin of phenotype variance.

422 Goodnight found that communities that exhibited heritable variation also tended to be small, integrated  
423 communities in which sampling effects had major consequences (Goodnight 2000, 2011). However,  
424 Swenson *et al.* did not observe any effect of the size of the sample used to create the offspring  
425 generation (6.0 vs. 0.06 g of soil); they interpreted this result as meaning that initial differences that  
426 form the basis of the butterfly effect (Lorenz 1993) can be arbitrarily small. Two explanations might  
427 account for the disparity between our results and those of Swenson *et al.* First, sample sizes tested in  
428 Swenson *et al.* experiment (6.0 vs. 0.06 g of soil) could both have been too large to exhibit notable  
429 differences due to sampling effects. This hypothesis could be tested by studying a larger range of  
430 sample sizes. Second, population size has been identified as placing strong limits on artificial selection  
431 at the individual level (Robertson 1960; Roberts 1966), because more non-optimal alleles can be fixed  
432 by drift when population size decreases. In our experiment, we observed a decrease in community  
433 biomass over time (Fig. 2c), likely to result from a decrease in population size. Over time, the  
434 increasing effect of drift could have taken precedence over the ecosystem's stochastic dynamics and  
435 explain our results. This hypothesis could be tested by choosing longer generation time, to ensure a  
436 constant population size and drift effect.

437

438

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595

596 **Figure legends**

597

598 **Figure 1** Experimental methodology. The control and treatment each contained six lines  
599 of 30 communities, which served as replicates. To create each line, we filled 30 wells of a 96-  
600 well microplate with a 1/20-diluted LB medium; we then inoculated each well with stock  
601 from a natural, complex microbial community. After a 24-hour incubation period, the CO<sub>2</sub>  
602 emitted by each community was measured. In the selection treatment lines, the three  
603 communities with the lowest CO<sub>2</sub> emissions were then selected and used to inoculate 30 new  
604 wells and thus formed the next generation. In the control lines, the three parental communities  
605 were randomly selected. This cycle was repeated for 20 generations.

606

607 **Figure 2** Changes in mean relative community properties in response to selection.  
608 Differences in community **a)** CO<sub>2</sub> emissions, **b)** carbon assimilation yield, and **c)** biomass  
609 production. To determine the effects of artificial selection, the mean values for the control  
610 were subtracted from the mean values for the treatment. The R<sup>2</sup> and P-values were determined  
611 using linear models. A regression line is depicted if the slope of the regression was  
612 significantly different from zero. Dashed line: control; solid line: selection treatment. See the  
613 Supplementary Materials and Methods section for a detailed explanation of how carbon  
614 assimilation yield was calculated.

615

616 **Figure 3** Community microbial diversity at the end of the selection experiment:  
617 **a)** correlation circle for the correspondence analysis—each box corresponds to one  
618 T-RFLP-defined genetic unit and **b)** barycenters for the control (C) and selection treatment  
619 (S) groups. Each point represents one of the six lines found in each group. The control and  
620 treatment communities differed significantly in composition (Monte Carlo test: P = 0.002).  
621 Note that certain genetic units were present in some but not all selection treatment lines and  
622 absent from the control lines (e.g., X197, X235, X361) or vice versa (e.g., X114, X209,  
623 X240); some were also more common in the treatment than in the control (e.g., X187, X230,  
624 X364) and vice versa (e.g., X173, X185, X205).

625

626 **Figure 4** Structure of the overall co-occurrence networks and values of the related  
627 indices. The co-occurrence matrices of the T-RFLP-defined genetic units present after 21  
628 generations were used to build interaction networks for **a)** the control (C) and **b)** the selection  
629 treatment (S). When two dots are connected by lines, it means that the abundances of the  
630 genetic units were significantly correlated (Spearman correlation coefficient;  $N = 6$ ;  $P < 0.05$ ).  
631 The interaction networks were used to calculate **c)** average degree and **d)** average  
632 betweenness for the two treatments. The networks were bootstrapped (200 random samples  
633 from each group's pool of genetic units) to determine **e)** average connectance and **f)** average  
634 connectedness. The values of these indices were compared for the control and selection  
635 treatment using Wilcoxon rank-sum tests (employing a continuity correction for non-  
636 parametric distributions). See the Supplementary Materials and Methods section for a full  
637 description of how the indices were calculated.

638

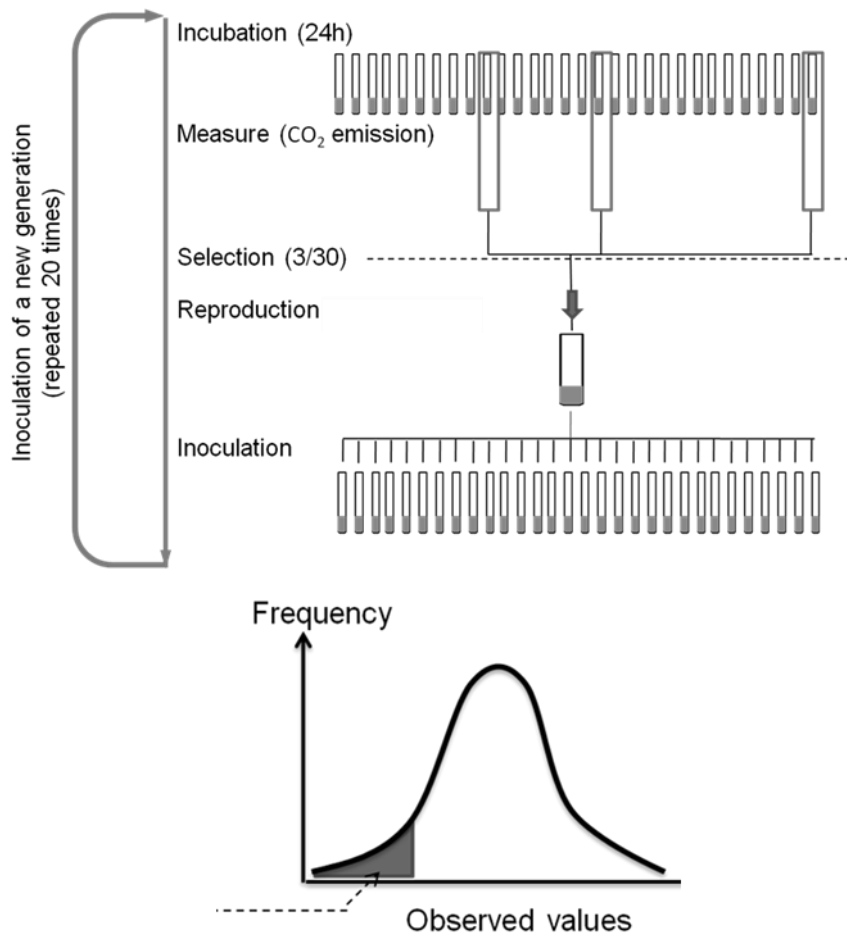
639 **Figure 5** Linear regression of the CO<sub>2</sub> emissions of the 30 offspring communities as a  
640 function of the mean CO<sub>2</sub> emissions of the three artificially selected parental communities (all  
641 generations included). The data were corrected by accounting for the decrease in CO<sub>2</sub>  
642 emissions in the control and standardized to get a slope, which was equal to heritability *sensu*  
643 *stricto* ( $h^2$ ). Regression equation:  $y = 0.52x$  ( $P < 2 \times 10^{-16}$ ,  $N = 3600$ ).

644

645

646 **Figures**

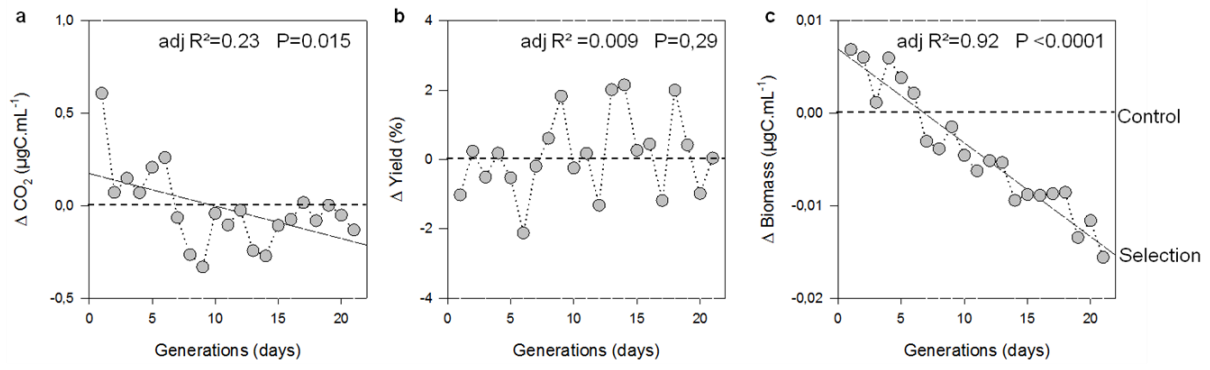
647 Figure 1



648

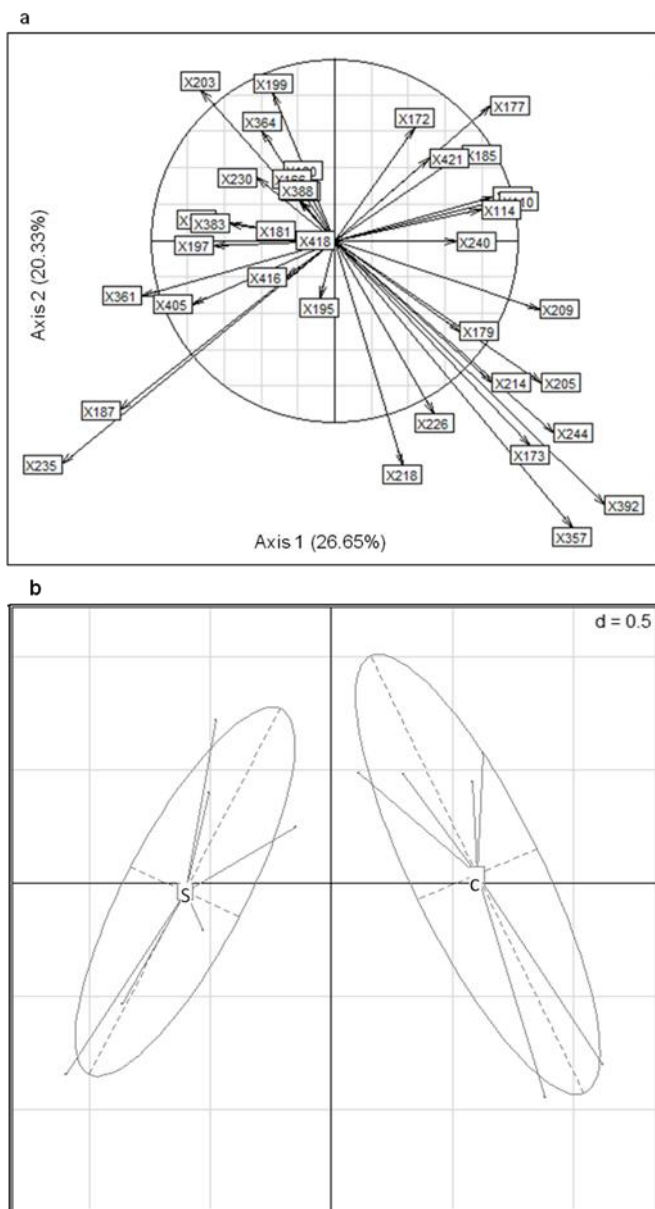
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650 Figure 2

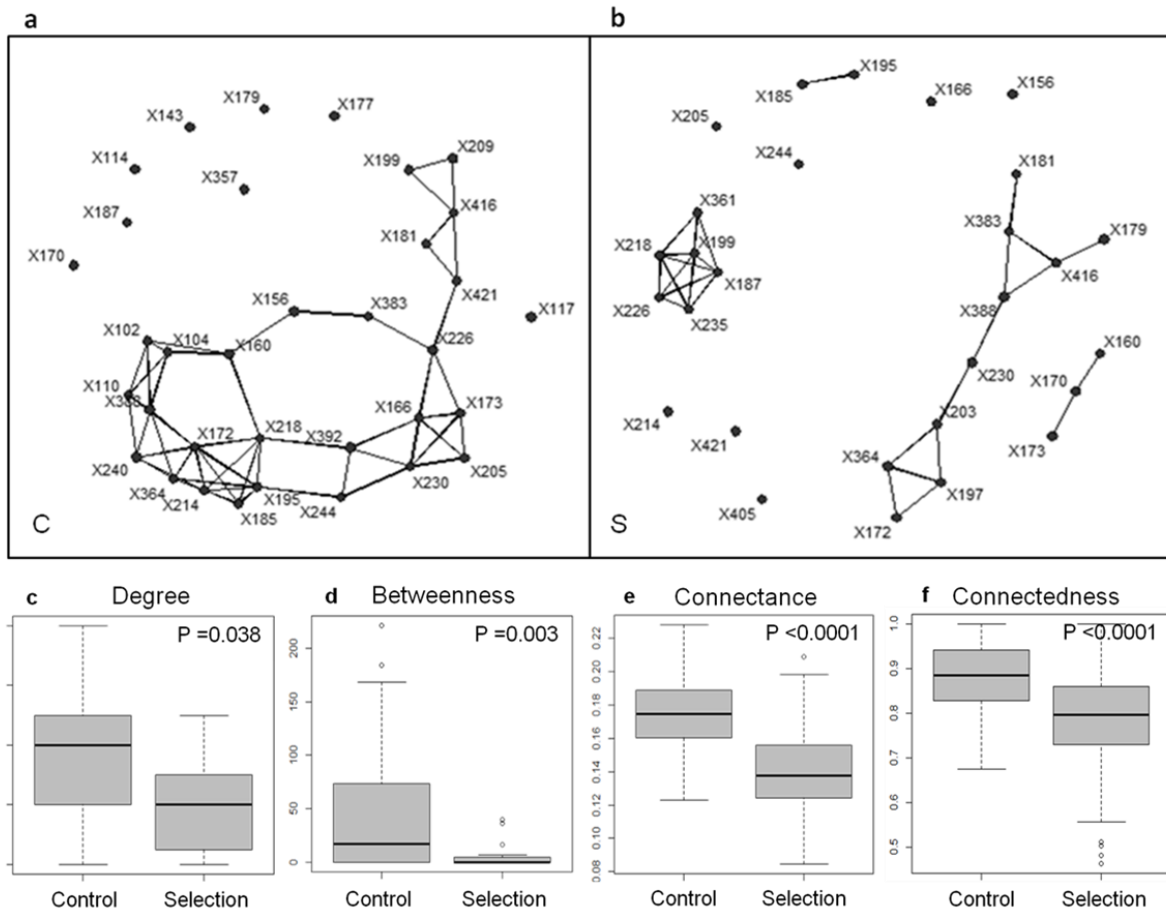


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652 Figure 3

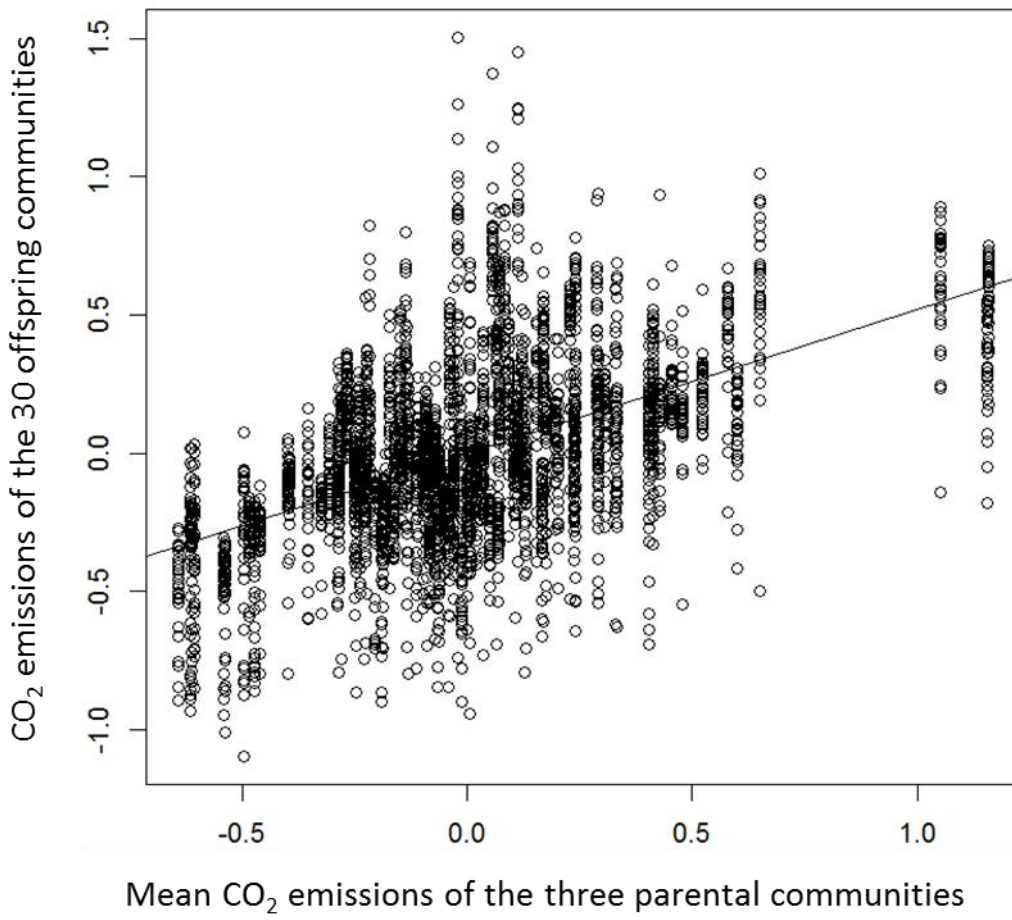


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656 Figure 5



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Inoculation of a new generation  
(repeated 20 times)

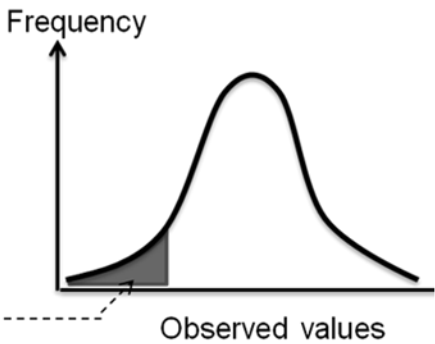
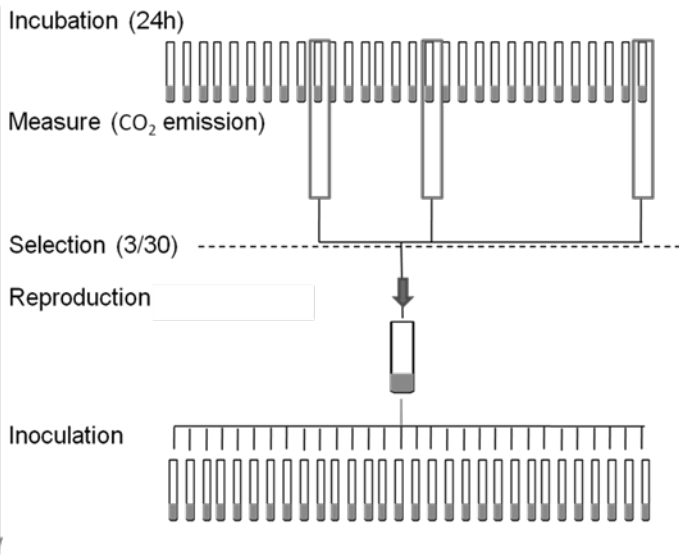


Fig.1

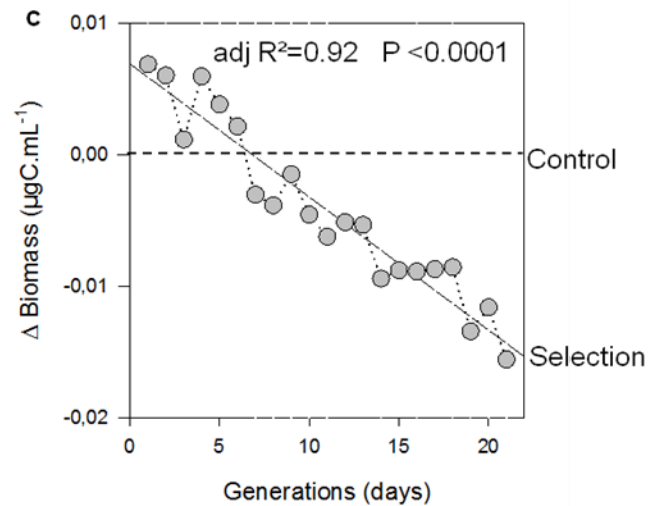
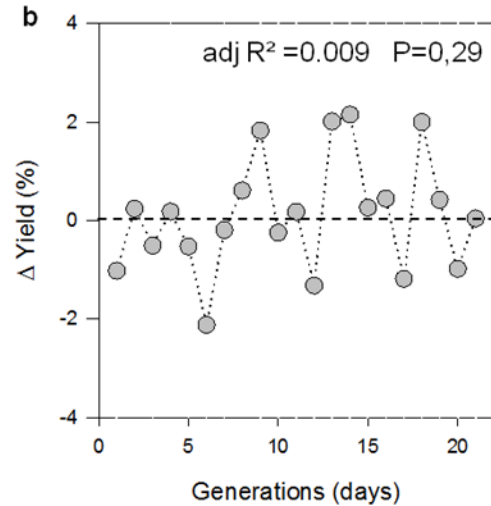
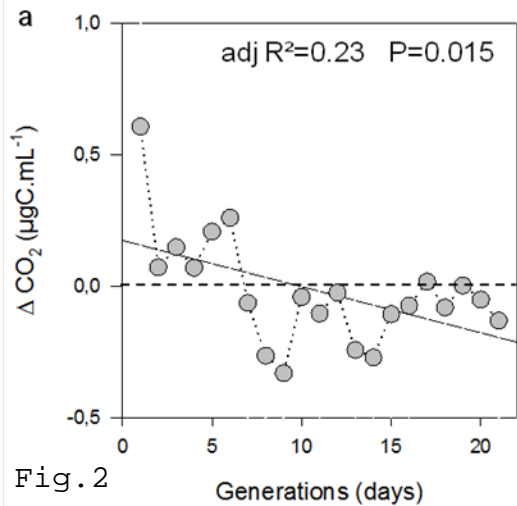
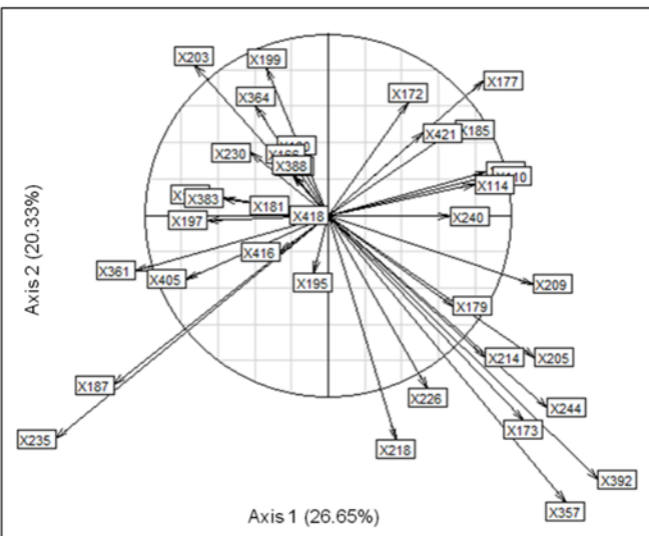
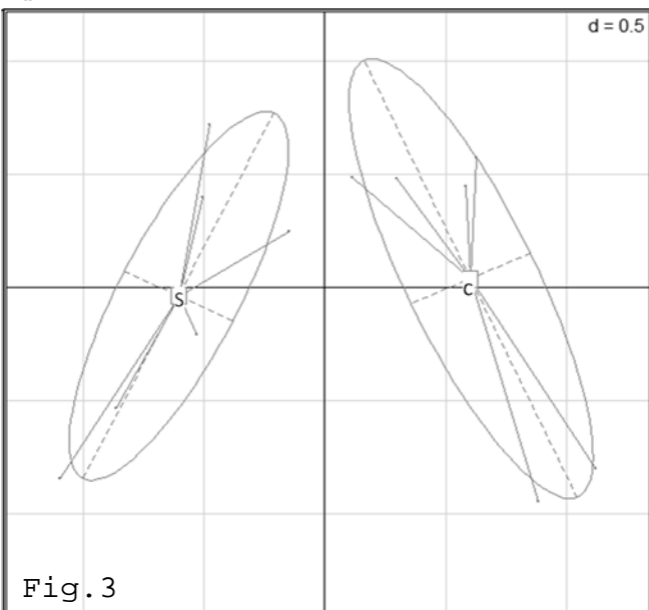
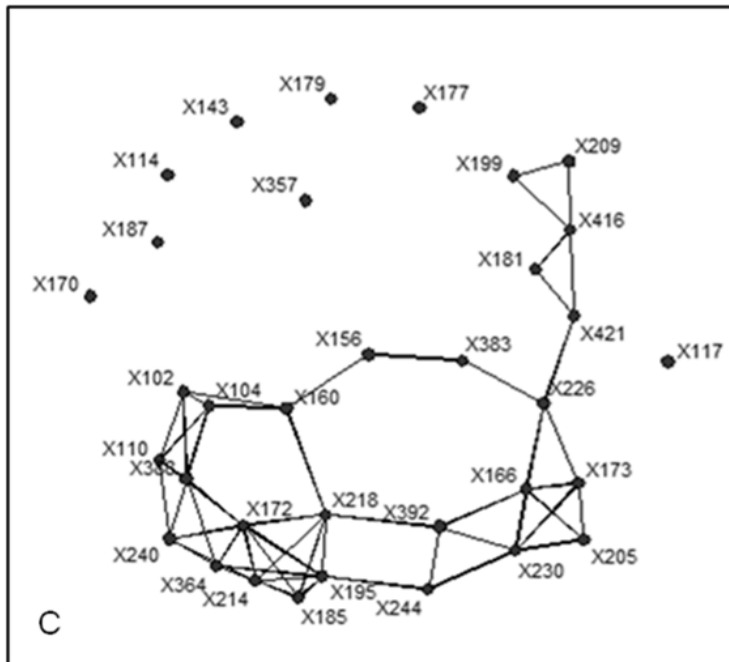
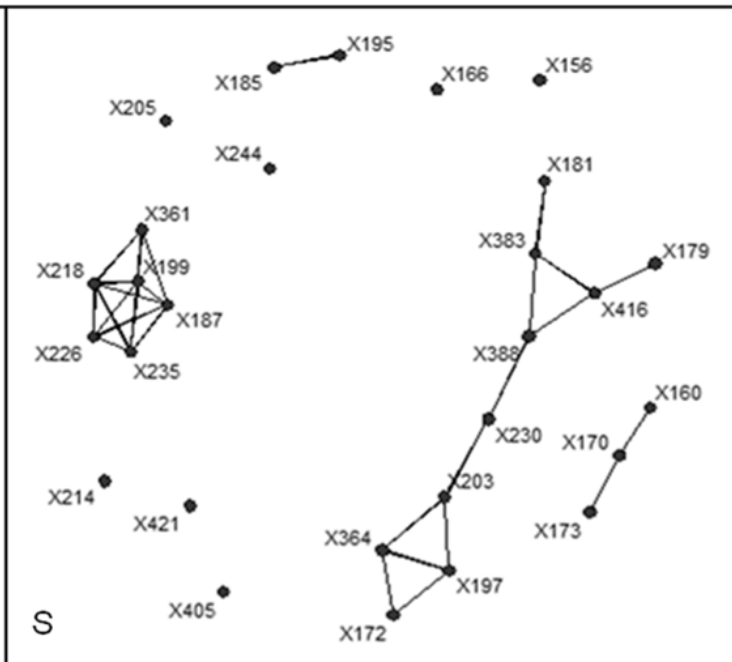
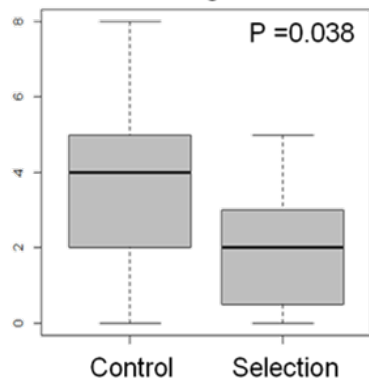
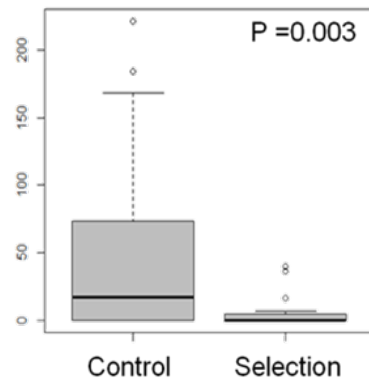
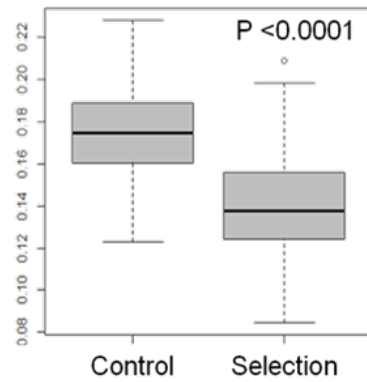
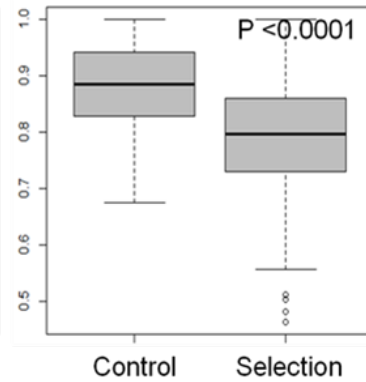


Fig. 2

**a****b****Fig. 3**

**a** Fig. 4**b****c** Degree**d** Betweenness**e** Connectance**f** Connectedness

CO<sub>2</sub> emissions of the 30 offspring communities

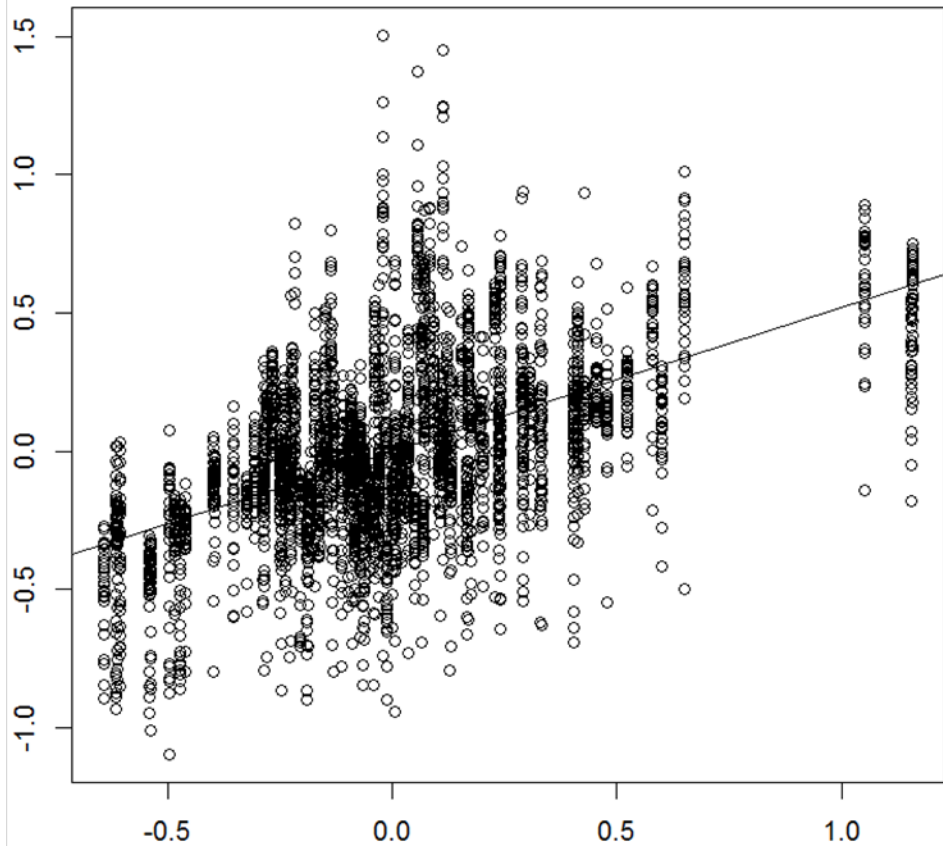


Fig. 5 Mean CO<sub>2</sub> emissions of the three parental communities