Levels and limits in artificial selection of communities

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TITLE

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AUTHOR CONTRIBUTIONS

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

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

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Abstract

Artificial selection of individuals has been determinant in the elaboration of the Darwinian theory of natural selection. Nowadays, artificial selection of ecosystems has proven its efficiency and could contribute to a theory of natural selection at several organization levels. Here, we were not interested in identifying mechanisms of adaptation to selection, but in establishing the proof of principle that a specific structure of interaction network emerges under ecosystem artificial selection. We also investigated the limits in ecosystem artificial selection to evaluate its potential in terms of managing ecosystem function. By artificially selecting microbial communities for low CO_2 emissions over 21 generations (n = 7,560), we found a very high heritability of community phenotype (52%). Artificial selection was responsible for simpler interaction networks with lower interaction richness. Phenotype variance and heritability both decreased across generations, suggesting that selection was more likely limited by sampling effects than by stochastic ecosystem dynamics.

INTRODUCTION

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

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

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

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

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

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

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

To test our hypotheses, we repeatedly selected for ecosystems with low CO_2 emissions, over 21 selection events hereafter referred to as "generations". The control (random selection) and selection treatment (selection for low CO_2 emissions) each contained 6 independent lines of 30 communities apiece, which allowed us to test the effects of ecosystem selection in a statistically sound way (Fig. 1). For each generation, we also determined community biomass and carbon assimilation yield. To determine if a part of the community phenotype variance could be due to changes in community structure, we looked for non-random changes in community composition and ecological network

structure in lines of the control and selection treatment. In this aim, microbial community composition was determined by characterizing the T-RFLP-defined genetic units present in the last generation. To reveal ecological interaction network structure, we used co-occurrence patterns (see Faust & Raes (2012) for a detailed discussion); we built co-occurrence networks using genetic-unit-based correlation matrices and were thus able to explore how the structure of ecological interactions responded to ecosystem selection. To identify if the limits in ecosystem artificial selection were due to sampling effect or ecosystem dynamics, we confronted the two opposite predictions on the sign of the correlation between variance and heritability. In this aim, we calculated the heritability of CO_2 emissions at the ecosystem level by (i) regressing emissions by offspring communities against mean emissions by artificially selected parental communities and (ii) using the breeder's equation (Goodnight 2000).

MATERIALS AND METHODS

Artificial selection experiment

An initial source ecosystem was obtained from the outlet of the Valenton water treatment plant (France) in April 2012. It was stored at 4 °C for 3 weeks and then, before the experiment, a 250-ml sample was incubated at 25 °C for 12 h to allow for acclimation to experimental conditions. Terminal restriction fragment length polymorphism (T-RFLP) analysis (see below) revealed that this sample contained an initial microbial community composed of 55 genetic units (Shannon index = 1.61). The experiment involved a control and a selection treatment, which were each made up of 6 independent lines of 30 communities apiece (Fig. 1). In the control, three communities were randomly chosen from each generation of each line; these parental communities were then pooled to produce the offspring communities of the next generation. In the selection treatment, the three communities with the lowest CO_2 emissions were selected, and the same procedure was followed. The experiment spanned 21 generations, producing a total of 7,560 communities. Our micro-ecosystems consisted of 50 µl inocula taken from the initial source ecosystem (about 10^5 CFU ml^{-1}) to which 750 µl of sterile liquid medium (1/20 diluted LB: 10 g l^{-1} Trypton, 15g l^{-1} TSB, 500 mg l^{-1} of yeast extract, pH=7.3) was added; they were cultivated in 96-deep-well microplates. In each plate, six control wells were filled with 800 µl of sterile liquid medium to detect any contamination. The microplates were incubated at 25 °C in the dark for 24 h; this time period was defined as the generation time. CO_2 emissions and microbial biomass were measured at the end of each 24-hour period. The new generation was created by taking 50 µl from each pool of control or artificially selected communities.

Quantification of ecosystem properties

As stated above, communities were selected based on their CO_2 emissions. CO_2 measurements were performed using the MicroResp™ system (Campbell *et al.* 2003), adapted for use with aquatic

communities (Tlili *et al.* 2011). The CO_2 emissions of each community were quantified by examining the relative change in the cresol red indicator dye suspended above each well of the 96-well plates. After a 24-hour incubation period, the absorbance of the indicator dye was measured at 570 nm using a microplate reader (Synergy HT, BioTek, USA); the values obtained were converted into estimates of CO_2 emissions using a calibration curve previously established at 25 °C (Lerch *et al.* 2013). Each community's microbial biomass was estimated using a 200-µl sample. Samples were placed in the wells of a 300-µL microplate and their absorbance at 600 nm (Synergy HT, BioTek, USA) was measured to assess the density of bacterial cells (cells ml^{-1}). Cell density was converted to C biomass (referred to as C_{Bio} below, µg C ml^{-1}) assuming 10^{-12} g wet weight per cell, a water content of 70%, and a C content as 40% of dry weight (Bratbak & Dundas 1984). The metabolic efficiency of the microbial communities was estimated using carbon assimilation yield (*Y*), which was calculated as follows:

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

where C_{Bio} and C_{Min} are the amounts of assimilated and mineralized carbon, respectively (Lerch *et al.* 2007a).

Community composition

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

$$H' = \sum_{i=1}^{S} p_i \ln(p_i)$$

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

Statistical Analysis

Statistical analyses were performed using the following variables quantified for each generation: (i) absolute CO_2 emissions of the control and treatment lines; (ii) relative CO_2 emissions of the six selection treatment lines (mean of each treatment line minus the overall control mean); (iii) mean relative CO_2 emissions of the selection treatment (overall treatment mean minus the overall control mean). Biomass and carbon assimilation yield were similarly quantified, transformed, and analyzed.

Data normality was tested using the *nortest* package in R (Gross & Ligges 2012). We found that the residuals of relative CO_2 emissions, biomass, and carbon assimilation yield were normally distributed, but those of the absolute values of these variables were not. The former were consequently analyzed using linear models (*stat* package in R), while the latter were examined using generalized linear mixed models employing restricted maximum likelihood (REML, *nlme* package in R (Pinheiro *et al.* 2013) Time (generation), treatment, and their interaction were the fixed effects; line and microplate position in the incubator were the random effects.

The T-RFLP profile data were analyzed using correspondence analysis (a*de4* package in R (Chessel *et al.* 2004) An intergroup constrained analysis coupled with a Monte Carlo permutation test was performed to examine differences in community composition between the control and the selection treatment. The data were also used to build co-occurrence networks (i.e., comprising direct and indirect interactions). Using the abundance of the different genetic units, we calculated Spearman correlation coefficients for different genetic unit pairs and constructed a correlation matrix. Only significantly correlated pairs were included in the networks ($P \leq 0.05$) (Barberan *et al.* 2012). Distinct positive and negative co-occurrence networks were built for the control and selection treatment by

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

RESULTS

Artificial selection

Artificial selection was successful: ecosystems from the selection treatment emitted significantly less CO_2 than those of the control. Over the experiment's 21 generations, CO_2 emitted in selection treatment decreased by 0.253 µg C ml^{-1} (-60%) and only by 0.142 µg C ml^{-1} (-38%) in the control (SI Fig. 1). Three of the six treatment lines emitted significantly less CO_2 over time relative to the control (SI Fig. 2). Mean overall CO_2 emitted by the selection treatment decreased significantly more than that emitted by the control ($R^2 = 0.23$, $P = 0.015$) (Fig. 2a).

Ecosystem function

Relative carbon assimilation yield did not change over time in any of the treatment lines (SI Fig. 3) or in the group as a whole ($R^2 = 0.009$, $P = 0.29$) (Fig. 2b). In contrast, relative biomass production declined over time in each of the selection treatment lines (SI Fig. 4) and in the selection treatment as a whole ($R^2 = 0.91$, $P < 0.001$) (Fig. 2c). The lower CO_2 emissions of the treatment communities were thus due to lower biomass production. The greater R^2 value for biomass (0.91) as compared with the selected CO_2 emissions (0.23) can be explained in two ways: (i)biomass measurements (made using optical density) were more accurate than the CO_2 measurements (made using the MicroResp™ system) (Campbell *et al.* 2003) or (ii)biomass values tend to integrate temporal variation, unlike CO_2 emissions.

Community structure

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

stronger decrease in CO_2 emissions in the selection treatment. Indeed, genetic diversity was similar in the control and selected communities (specific richness of 20 and 18 genetic units and Shannon index of 0.87 and 0.83 for the control and selection treatment respectively). The treatment explained 23% of the variance in community composition (constrained correspondence analysis; axis 1: 27%, axis 2: 20%, Monte Carlo test: P = 0.002) (Fig. 3). Despite an important variation in the different lines within the control and selection treatment, species composition differed significantly between the control and the treatment.

Ecological network structure

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

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

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

Ecosystem-level heritability

Heritability has been defined as the covariance between average effects and average excess (Fisher 1930), or as the proportion of total variance that can contribute to a response to selection (Goodnight 2000). Heritability at the ecosystem level can be calculated using the slope of the regression between the mean trait values of the selected parental ecosystems and the trait values of the offspring ecosystems (Goodnight 2000). Accounting for the decrease in CO_2 emissions observed in the control, we found a very high overall heritability (h^2) of 51.9±1.4% (mean±s.e.) ($P < 2 \times 10^{-16}$, $N = 3600$) (Fig. 5). For lines 1 to 6 ($N = 600$), h^2 equaled 61.7±3.9% ($P < 2 \times 10^{-16}$), 70.4±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

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DISCUSSION

Statistically sound evidence that ecosystems can be artificially selected

Artificial selection of ecosystems was successful. Decreased CO_2 emissions were observed in both the selection treatment and the control because biomass declined over time, probably due to a dilution effect. However, the decrease in emissions was significantly greater in the selection treatment than in the control. Previous studies demonstrating artificial selection of complex ecosystem properties were lacking statistical robustness because of an absence of line replication (Swenson *et al.* 2000b) or synthetic regression analysis (Swenson *et al.* 2000a). Here, with a large data set obtained using 7,560 ecosystems (12 independent lines of 30 ecosystems each allowed to run for 21 generations), we have clearly demonstrated that artificial selection can lead to a statistically significant difference in ecosystem properties.

Levels of selection

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

In our study, we were not able to analyze all possible sources of variance, but our results provide several new pieces of knowledge regarding the nature of variance of ecosystem phenotype. Firstly, microbial ecosystem selection does not select for a single genetic species; indeed, at the end of the experiment, selected communities were not comprised of one or a few species. Instead, species richness was equally high in the selection treatment and the control. Mean numbers of genetic units and Shannon index values were similar for the control and selection treatment. However, rare species that could not be detected with our molecular methods might have been impacted. Secondly, changes in community composition are an ecosystem response to selection. Although a large amount of variance resulted from sampling effects and the lines' different evolutionary trajectories, the control and treatment groups nonetheless differed dramatically in community composition and structure (Fig. 3). A significant effect of artificial selection on microbial community composition has also been observed for plant trait-associated microbiomes obtained in multigenerational selection experiments (Lau & Lennon 2012; Panke-Buisse *et al.* 2015). Thirdly, specific ecological network patterns, especially interaction richness, can be selected for: co-occurrence network analysis showed that ecological network structure was very different in the control versus the selection treatment (Fig. 4), especially with regards to interaction richness. Since lower interaction richness may be associated with lower rates of ecosystem function (Snyder *et al.* 2006; Hoehn *et al.* 2008; Tylianakis *et al.* 2010), it is likely that selection resulted in reduced CO_2 emissions by reducing interaction richness. It is thus unlikely that individual organism-level selection could have acted to shape ecological co-occurrence patterns, which emerge at the community level.

Our results call for epistemological considerations. First, in ecosystem artificial selection experiments, the nature of the selected entities (either community or ecosystem) depends on the amount of abiotic (even if biogenic) material transmitted with the inoculum, and its functional consequences. However, it was not possible to assess the role played by this abiotic material in our experiment. Secondly, the level of the selected unit was methodologically defined by the generation time and the volume of ecosystems. Reproduction is a step of the experimental protocol. This is responsible for a disjunction 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_2 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_2 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).

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

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

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

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594

595

Figure legends

Figure 1 Experimental methodology. The control and treatment each contained six lines of 30 communities, which served as replicates. To create each line, we filled 30 wells of a 96-well microplate with a 1/20-diluted LB medium; we then inoculated each well with stock from a natural, complex microbial community. After a 24-hour incubation period, the CO_2 emitted by each community was measured. In the selection treatment lines, the three communities with the lowest CO_2 emissions were then selected and used to inoculate 30 new wells and thus formed the next generation. In the control lines, the three parental communities were randomly selected. This cycle was repeated for 20 generations.

Figure 2 Changes in mean relative community properties in response to selection. Differences in community **a**) CO_2 emissions, **b**) carbon assimilation yield, and **c**) biomass production. To determine the effects of artificial selection, the mean values for the control were subtracted from the mean values for the treatment. The R^2 and P-values were determined using linear models. A regression line is depicted if the slope of the regression was significantly different from zero. Dashed line: control; solid line: selection treatment. See the Supplementary Materials and Methods section for a detailed explanation of how carbon assimilation yield was calculated.

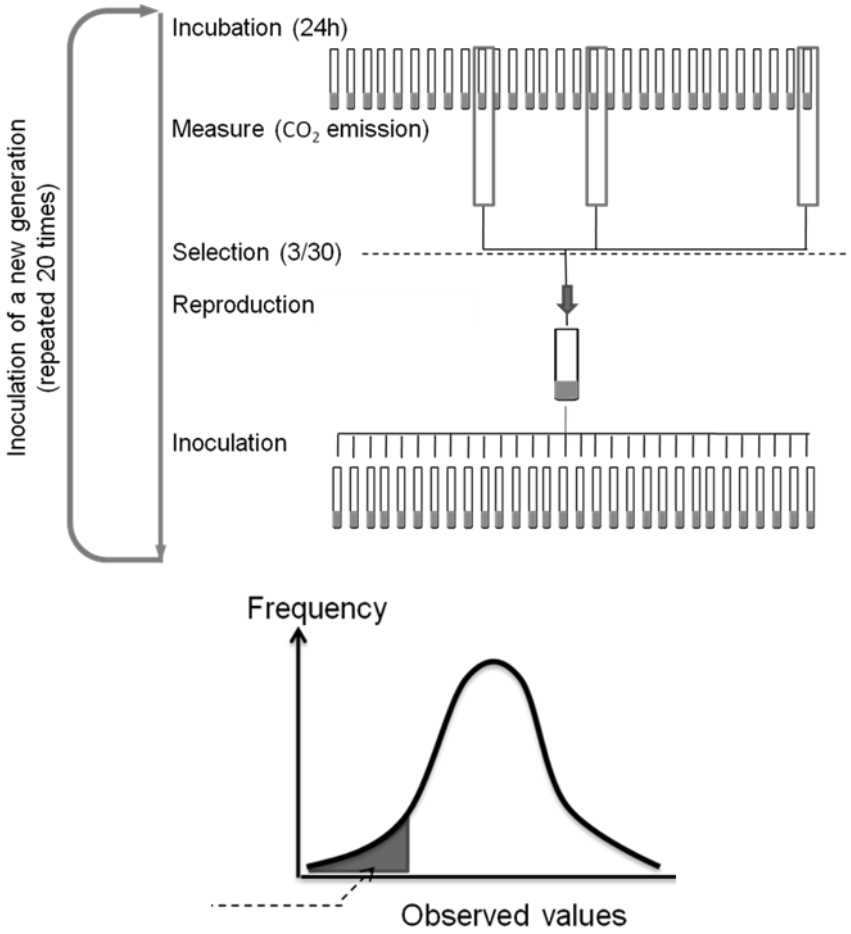
Figure 3 Community microbial diversity at the end of the selection experiment: **a**) correlation circle for the correspondence analysis—each box corresponds to one T-RFLP-defined genetic unit and **b**) barycenters for the control (C) and selection treatment (S) groups. Each point represents one of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

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

Figure 5 Linear regression of the CO_2 emissions of the 30 offspring communities as a function of the mean CO_2 emissions of the three artificially selected parental communities (all generations included). The data were corrected by accounting for the decrease in CO_2 emissions in the control and standardized to get a slope, which was equal to heritability *sensu stricto* (h^2). Regression equation: $y = 0.52x$ ($P < 2 \times 10^{-16}$, $N = 3600$).

646 **Figures**

647 Figure 1



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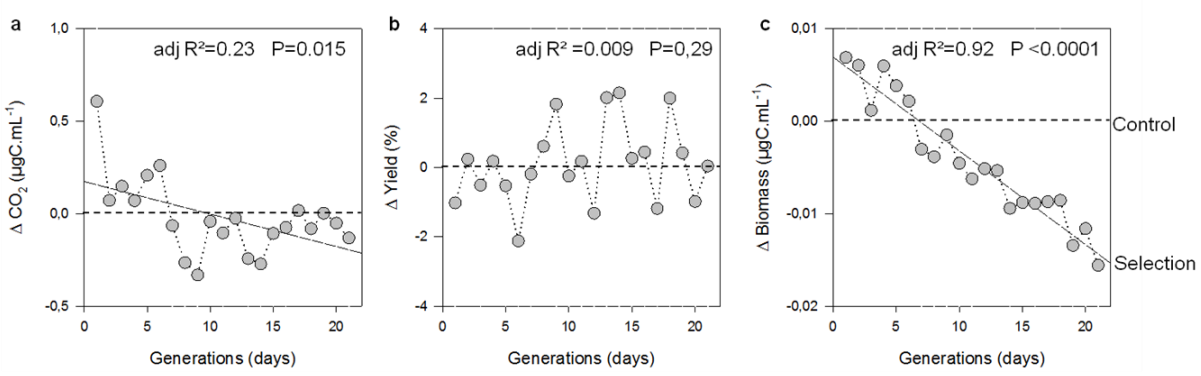
650 Figure 2



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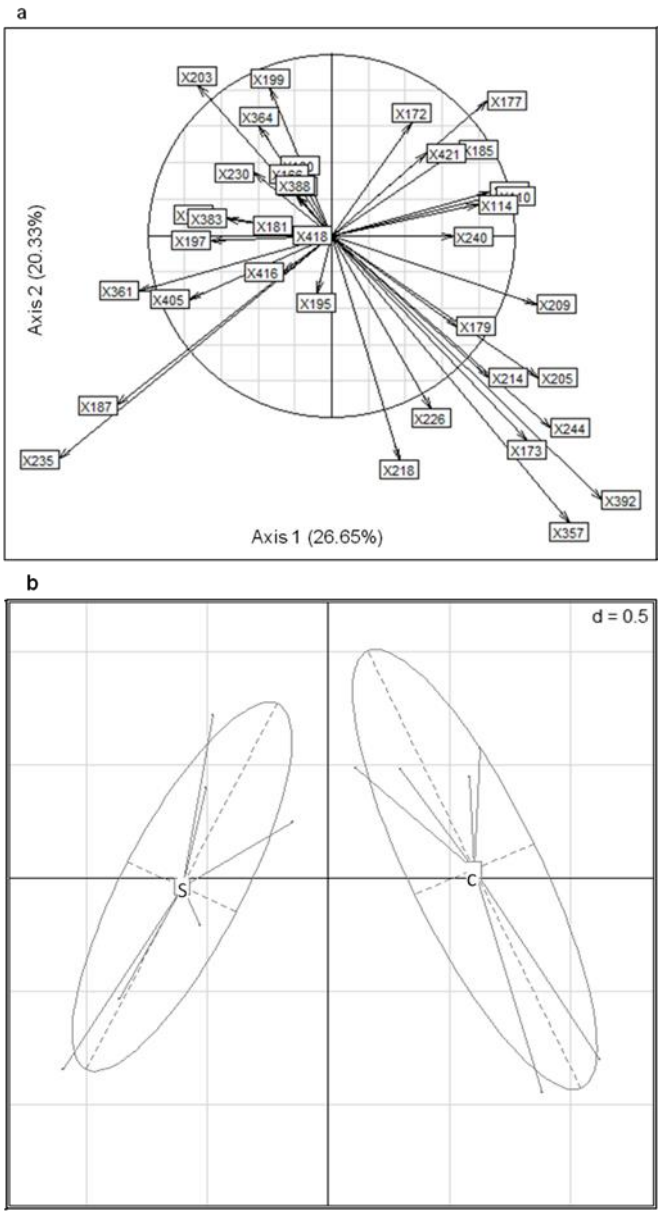
652 Figure 3



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654 Figure 4

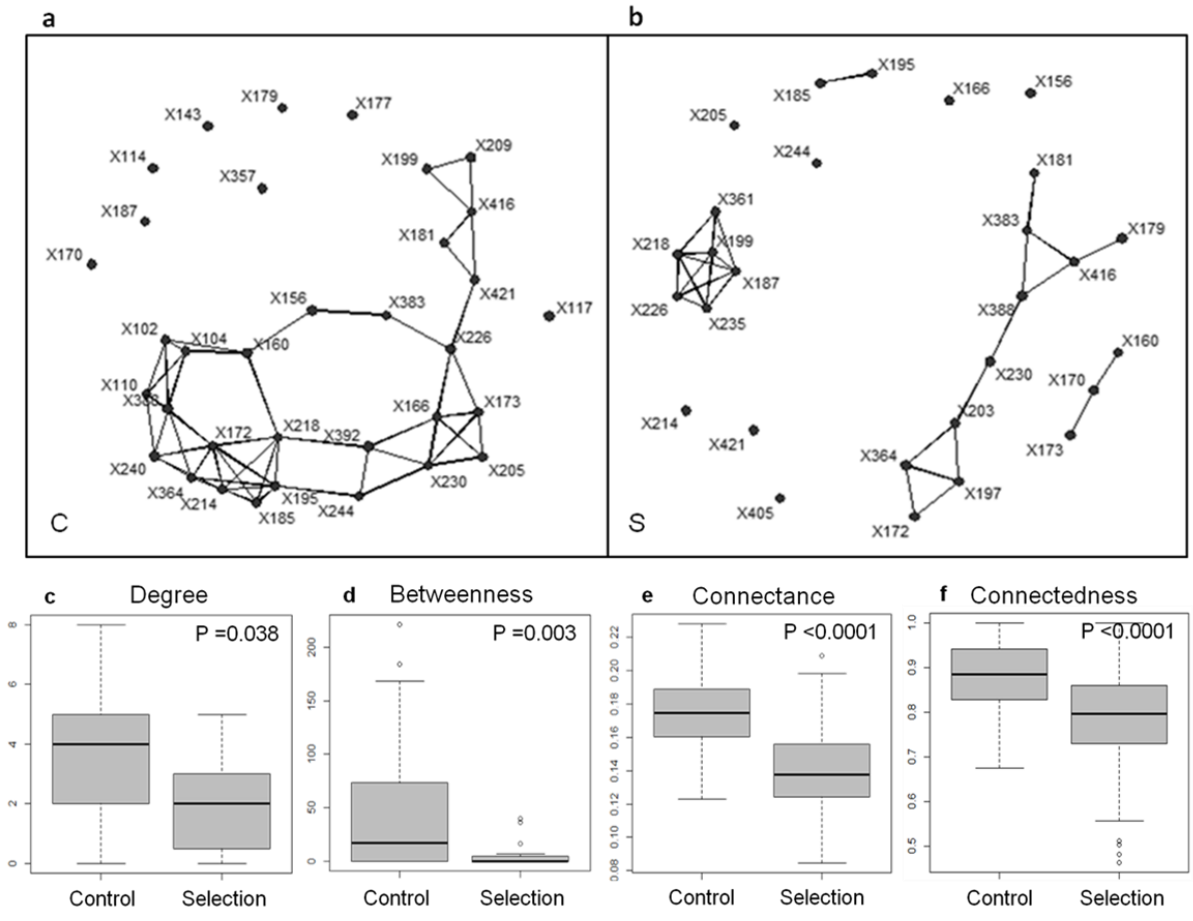

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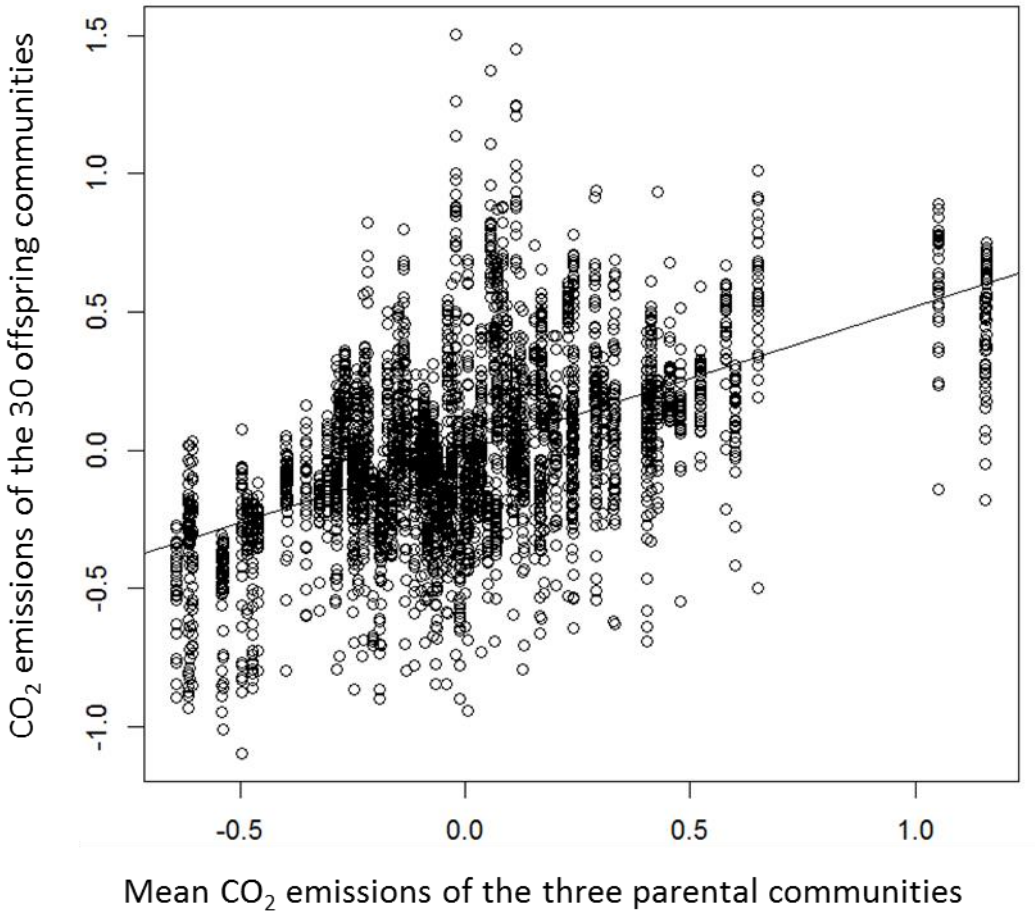
Figure 5


CO_2 emissions of the 30 offspring communities
1.5
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
Mean CO_2 emissions of the three parental communities

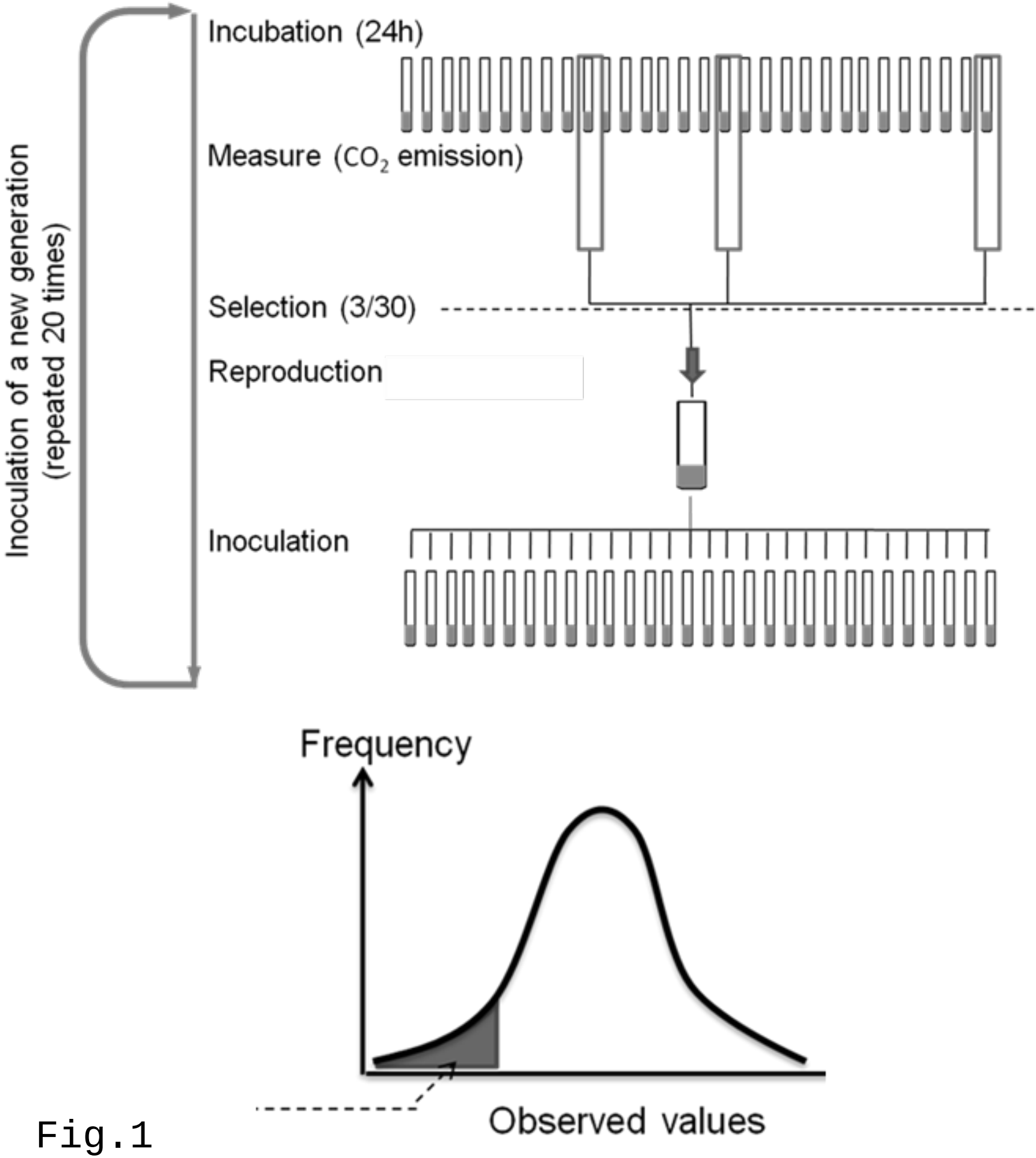

Inoculation of a new generation
(repeated 20 times)
Incubation (24h)
Measure (CO_2 emission)
Selection (3/30)
Reproduction
Inoculation
Frequency
Observed values

Fig.1

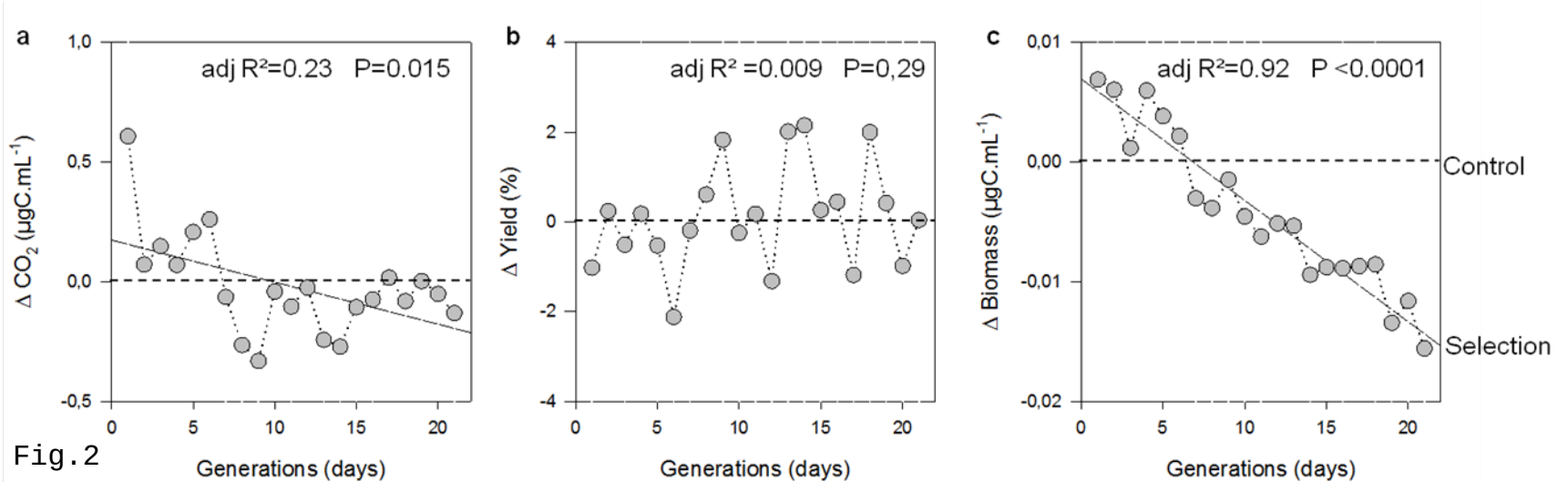
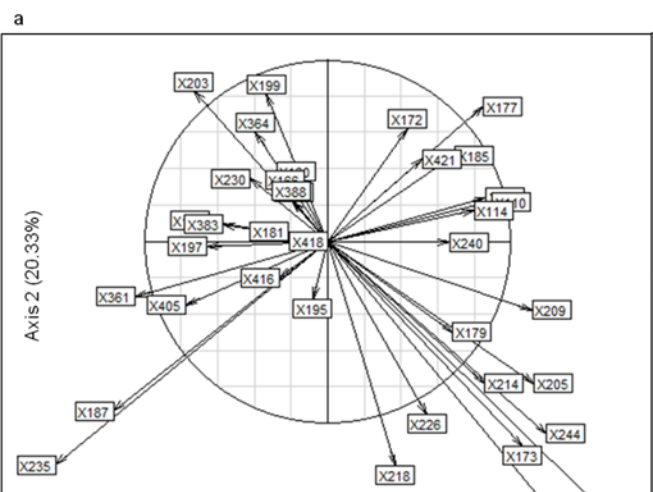

a
adj R²=0.23 P=0.015
Δ CO2 (µgC.mL-1)
Generations (days)
b
adj R² =0.009 P=0,29
Δ Yield (%)
Generations (days)
c
adj R²=0.92 P <0.0001
Δ Biomass (µgC.mL-1)
Control
Selection
Generations (days)

Fig.2


a
X203
X199
X364
X172
X177
X421
X185
X230
X388
X114
X383
X181
X197
X418
X240
X361
X405
X416
X195
X209
X179
X214
X205
X187
X226
X244
X173
X235
X218
X392
X357
Axis 2 (20.33%)
Axis 1 (26.65%)

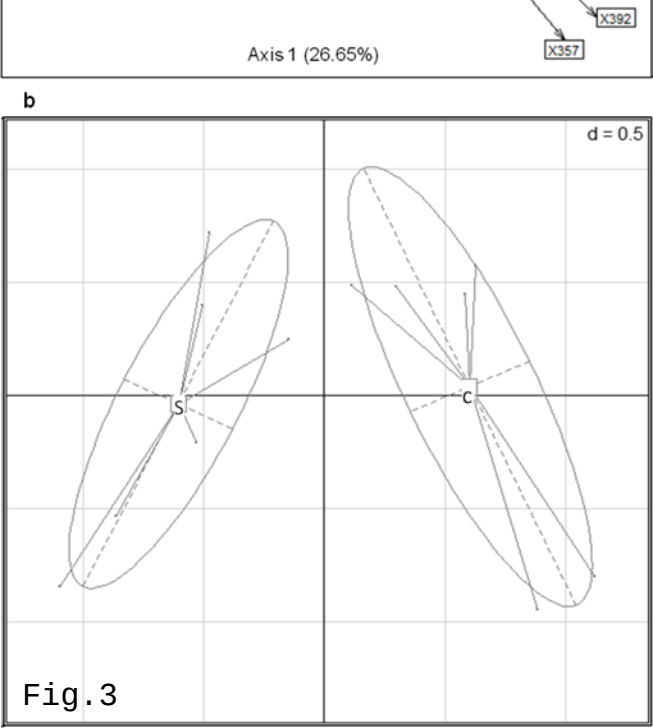
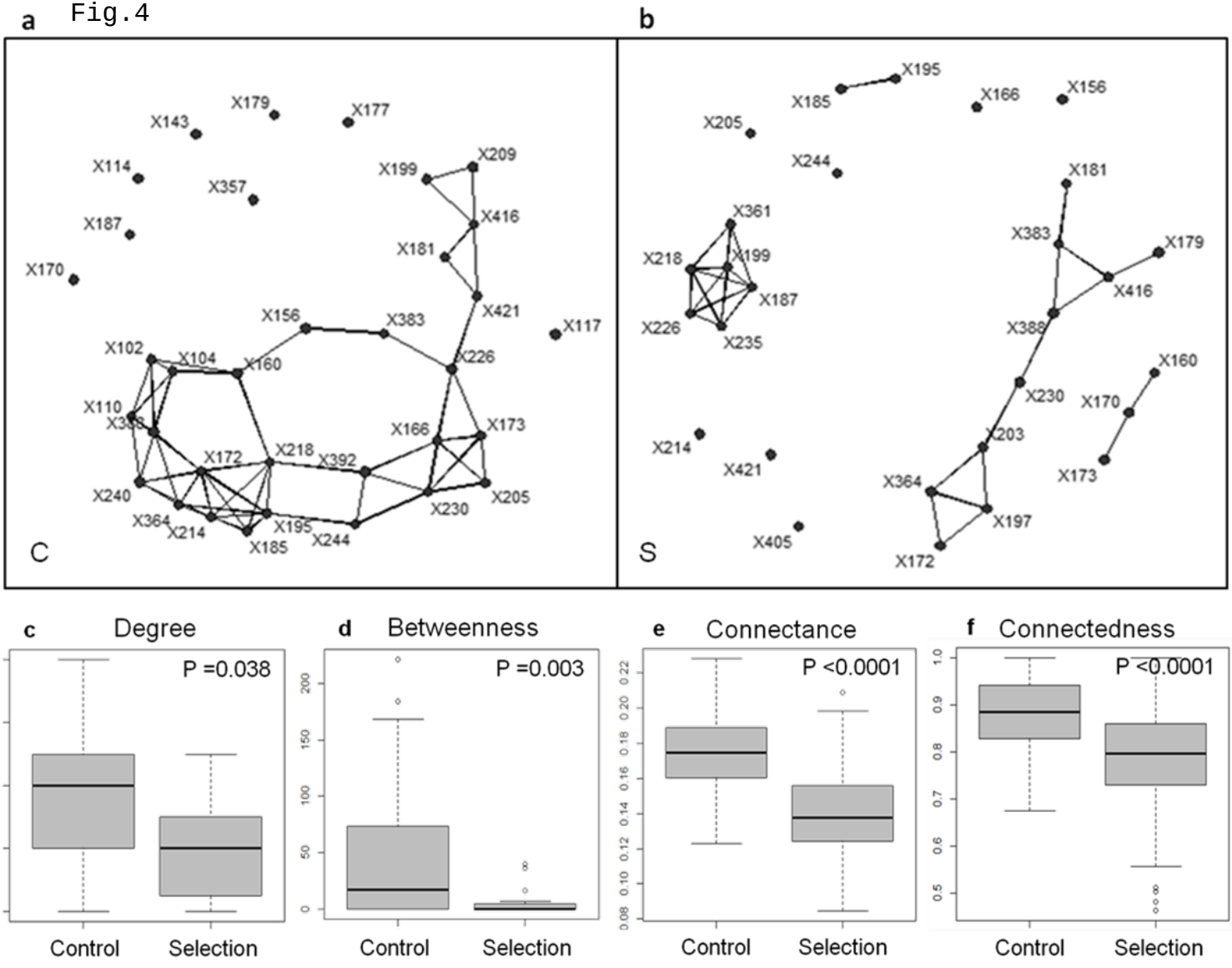

b
d = 0.5
S
C

Fig.3

Fig.4


a
b
X179
X177
X143
X114
X357
X199
X209
X416
X187
X181
X170
X421
X156
X383
X117
X102
X104
X160
X226
X110
X166
X173
X172
X218
X392
X240
X205
X364
X214
X195
X230
X185
X244
C
X195
X185
X166
X156
X205
X244
X181
X361
X383
X179
X218
X199
X416
X187
X388
X226
X235
X160
X230
X170
X214
X203
X421
X173
X364
X197
X405
X172
S
c
Degree
P =0.038
Control
Selection
d
Betweenness
P =0.003
Control
Selection
e
Connectance
P <0.0001
Control
Selection
f
Connectedness
P <0.0001
Control
Selection

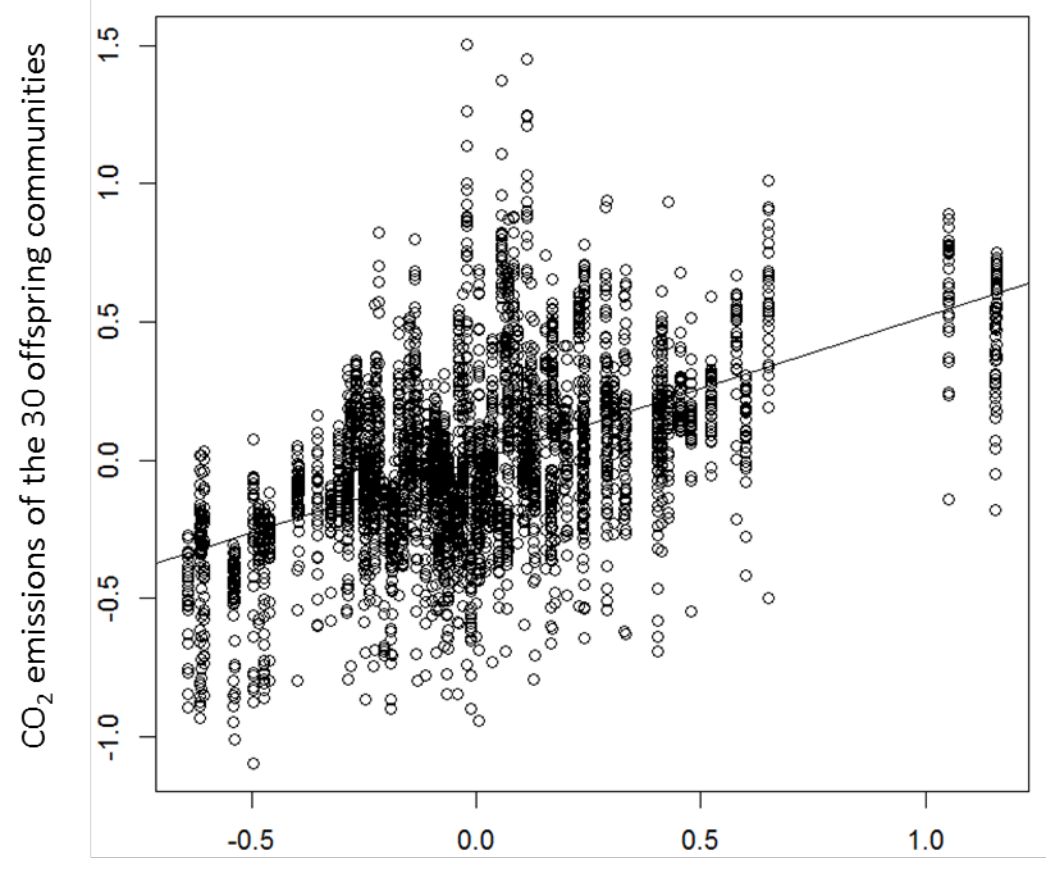


Fig. 5 Mean CO_2 emissions of the three parental communities