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Artificial selection of microbial communities: the effect of diversity and the role of interspecific interactions

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List of abbreviations

ANCOVA: analysis of covariance
ANOVA: analysis of variance
ARISA: automated ribosomal intergenic spacer analysis
a.u.: arbitrary unit
bp: base pair
CI: confidence interval
DNA: deoxyribonucleic acid
dsDNA: double stranded DNA
ESV: exact sequence variant
FMT: fecal microbiota transplant
HSD: honestly significant difference
LB: lysogeny broth
NGS: next-generation sequencing
NMDS: non-metric multidimensional scaling
OD: optical density
OTU: operational taxonomic unit
PCA: principal component analysis
PCR: polymerase chain reaction
PERMANOVA: permutational multivariate analysis of variance
pH: potential of hydrogen
RCF: relative centrifugal force
RNA: ribonucleic acid
rRNA: ribosomal RNA
SE: standard error
rpm: revolutions per minute
TSB: tryptic soy broth

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INTRODUCTION

I. THE ORGANIZATION IN COMMUNITY

1. What is a community?

In ecology, the simplest way of defining a community is to consider it as the collection of organisms that can be found in a particular space (Morin 2009). Most of the definitions of an ecological community also include co-occurrence of the organisms in time and the existence of interactions between the species (Stroud et al. 2015). One detailed definition is this of Whittaker (1975): "an assemblage of populations of plants, animals, bacteria and fungi that live in an environment and interact with one another, forming together a distinctive living system with its own composition, structure, environmental relations, development, and function". Behind the differences in the way of defining a community, there are two historical perceptions of what a community is that are referred to as ***the organismal*** (Clements 1916) ***and the individualistic*** (Gleason 1926) ***nature of the communities***. Indeed, while studying plant succession, Clements (1916) proposed that, due to the interactions between the species, communities had their own development analogously to organisms. In 1926, Gleason proposed another vision in which a plant community is the assemblage of species that are able to persist in given environmental conditions. Thus, on the one side, a community is mainly defined through the interactions between the species and can be viewed as an entity in itself and, on the other side, a community is mainly the result of interactions between each species and its environment, and can be considered as an assemblage of species (Ovaskainen and Nerea 2020). This old divergence has had an influence on the following ecological theories and still has one on the way the communities are considered and studied depending on the research fields. A recent study proposed a modelling approach to answer the question whether communities are "superorganisms or loose collections of species" (Liautaud et al. 2019). Both the organismal and the individualistic organizations of the community were predicted to occur depending on the strength of the interactions between the species and on their dispersal rate and distance. Thus, these two ways of conceptualizing a community can be imagined as the extremes of a continuum and both ***species-species interactions*** and ***species-environment interactions*** must not be neglected when studying a community. A short and complete definition of a community could be: "the species co-occurring in space and time that interact together and with their environment". The latter part of this definition also falls in the field of ecosystem ecology which focuses mainly on energy and material flows but which is closely linked to community ecology as the biotic and abiotic components of an ecosystem interact (Morin 2009). In practice, there are four main ways of identifying a community (Morin 2009): ***physically*** (i.e. the boundaries of the community are these of the habitat), ***taxonomically*** (i.e. the community is defined by the presence of one or few dominant species), ***statistically*** (i.e. through correlation between species abundance over space and time), ***interactively*** (i.e. the community is composed of the species whose abundances are influenced by their

interactions). The concept of community was established to describe assemblages of plants or animals, but it can also be used for microbes (i.e. Bacteria, Archaea, protists, fungi) and for multi-kingdom assemblages. At the end of the 20th century, the gap between microbial ecology and ecology started to be filled. Indeed, microbial models can be good candidates to test for ecological theories (Jessup et al. 2004) and, conversely, the theories drawn from the study of plants or animals can be useful to understand microbial ecology (Prosser et al. 2007). If we go back to the definition of a community, beyond the fact that the concept of "species" is different between microbes and other kingdoms (Kirchman 2018), microbial communities can be defined similarly to plant or animals communities. In this work, we will focus on empirical studies about microbial communities, and more particularly bacterial communities, and we will mobilise theories that were not drawn specifically from the study of microbes.

2. The emergence of community intrinsic properties

It is increasingly recognized that focusing on the ***interspecific interactions*** when studying a community is particularly relevant as they can give rise to properties that are unique to the organization in community (Gorter et al. 2020). A ***community intrinsic property*** or emergent property can be defined as such when not predictable from the characteristics of the species composing the community or from the sum of their characteristics (Madsen et al. 2018; Bengtsson-Palme 2020). As an example, it has been shown that biofilm formation was enhanced in multispecies assemblages of bacteria as compared to the biofilm formation capacity of the species in monocultures (Røder et al. 2015). As illustrated in Figure 0.1, the emergence of community intrinsic properties relies on the existence of interspecific interactions and one can argue that it is precisely what makes a community. Pairwise interactions are classically defined based on the nature of the effect of the interaction on the involved species i.e. positive, neutral or negative (Faust and Raes 2012). In microbial communities, as in plants or animals, negative interactions can occur through competition (-/-) or amensalism (0/-) and positive interactions can occur through mutualism (+/+) or commensalism (0/+). Predation and parasitism (+/-) also occur in microbial communities that include protists and bacteria (Sherr and Sherr 2002) or bacteriophages

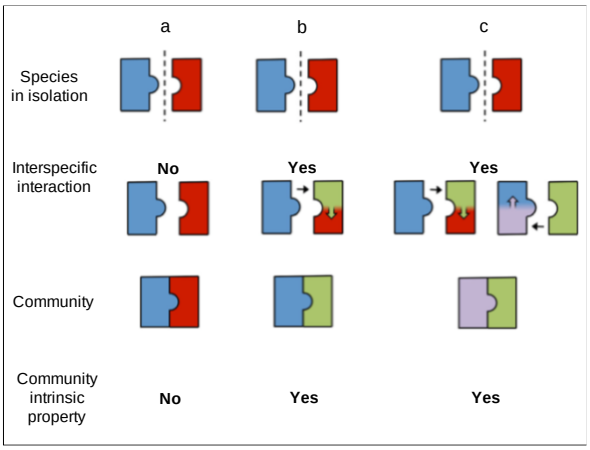

Figure 0.1 Schematic representation of the concept of community intrinsic property. The puzzle pieces represent two species (a blue and a red) and their assemblage represents a community. Colours represent phenotypes (e.g. biomass, excretion of an antibiotic, metabolic rate). Black arrows represent interspecific interactions. The outcome of the assemblage of the two species can be **a)** predictable from the phenotype of the species in isolation as there is no interaction, **b)** unpredictable as a species expresses a different phenotype when assembled with the other, **c)** unpredictable as both species express different phenotypes when assembled than when isolated. Adapted from Madsen et al. 2018 in Current Opinion in Microbiology.

and bacteria for example (Labrie et al. 2010). Among these interactions, competition and mutualism (or cooperation) have received much attention as they can allow a better understanding of species co-existence or not (Hillesland and Stahl 2010; Freilich et al. 2011; Foster and Bell 2012; Ghoul and Mitri 2016). Indeed, such pairwise interactions will influence the outcome of species assemblage but, in natural, i.e. complex, communities it is more likely that the community intrinsic properties will also be influenced by higher order interactions i.e. interactions that occur between more than two species. The occurrence of these interactions would complexify our ability to predict community properties. In fact, what emerges from the literature is that community intrinsic properties, such as the overall metabolic rate, can be predicted to a certain extent at low complexity levels but the higher the number of species in a community, the lower the predictability (Guo and Boedicker 2016; Friedman et al. 2017; Sanchez-Gorostiaga et al. 2019). Indeed, in a three-species community, pairwise interactions can allow predicting community property (Friedman et al. 2017) but, as long as there are more species in the community, a knowledge of higher order interactions is needed to predict the outcome of multispecies assemblage. Community properties are even more challenging to predict as they depend on the abiotic environment (Abreu et al. 2020) and can fluctuate along with fluctuations of the environmental conditions. Furthermore, there can be a complex interplay between the abiotic and the biotic conditions as the abiotic environment can influence species and interspecific interactions but, the species can also modify the abiotic environment in which they are living through nutrient acquisition or ecosystem engineering (i.e. changes in the chemical or physical conditions respectively; Matthews et al. 2014).

3. The diversity-functioning relationship

The traits that are specific to the presence of multiple species can also be considered as emergent properties of the communities (Konopka 2009). What is of particular interest is ***community diversity***. It allows a description of which species are present in the community and in which proportions. A lot of work has been conducted on plant communities to study the effect of community diversity on ecosystem functioning (Hooper et al. 2005). The idea that emerged from these studies is that an increase in community diversity can lead to an increase in the level of a function. This can occur through two main categories of mechanisms: ***complementarity/facilitation effects*** and ***selection effects*** (Loreau et al. 2001). Complementarity is allowed by niche differentiation: different species can have

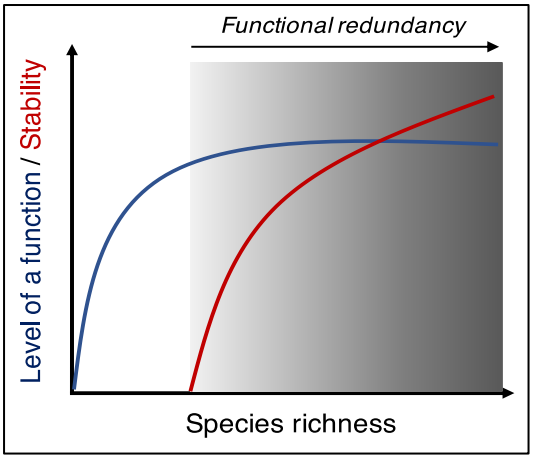

Figure 0.2 The theoretical relationship between species richness, functional redundancy and ecosystem function and stability. The level of a function increases with the increase in species richness through complementarity, facilitation and/or selection effects and reach a plateau when there is functional redundancy between the species. Community stability (i.e. resistance and resilience) increases with the increase of functional redundancy between species. Adapted from Konopka 2009 in The ISME Journal.

different resource use which can lead to a better use of the pool of resources in a given environment. This is of particular interest in the field of microbial engineering, as communities – called consortia in this field – can outperform what is done by isolated species (Brenner et al. 2008; Hays et al. 2015; Lindemann et al. 2016). As an example, the degradation of lignocellulose (Puentes-Téllez and Falcao Salles 2018) or of polycyclic aromatic hydrocarbons (Wu et al. 2013) is more efficient when performed by consortia rather than isolated strains due to the panel of metabolic capabilities that can be found in the different species. Selection effects occur through the increase in the probability of observing the dominance of a species with a high level of a function when increasing the number of species in a community (Loreau et al. 2001). Negative selection effects (i.e. dominance of a species with a low level of a function) can also occur and limit the positive effect of diversity on the considered function (Jiang 2007). In microbial communities, as in plant communities, experimental studies have shown that the diversity-functioning relationship is saturating (Bell et al. 2005; Fetzer et al. 2015). This is due to ***functional redundancy***: at one point, increasing species richness will not induce an increase in the level of a function as the functional diversity does not increase anymore (i.e. many functions can be performed by multiple species; Louca et al. 2018). This functional redundancy is of particular interest in community stability (i.e. another community emergent property). Indeed, the higher the functional redundancy, the lower the risk of losing of function through the loss of a species (Naeem 2008) and the lower the variability in a function. This is referred to as the ***insurance hypothesis*** (Yachi and Loreau 1999). The link between community diversity and community functioning is summarized in Figure 0.2.

To sum up, microbial communities are multi-species assemblages in which species interact together and with their abiotic environment which can lead to the emergence of community intrinsic properties. This can give rise to an increase in the level of a function as compared to what is observed from species in isolation. Community-level properties cannot be easily predicted but, it is often observed that an increase in community diversity increases community functions and stability. The existence of community-level properties makes relevant the consideration of a possible evolution *of* the communities rather than only an evolution *in* the communities.

II. MICROBIAL COMMUNITY EVOLUTION

1. Communities as selection units

The concept of evolution through ***natural selection*** dates back to Darwin. In 1859, he stated that natural selection is the principle "by which each slight variation, if useful, is preserved". It implies that there must be phenotypic variation, which must be associated to a differential in fitness, itself being heritable (i.e. possibly transmitted from the parents to offspring; Lewontin 1970). While enunciating these three conditions for natural selection to occur, I deliberately did not specify the level of biological organization at which they must be fulfilled as Lewontin himself proposed that it could occur from the

molecule to the community level. Indeed, this is the purpose of a long debate that lasted over decades (Gliddon and Gouyon 1989) and about which there are still strong divergences. One point that specifically failed to bring consensus is the pertinence of the ***group level*** in the selection process (Leigh Jr 2010). There is not one group level selection theory but several, and notably one that invoked cooperation between the individuals for the good of the group (Wynne-Edwards 1962) which has been later considered as non-relevant because the conditions for it to occur would be extremely rare (West et al. 2007). Group-selection is sometimes still assimilated to this theory which creates confusion about the validity of the other theories that have been proposed. Many of them have been developed in the aim of understanding ***the evolution of altruistic traits*** (i.e. behaviours that are costly to express for an individual and beneficial to the other individuals; West et al. 2007) and relied on the idea that an altruistic trait could evolve within a group given that this group is small and slightly connected with the others (Wilson 1973). Then, Wilson (1975) proposed a theory based on the distinction between the smaller level at which individuals spend the most of their life cycle (called the ***trait-group***) and the higher level at which individuals mate (called the deme). What happens at the trait-group level would influence the contribution of the different groups to the common pool of individuals at the deme level and the following formation of new trait-groups so that an altruistic trait could be maintained (Wilson 1975). Another well-known theory is this of the ***superorganism***. It is based on the idea that a group is functionally organized as an individual: as same as individuals can be seen as groups of genes, a superorganism can be seen as a group of individuals (Wilson and Sober 1989). As a consequence, natural selection could occur at the superorganism level and adaptation (i.e. the response to selection) would occur on the group phenotype (Gardner and Grafen 2009).

Beyond these different theories, what is commonly admitted is that group-level selection was probably involved in the major transitions of evolution (e.g. the transition from independent replicators to chromosomes, from Prokaryotes to Eukaryotes; Okasha 2006; Leigh Jr 2010). However, among the authors that consider group-level selection as relevant, there is still discussion about its importance. In the framework of the ***multilevel selection theory***, group-level selection is supposed to occur simultaneously to selection at lower levels of biological organization (Okasha 2006) and it is likely that the selection pressures could be in opposite directions, giving rise to conflicts between the levels of selection (Wilson and Sober 1989). Another possibility is that group-level selection would occur under very specific conditions when there is no within-group selection (Gardner and Grafen 2009). In the theories discussed above, the main idea is that natural selection occurs through a differential reproduction of biological entities (either individuals within a (trait-)group or a group itself). There is one recent theory, called "It's the song not the singer" (ITSNTS), that proposed that not only biological entities but also processes can be the units of selection (Doolittle and Inkpen 2018). Thus, new ideas are still feeding the theory of evolution by natural selection so that the debates on the levels and units of selection is still alive. In this work, we will base on the definition of group selection enunciated by Wade (1978): "group selection is defined as that process of genetic change which is caused by the

differential extinction or proliferation of groups of organisms". For a differential in extinction or proliferation between groups to exist, the three conditions enunciated by Lewontin (1970) must be fulfilled: *i*) between-group ***variation*** linked to *ii*) a differential in ***reproduction*** which is *iii*) ***heritable***. *In natura*, the fulfilling of these conditions could occur under the influence of the abiotic environment which could promote the discretization of groups and their reproduction through dispersion events, this is referred to as ecological scaffolding (Black et al. 2020). In the laboratory, it is possible to apply group level artificial selection through the compartmentalization of groups of individuals and the selection of the best performing according to a pre-defined function. The first studies involving group selection were conducted at the population or community level on plants (Goodnight 1985) or animals (Wade 1976, 1977; Goodnight 1990a; Craig and Muir 1996) and showed that a population or ***community phenotype*** (i.e. a trait measured at the population or community level) can change under artificial selection at the group level.

2. Artificial selection of microbial communities

Following the first experimental studies of artificial selection at the group level on plants and animals, Swenson and colleagues performed several experiments on microbial communities[1] (Swenson et al. 2000a, b). The principle of ***microbial community artificial selection*** is presented in Figure 0.3. There are three main steps: *i*) the establishment of a population of units, *ii*) the phenotyping according to the targeted property and *iii*) the selection and propagation of the best performing units. Swenson and colleagues applied this procedure targeting three different properties: the pH of aquatic ecosystems, the degradation of the toxic compound 3-chloroaniline and the above-ground biomass of *Arabidopsis thaliana* while selecting on pond water microbial communities in the two first cases and on soil microbial communities in the latter case. These first experiments on microbial communities showed encouraging results as the selected properties responded to selection. However, the responses were unstable and lost at certain generations giving erratic profiles of the targeted function changes over time (Swenson et al. 2000a, b) and suggesting that improvements could be done to increase the efficiency of the artificial selection. In the following years, several modelling approaches have been conducted and allowed exploring the mechanisms involved in the community response to selection (Penn 2003; Penn and Harvey 2004; Williams and Lenton 2007). These approaches were based on Lotka-Volterra equations and all showed that there was a community response to artificial selection, confirming what

[1] In the studies of Swenson et al. (2000a, b), the authors referred to artificial selection of ecosystems. Indeed, it is relevant to consider the ecosystem level as the properties that are selected for, e.g. pH, are the result of the ecosystem functioning. However, even if the phenotyping occurs at the ecosystem level, the variation between the units of selection is primarily due to the biotic component, i.e. the community, as in these experiments the environmental conditions are maintained constant. What is selected for is the biotic component (with its potential effect on the abiotic component) and this is what the experimenter aims to transmit to the next generation of units. Thus, in this work we will refer to community-level selection.

was suggested experimentally. Williams and Lenton (2007) showed that the response of the community to selection was often not explainable by a response at the individual level only, which confirmed that community-level selection can be relevant. They also showed that whether the response involved the interactions between species or not depended on the targeted property (i.e. some properties are more susceptible to be achieved by a unique species than others) and on the reproduction method i.e. whether the parents are mixed (=***migrant pool method***) or kept separated (=***propagule method***). What was also highlighted through these modelling approaches is that the variation needed for selection to act can be brought by genetic changes in the species composing the community but also by changes in community composition (Penn 2003; Penn and Harvey 2004), which is specific to the selection at the community-level. The outcome of the artificial selection would depend on the balance between ***variation*** and ***heritability***: variation is needed but, only heritable variation will contribute to the response to selection (Penn and Harvey 2004). In the 2000s, the studies dealing with artificial selection at the community-level were restricted to the studies cited above. It is only in the last decade that the number of studies in this field started to be significant. Artificial selection was applied on microbial communities for decreased CO_2 emissions (Blouin et al. 2015), increased chitinase activity (Wright et al. 2019), increased amylase activity (Chang et al. 2020) and increased cross-feeding potential (Chang et al. 2020). In the same way of the experiment of Swenson et al. (2000b) in which they selected the soil microbial communities for an increase in the associated plants' biomass, several studies reported artificial selection on host-associated microbes. This is now referred to as ***microbiota*** (Jacquiod et al. 2021) ***or microbiome selection*** (Mueller and Sachs 2015). Four studies of this kind have been undertaken, three of them targeting a plant trait: the time of flowering (Panke-Buisse et al. 2015), salt-tolerance (Mueller

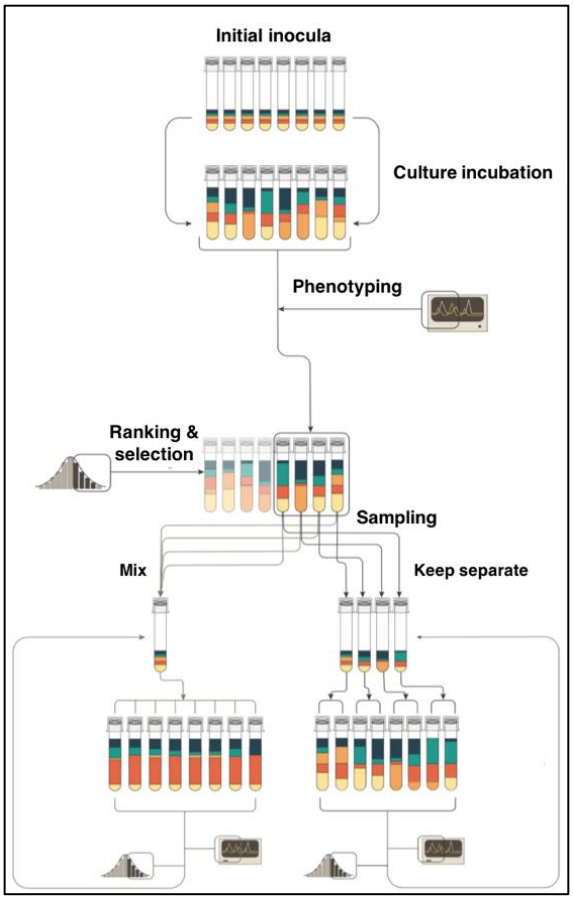

Figure 0.3 The procedure of the artificial selection of microbial communities. The first step is to establish a population of selection units i.e. a population of communities, also referred to as a metacommunity. Initially, the communities are supposed to be identical in their composition (colours represent different species). There are grown under controlled environmental conditions for a pre-determined duration during which differences can arise between the communities (e.g. in the species relative abundances). At the end of the growth phase, the communities are phenotyped according to the targeted property (e.g. a given enzymatic activity). The best performing communities according to the desired property are identified and selected to create a next generation of units. This step corresponds to a reproduction of the communities which occurs through the sampling of the parents and the inoculation of new medium to create the offspring communities. The parents can either be kept separated from each other or mixed before creating the offspring communities. Then, a new selection cycle (i.e. the repetition of all of the previously mentioned steps) can start. Adapted from Borchert et al 2021 in Trends in Microbiology.

et al. 2019), leaf greenness (Jacquiod et al. 2021), and one of them targeting the time of eclosion of fruit flies (Arora et al. 2020). Along with these experimental studies, three other studies based on modelling approaches contributed to the increase in the knowledge in the field of community-level artificial selection (Xie et al. 2019; Doulcier et al. 2020; Chang et al. 2021). What emerges from this increasing body of literature is that many factors limit the efficiency of the community-level artificial selection, which gives rise to unstable changes in the targeted properties, as observed by Swenson and colleagues. One of the main issues is related to a lack of heritability: the variation selected for in the parental community is often not reliably transmitted to the offspring communities (Blouin et al. 2015; Xie et al. 2019; Chang et al. 2020; Jacquiod et al. 2021). This can have different causes such as small variations in the environmental conditions (Xie et al. 2019), the absence of stability of the community (Jacquiod et al. 2021; Chang et al. 2021) or the sampling effect occurring when passaging the communities (Blouin et al. 2015; Xie et al. 2019). Another issue that was reported is the decrease in variance and heritability over time which lowers the impact of the selection throughout the experiment (Blouin et al. 2015; Chang et al. 2020). Furthermore, there is evidence that artificial selection at the community-level could have limited effects when other selection pressures are at stake (Arora et al. 2020), as predicted by the theory (Wilson and Sober 1989). Understanding how to deal with these different limitations will improve the efficiency of artificial selection of microbial communities and microbiomes.

Artificial selection at the community level addresses a variety of questions regarding the potential for a community to be a selection unit but it is also a promising tool to engineer microbial communities for applied purposes. ***Microbial community and microbiome engineering*** receive much attention as microorganisms play key roles in plant and animal – including human – health (Mueller and Sachs 2015), in environment depollution (Borchert et al. 2021) and in pharmaceutical, chemical and food industry (Demain 2000) notably. Apart from artificial selection, there are several ways of engineering microbial communities. One possibility is to engineering the microbial communities through bottom-up approaches which would rely on the design, building and manipulation of synthetic communities in order to perform a desired function (Lawson et al. 2019). These approaches are based on a priori choices of the species and the metabolic pathways that are supposed to lead to the expression of the desired function (Lawson et al. 2019) which requires specific knowledge on the species and their interactions (e.g. to ensure complementarity between the species and optimization of resource consumption; Hays et al. 2015; Tsoi et al. 2019). Another possibility is to engineer the communities through top-down approaches. These approaches rely on the control of extrinsic factors to manipulate the ecosystem processes (physical, chemical, biological) and promote the desired function (Lindemann et al. 2016; Lawson et al. 2019). The artificial selection of microbial communities is one of the possibilities to engineer the communities through a top-down approach. Contrary to bottom-up approaches, it can be applied without any *a priori* knowledge on community composition or functioning so that it can allow finding combinations of genes that perform well regarding the desired property and that would not have been found otherwise.

To sum up, from a theoretical standpoint, the conditions for selection to occur can be fulfilled at the community level under laboratory conditions. As a consequence, artificial selection at the community level can be performed and many studies have shown promising results even if there are still limitations notably regarding the heritability of the community phenotype. A potential lever to address the encountered limitations could be the integration of the potential effects of community functioning on community evolution. It could open the way to the use of the artificial selection as a tool for microbial community engineering.

III. INTERPLAY BETWEEN ECOLOGY AND EVOLUTION

1. Eco-evolutionary dynamics

It is increasingly recognized that the ecological and evolutionary dynamics of the communities are closely linked to each other. As exposed by Mittelbach and McGill (2019), the effect of ecology on evolution has been considered for a long time since the ecological conditions build the selection pressures to which the organisms are submitted. Later in time, the effect of evolution on ecology has also gain interest as, contrary to what was commonly thought, both can occur at the same timescales (Mittelbach and McGill 2019). This is especially true for organisms that have large population sizes and short generation time such as microbes (Rainey et al. 2000). This observation has led to the concept of ***eco-evolutionary dynamics*** which relies on the idea that ecological and evolutionary dynamics have reciprocal effects on each other over ecological time-scales (Fussmann et al. 2007). The eco-evolutionary dynamics lie at the intersection of ecology and evolution as illustrated in Figure 0.4. These dynamics have first been evidenced in predator-prey systems. As an example, Fussmann et al. (2003) have shown that the observed dynamics of a rotifer-alga community was predictable by a modelling approach only if the evolution of the rotifer was taken into account. In this case, the abiotic environment (a chemostat) exerted a selection pressure on the rotifer that favoured an asexual reproduction over a sexual one, this evolutionary change influenced the rotifer population dynamics and the community dynamics (Fussmann et al. 2003). When there is a complex interplay

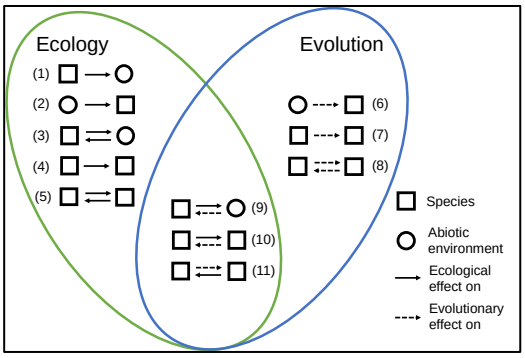

Figure 0.4 Intersection between ecology and evolution. In ecology, a species can modify its environment (1), be influenced by its environment (2) or modify its environment and in turn be influenced by it (3). Interactions can also occur between species, either unidirectionally (4) or bidirectionally (5). In evolution, the environment can exert a selection pressure on a species (6), as well as the presence of another species (7) and species can respond reciprocally to the presence of each other through co-evolution (8). Simple examples of eco-evolutionary dynamics would include: an ecological effect of a species on its environment that will in turn affect its evolution (9), an ecological interaction between species that will influence species evolution (10), an evolutionary response of a species under the selection pressure exerted by another species that will in turn influence the interspecific interaction (11). This is a non-exhaustive list of simple cases that involve only pairwise interactions. The eco-evolutionary dynamics can involve very complex feedback loops. Adapted from Matthews et al 2014 in Ecological monographs.

between ecological and evolutionary dynamics, the authors refer to ***eco-evolutionary feedbacks***. It can be defined as "the cyclical interaction between ecology and evolution such that changes in ecological interactions drive evolutionary change in organismal traits that, in turn, alter the form of ecological interactions" (Post and Palkovacs 2009).

There is a large body of literature that theorizes and experimentally illustrates the complex interplay between ecology and evolution, particularly with respect to ***community diversity*** and ***interspecific interactions***. Both of these community components have been proven to be involved in eco-evolutionary dynamics or feedbacks. Indeed, the evolution can influence community assemblage (Harcombe 2009), community composition (Meroz et al. 2021) or community structure (Celiker and Gore 2014) through adaptations that affect species co-existence for example (Harcombe 2009). On the other side, community diversity can influence the evolutionary dynamics: when multiple species are present in a community, changes in species relative abundances are more likely to occur than genetic changes within species (de Mazancourt et al. 2008; Barraclough 2015). Furthermore, increasing the number of species in a community could lead to a reduced population size within each species which could influence the evolutionary rate of the species or give rise to a different balance between the evolutionary process (*e.g.* selection and genetic drift; de Mazancourt et al. 2008).

The interspecific interactions, as well as the interactions of the species with their abiotic environment, can also play a role in the evolutionary dynamics. This has been illustrated in the study of Andrade-Domínguez et al. (2014) in which a yeast and a bacteria were grown in co-culture. At the beginning of the experiment, the yeast excreted both bacterial growth promoters and a bacterial growth inhibitor, then, the bacterial growth promoters were depleted, and resistance evolved in the bacterial species which, in turn, influenced the growth capacity of the yeast along with environmental changes. This study showed with a simple two-species community how a complex interplay between ecological interactions and evolutionary responses could affect community dynamics. Thus, the effects of the interspecific interactions in even more complex communities should be difficult to predict. From a theoretical standpoint, it is believed that the interspecific interactions could promote species evolution through the increase of the selection pressures (O'Brien et al. 2013; Barraclough 2015) but, at the same time, trade-offs could occur between the adaptation to the presence of multiple species (O'Brien et al. 2013) or between the adaptation to the presence of other species and to the abiotic environment (Barraclough 2015) and potentially lower the evolutionary rates of the species. Furthermore, an influence of interspecific interactions on the population sizes of the different species or the occurrence of very specific interactions such as horizontal gene transfer could influence the evolution of the communities in ways that are difficult to predict (Barraclough 2015).

2. Eco-evolutionary dynamics in community-level artificial selection experiments

In the field of the artificial selection of microbial communities, many authors consider changes in species relative abundances and in interspecific interactions over the selection cycles as part of the community evolution (Goodnight 2000; Penn and Harvey 2004; Williams and Lenton 2007; Blouin et al. 2015). Thus, even if this approach does not require any knowledge on community composition or functioning, there is an interest in understanding how community ecology will impact community evolution. First, it has been suggested that the ***ecological dynamics*** of the community within a selection cycle can be a key determinant of the outcome of the artificial selection (Wright et al. 2019; Jacquiod et al. 2021; Chang et al. 2021). Indeed, Wright et al. (2019) showed that the ecological succession in the microbial community affected their ability to select efficiently for enhanced chitinase activity. This function was mainly associated to Gamma-proteobacteria but, over the ecological succession, they were replaced by Alpha-proteobacteria that were not able to degrade chitin. Thus, when the transition from one selection cycle to the other occurred when the community was dominated by the Alpha-proteobacteria, the selection was not effective (Wright et al. 2019). Other authors pointed out the ***need for stability*** in community structure for artificial selection to work. Indeed, it is likely that an ecological succession occurs each time offspring communities are created from the sampling of the parent communities. Chang et al. (2021) suggested that, at the end of this ecological succession, the communities are in "an adult state" which allows the reliability of their reproduction. The communities are "generationally stable" when the succession in the offspring communities is identical to this of their parents. In the study of Chang et al. (2021), a modelling approach indicates that more than five selection cycles are needed for this stability to be reach. Accordingly, in an experiment of plant's microbiome selection, Jacquiod et al. (2021) showed that the bacterial community structure varied greatly among the selection cycles for the first five cycles and that, once community was stabilized, offspring and parent phenotype (i.e. leaf greenness of the plants) were positively correlated.

The interspecific interactions can also influence the outcome of the evolution through selection at the community level. As discussed earlier, they can be involved in the community response to selection as, in many cases, the community evolutionary response is not predictable from the evolutionary responses of the organisms composing this community (Williams and Lenton 2007). To go further, there is evidence from a study on beetle communities (Goodnight 1990b), that a community can respond to artificial selection without any detectable change in the species composing the community which suggests that the selection can act directly on the interactions. With a modelling approach on collectives of red and blue particles, Doulcier et al. (2020) showed that the selection for a collective level phenotype – a purple colour - could affect the phenotype of the particles within the collectives. This change in particle phenotype gave rise to changes in the interactions between the particles which promoted the parent/offspring resemblance i.e. whatever the phenotype of the offspring just after the transfer, the collective developed a similar phenotype to the parent which did not occur at

the beginning of the procedure (Doulcier et al. 2020). These results suggested that the evolution of the interactions could be a key determinant of heritability at the community level.

To sum up, community ecology and evolution can occur at the same timescales and be closely linked to each other. Complex interactions between ecological and evolutionary dynamics lead to the necessity of integrating ecology when studying evolution and conversely. In artificial selection experiments, changes in community structure over generations can be considered as evolutionary responses at the community level. Several studies have demonstrated that community structure and interspecific interactions can determine the outcome of the artificial selection.

IV. AIMS AND OBJECTIVES OF THE THESIS

The aim of this work is to contribute to the field of the artificial selection at the community level, notably through the study of community functioning and evolution, and to shed light on the knowledge that this field can bring to the larger field of the community eco-evolutionary dynamics. To that end, our objectives are *i*) to address how to improve the efficiency of the artificial selection procedures and *ii)* to investigate the determinants of the community evolutionary responses. These objectives are addressed in three chapters:

- **Chapter 1**: Effect of the reproduction method in an artificial selection experiment at the community level
- **Chapter 2**: Species richness influences the evolution of synthetic bacterial communities under artificial selection
- **Chapter 3**: The central role of the interspecific interactions in the evolution *in* and *of* the microbial communities

In the first chapter, we addressed how the step of the reproduction of the communities can influence the outcome of the artificial selection. We applied an artificial selection procedure on complex soil microbial communities grown under laboratory conditions and asked whether the use of the migrant pool or propagule strategy (i.e. mixing the parental communities or not respectively) to create the offspring generation can influence the efficiency of directional or stabilizing selection for biomass production.

In the second chapter, we investigated how the community diversity would influence the artificial selection efficiency. We worked on synthetic bacterial communities varying for their richness level and targeted a high biomass production through artificial selection. We characterized community growth dynamics, metabolic profile and evolutionary response and asked whether increasing community richness would positively influence community functioning and the efficiency of the artificial selection.

In the third chapter, we focused on the role of the interspecific interactions in community evolution. We studied two-species communities that stemmed from the artificial selection experiment designed for Chapter 2. We assessed the evolutionary responses of communities and species composing these communities and investigated the role of the evolution of the interactions in the community evolutionary response.

The study presented in chapter 1 was conducted following the work of Blouin et al. (2015) in which a decrease in phenotypic variation was registered throughout an artificial selection procedure for a decrease in CO_2 emissions of microbial communities. The underlying idea when designing the study was that pooling the parental communities when creating the offspring communities could help maintaining a higher level of phenotypic variation than the use of a unique parental community. This work has been published as a brief research report in Frontiers in Ecology and Evolution in November 2019 (https://doi.org/10.3389/fevo.2019.00416).

CHAPTER 1: EFFECT OF THE REPRODUCTION METHOD IN AN ARTIFICIAL SELECTION EXPERIMENT AT THE COMMUNITY LEVEL

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Abstract

Selection at the group level is proposed to be an evolutionary process occurring in the context of multilevel selection *in natura*. In artificial selection experiments, selecting at the community level can allow finding multispecies assemblages that are more efficient than a single species at solving a given problem. In such procedures, the main difficulty is to find a balance between variation and heritability, which are both essential for selection to act. The aim of our study was to determine if the way of creating offspring units of selection from parental units, called "reproduction method," could influence artificial selection efficiency through a differential in the variation/heritability balance. Selecting microbial communities depending on their biomass production and propagating them either one by one or in a mix of three communities, we showed that the effect of the reproduction method was not maintained over time with a loss of the effect of artificial selection on community phenotype at certain cycles and a very low heritability. However, mixing parental communities was more efficient at increasing biomass production than using a single parental community (+5% of biomass). We discussed the role of differences in community richness and structure in explaining these results.

Keywords: community composition, experimental evolution, level of selection, microbial communities, propagule and migrant pool reproduction methods, species richness

INTRODUCTION

From a theoretical standpoint, the three conditions stated by Lewontin (1970) for selection to occur, i.e., *i)* the existence of phenotypic variation, *ii)* the existence of a link between this variation and a differential in fitness, *iii)* the heritability of the variation, can be completed at the group level. But group selection and its role as an evolutionary process *in natura* have been controversial especially because they are difficult to demonstrate (Okasha 2006; West et al. 2007; Leigh Jr 2010). By contrast, artificial selection at the group level in experimental conditions has been proven effective in changing a phenotypic trait of the group but also in changing the fitness of the individuals composing this group (Goodnight and Stevens 1997).

The first experimental results of group selection in beetle populations of *Tribolium castaneum* and in communities of *T. castaneum* and *T. confusum,* gave a stronger response to selection than predicted by modelling (Wade 1978; Goodnight and Stevens 1997). Indeed, group selection may have an effect on variance components that are not involved at lower levels (e.g., individuals, genes). For example, if the considered selection unit is a population, genetically-based among-individuals interactions can be selected. If we consider higher levels such as communities, selection at higher levels than the population can occur through genetically-based between-species interactions, such as syntrophy (obligately mutualistic metabolism; Morris et al. 2013).

Artificial selection at the group level is of particular interest because it allows the selection of combinations of organisms, and hence indirectly combinations of genes, that would not have been discovered otherwise. Many applications could be considered, especially on microbial ecosystems for improving the degradation of toxic compounds, such as 3-chloroaniline (Swenson et al. 2000a), for which syntrophic interactions between species often take place. Other experiments of community level artificial selection have proven to be efficient for example at reducing CO_2 emissions (Blouin et al. 2015) or modifying the flowering date of *Arabidopsis thaliana* (Panke-Buisse et al. 2015). However, the long-term stability of the selected communities has not been investigated.

Usually in an artificial selection procedure, three successive steps take place: the identification of the units of interest according to the targeted phenotype, the selection of these units, and the creation of a new population of units from the selected ones. Then many questions arise about the efficiency of artificial selection as pointed out by Xie et al. (2019) when modelling artificial selection in a two-species community. Few of them have been addressed experimentally. Swenson et al. (2000b) investigated the effect of the size of the sample used to create a new population from the selected parents, and more recently, Wright et al. (2019) discussed the effect of the incubation time on the expression of the targeted phenotype. In this study, we investigated the effect of the reproduction method, which has been tackled in a model (Williams and Lenton 2007), but not in experiments. By analogy with the artificial selection process on sexually reproducing organisms, the "reproduction method" refers here to the way of creating new experimental units from parental units. Because we work with microbial communities, we

can consider two possibilities: *i)* offspring units all derive from a unique parental unit, or *ii)* they derive from the combination of several parental units. These two methods can respectively be regarded as asexual and sexual reproduction, and are referred to as "propagule method" and "migrant pool method" in the literature (Wade 1978; Williams and Lenton 2007; Goodnight 2011). Depending on the reproduction method, the efficiency of artificial selection (i.e., our ability to reach a targeted phenotype) might be impacted in ways that are difficult to predict.

Indeed, as group selection can act on intra- and interspecific genetically based interactions (Goodnight 2000), one could expect that the most efficient reproduction method would be the one that best maintains the interactions responsible for the phenotype of interest. In other words, the genes responsible for these interactions must be preserved from one cycle to the other. This refers to the concept of heritability (h^2) which is, in the narrow-sense, the proportion of total phenotypic variance related to additive genetic variance (i.e., not due to dominance, epistatic, or environmental variance; Visscher et al. 2008). A higher heritability is expected with the propagule method as offspring units are produced by a unique parental unit whereas the migrant pool method is more likely to disturb the among-individuals or between-species interactions potentially responsible for the community phenotype, thus leading to a decrease in selection efficiency (Williams and Lenton 2007).

However, as exposed by Penn (2003), artificial selection efficiency depends not only on heritability but also on phenotypic variation and on the balance between both of them. Phenotypic variation between offspring and parental units and within offspring units is supposed to occur mainly through sampling effect and stochastic ecosystem dynamic (e.g., demographic and genetic drift) (Wade 1978; Penn 2003). It is expected that mixing several parental units (migrant pool method), thought to be different by the action of the two phenomena aforementioned, maintains a higher inter-, and intragroup diversity over time which could enhance selectable variation by group selection. On the contrary, the propagule method is thought to give rise to an erosion of diversity over time due to the use of a unique parental unit to produce a new offspring population. Thus, a different balance between phenotypic variation and heritability (i.e., different selection efficiency) is expected between the two reproduction methods. Because the effect of these methods could vary according to the target of the selection, i.e., a high, low or stable value of a community phenotype (Williams and Lenton 2007), this hypothesis was tested in different selection contexts, using microbial communities as experimental units, and biomass production as targeted phenotype.

MATERIALS AND METHODS

Experimental selection

The microbial community providing the initial pool of communities selected in this experiment was extracted from a topsoil sample (a lawned area at the INRA centre in Dijon, France). We inoculated 1 g of soil in 10 ml of lysogeny broth (LB) medium diluted 1:5. After 24 h of cultivation (28° C, 130

rpm) and a decantation (30 s, 1 000 rpm), 1 ml of supernatant diluted in 100 ml of LB 1:5 was incubated at 28°C for 48 h (130 rpm) and a second incubation (48 h, 28°C) was conducted in a 96 well-microplate to let the community adapt to the experimental conditions (200 µl per well). The content of this microplate was pooled and diluted 20 times. It corresponded to the initial material on which the artificial selection was conducted. The experiment ran over 14 cycles. A cycle is defined as the incubation time between two selection events, which occurred through the inoculation of three microplates and their incubation at 28°C for 48 h. The 48 h duration was experimentally determined to select the microbial communities once the stationary phase has been reached to maximize the probability of selecting complex and stable interactions instead of selecting individual traits (e.g., fast growing microorganisms).

Selection treatments were based on the estimation of biomass production by optical density measurements (λ = 595nm) with a microplate reader (Thermomax, Molecular Devices, United States). Hereafter we will use "biomass production" to refer to the estimation of biomass production by optical density measurement. We considered three selection treatments. In two of them, we used directional artificial selection, targeting either an increase (High, H) or a decrease (Low, L) in biomass production. The third treatment corresponded to stabilizing selection, i.e., selection of communities with the closest biomass to the average (Stabilizing, S). In addition, communities were randomly chosen (Random, R) without any consideration of their biomass production as a control to assess the effect of experimental conditions on biomass production.

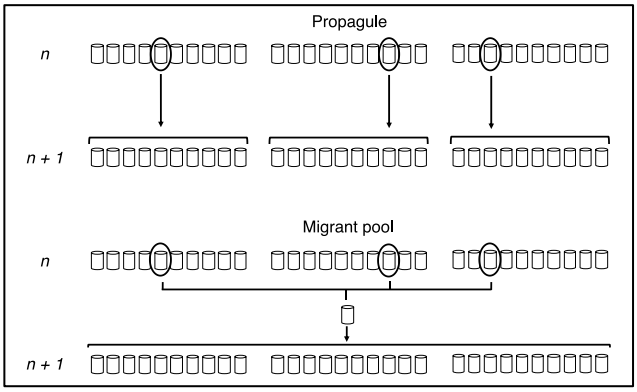

Figure 1.1 Reproduction methods. Propagule method corresponded to the selection of one parental community among ten at cycle *n* and the creation of ten new communities from this parental unit at cycle *n+1*. This protocol was repeated independently three times (*n* = 30, three distinct lines). Migrant pool method corresponded to the selection of three parental communities among thirty (one among ten, three times, to have a constant selection rate between the two reproduction methods; *n* = 30, one line). The three parental units were mixed and used to create thirty new communities at cycle *n+1*. The experiment ran over 14 cycles.

For each treatment aforementioned, the creation of a new cycle occurred through two different reproduction methods: once the parental communities have been selected, the offspring communities derived either from a single parental community (Propagule method, P) or from the mixing of three parental communities (Migrant pool method, M) (Figure 1.1). In the first case (P), the parental community was selected among ten, and this procedure was repeated three times (*n* = 30 per selection treatment, three distinct lines). With the second method (M), three parental communities were selected among 30 (*n* = 30 per selection treatment, one line).

For each new experimental cycle, 120 µl were taken from the well(s) corresponding to the selected community(ies), pooled or not depending on the treatment, and diluted 20 times with LB 1:5 to inoculate the microplates of the next cycle (200 µl per well).

Combining the selection treatments and the reproduction methods, there were eight different treatments in total (RP, RM, LP, LM, SP, SM, HP, HM).

Microbial community analysis

A microbial diversity analysis was conducted on the initial community and on communities from cycles 3, 7, 10, and 14 by ARISA, a community fingerprinting method (Fisher and Triplett 1999), using the primers 1522F and 132R. The pictures of the obtained gels were analyzed with ONE-Dscan (BD Biosciences, United States) giving a number of bands and the intensity of each band.

Heritability

In an artificial directional selection experiment, the heritability (h^2) can be estimated by the slope of the regression of the cumulative selection response to the cumulative selection differential: this is called realized heritability (Falconer and Mackay 1996 cited by Visscher et al. 2008; Roff 2012). The selection response (*R)* is given by the difference between the mean phenotype of the offspring units and the mean phenotype of the parental unit(s). The selection differential, *S*, is given by the difference between the mean phenotype of the selected unit(s) (parent(s) of the next cycle) and the mean phenotype of the entire population of units (selected and unselected units). h^2 was calculated for each line independently and multiple testing was taken into account with Bonferroni correction (Bland and Altman 1995). This calculation method was only proposed for directional selection (Roff 2012) so it was not used to characterize Stabilizing lines in which the treatment consisted in the reduction of the selection differential thus making the regression of the cumulative selection response to the cumulative selection differential unsuitable. In order to characterize the degree to which the parental phenotype influenced the offspring phenotype for Stabilizing, and even for Random lines, we calculated the sum of squares of the difference between offspring biomass production and the respective parental biomass production depending on the reproduction method all cycles together.

Statistical Analyses

Considering a possible block effect due to the distribution of selection treatments onto three 96 well-microplates, we corrected for each cycle the biomass production values with the values of communities belonging to the same line that were present and repeated over the three microplates. Biomass production was analyzed using the following linear mixed model:

$$Y_{ijkl} = \mu + cycle_i + selection\ treatment_j + reproduction\ method_k + (cycle \times selection\ treatment)_{ij} + (cycle \times reproduction\ method)_{ik} + (selection\ treatment \times reproduction\ method)_{jk} + (cycle \times selection\ treatment \times reproduction\ method)_{ijk} + LINE_l + \varepsilon_{ijkl}$$

where Y is biomass production, cycle is the effect of the selection cycle (df = 13), selection treatment is the effect of the selection treatment (df = 3), reproduction method is the effect of the reproduction method (df = 1), (cycle x selection treatment), (cycle x reproduction method), (selection treatment x reproduction method) and (cycle x selection treatment x reproduction method) are interaction effects, LINE is the random effect of the line, ε is the residual error. The analysis was performed with lmer function of *lmerTest* package (Kuznetsova et al. 2017) and r.squaredGLMM function of *MuMin* package (Bartoń 2019) in R version 3.6.1. The anova function within the *stats* package (R Core Team 2019) was used to describe the linear mixed model.

Biomass production, all cycles taken together, was analyzed with a two-way ANOVA (with selection treatment, reproduction method, and their interaction as factors) followed by a Tukey's HSD test (*stats* package in R version 3.6.1)

The ARISA data were analyzed using a non-metric multidimensional scaling (NMDS; *vegan* package in R version 3.6.1; Oksanen et al. 2018). A dissimilarity matrix was built with vegdist function and Bray-Curtis index, and NMDS was performed with metaMDS function.

RESULTS

Biomass variations over time

The linear mixed model showed that the cycle factor had a strong effect on biomass production (77% of the explained variance; mean square of the cycle factor divided by the total mean square excluding that of the residuals) as well as its interaction with the selection treatment (11%) and the three-way interaction: cycle- by-selection treatment-by-reproduction method (9%; Table S1.1). As shown in Figure S1.1, the effect of the cycle was not monotonic and a loss of the effect of artificial selection on community biomass was observed at certain cycles. A general decrease of biomass was observed at the beginning of the experiment in R, L and S lines and to a lesser extent in H lines (mean decrease of 28% between cycle one and six in R, L and S lines vs −11% in H lines).

Selection treatment

Biomass production was not always changing in the expected direction (Figure 1.2a, Figure S1.1). All cycles taken together, L lines produced significantly less biomass than H and R lines (Figure S1.2, −5.1 and −3.9%, respectively, $p < 2 \times 10^{-16}$). Stabilizing selection was not effective in maintaining an absorbance value over time (Figure 1.2a), which gave rise to mean values (all cycles taken together)

similar to those obtained in H lines in treatment M and to those obtained in L lines in treatment P (Figure 1.2b).

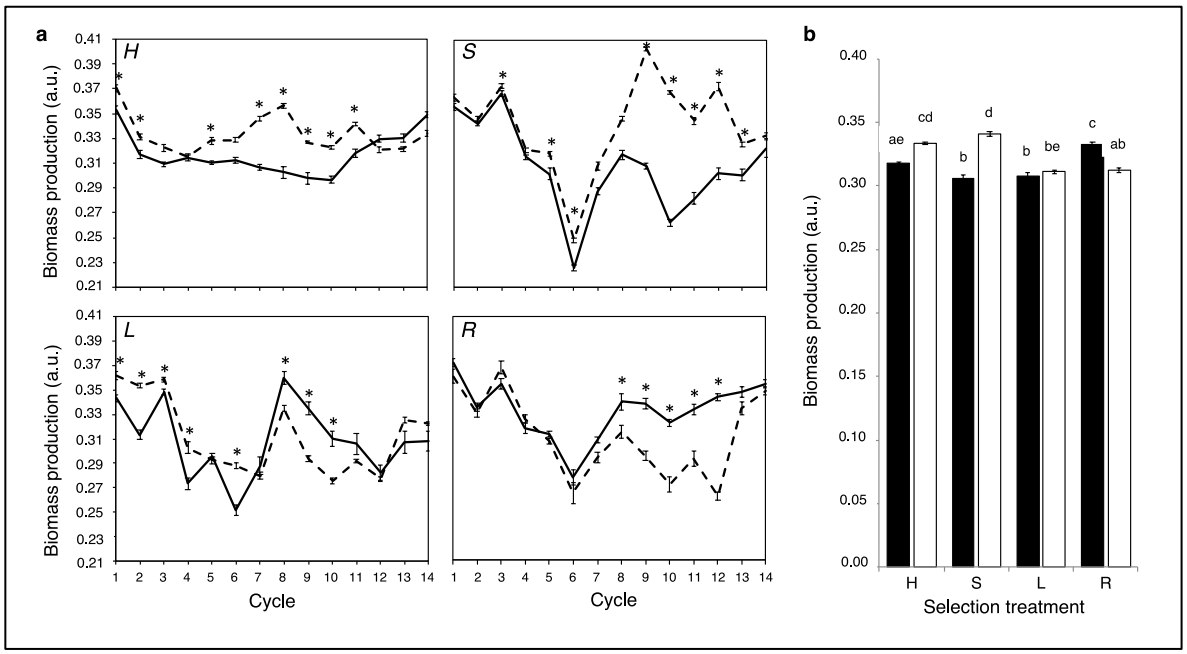

Figure 1.2 Biomass production. a) Over time. Mean biomass production of the 30 communities of propagule (solid lines) or migrant pool (dotted lines) reproduction method. Biomass production of the communities from propagule lines was averaged even if these communities belonged to three distinct lines as their profile were quite similar. Bars represent SE. Asterisks represent significant differences between the two reproduction methods in a given cycle and selection treatment (Tukey HSD test at each cycle; $p < 0.05$). Top left, High; top right, Stabilizing; bottom left, Low; bottom right, Random. **b)** All cycles together. Mean biomass production depending on the selection treatment and the reproduction method ($n = 420$). Black, propagule; white, migrant pool. H, High; S, Stabilizing; L, Low; R, Random. Bars represent SE. Different letters represent significant differences (Tukey HSD test; $p < 0.05$).

Reproduction method

Biomass production differed significantly between the two reproduction methods over time (Figure 1.2a, cycle*reproduction method Table S1.1). Indeed, there were transient phases of divergence during which HM and SM produced more biomass than HP and SP, respectively (difference of 4.48– 17.75% between HM and HP and of 1.68– 40.24% between SM and SP, depending on the cycle) and RM produced less biomass than RP (Figure 1.2a; difference of 7.22– 23.54% between RM and RP depending on the cycle). LP and LM were quite close to each other in terms of biomass production over the course of the experiment. Biomass was 5 and 11% higher using three parental communities (M) than a single one (P) with the selection treatments H and S, respectively (Figure 1.2b). However, the L selection treatment was not affected by the reproduction method (Figure 1.2b). In the Random lines (R), using a single parental community resulted in a higher biomass production than the one obtained when three parental communities were pooled.

Heritability

Our assessment of realized heritability suggested that h^2 depended on the selection treatment (H or L): in L lines, the slope of the regression of the cumulative selection response to the cumulative selection differential tended to be either positive or negative, depending on the line, but never significantly different from zero (Table S1.2). The slope was always positive in H lines, ranging from 0.02 to 0.15, although this was not significantly different from zero after correcting for multiple testing. Our results did not allow us to conclude on the effect of the reproduction method on h^2 because of a lack of statistical power (due to a low number of observations constrained by the number of cycles). However, all selection treatments taken together, the sum of the squares of the difference between parents and offspring biomass production was significantly higher in migrant pool lines than in propagule lines (0.05 and 0.02, respectively, on average; Mann-Whitney U test: W = 2,036, df =1; $p=7.8\times10^{-8}$).

Community structure

Communities from the different lines were originating from the same initial pool and remained close from each other at the third cycle in terms of composition (Figure 1.3). Then, they diverged between cycles three and seven. At cycle 7, and to a lesser extent at cycle 10, the divergence of the microbial communities seemed to be mostly driven by the selection treatment (Figure 1.3). Moreover, the relative position of the centroids of the two reproduction methods tended to diverge over time, with no more overlap between the two ellipses at cycle 14 (Figure 1.3). For a given selection treatment, M communities were closer from each other than P communities as they were stemming from the same line of 30 communities. At the 14th cycle, P communities grouped at the center of the graph whereas M communities of the L, H and S selection treatments were located at the periphery, indicating a stronger divergence due to the selection treatment in M than in P.

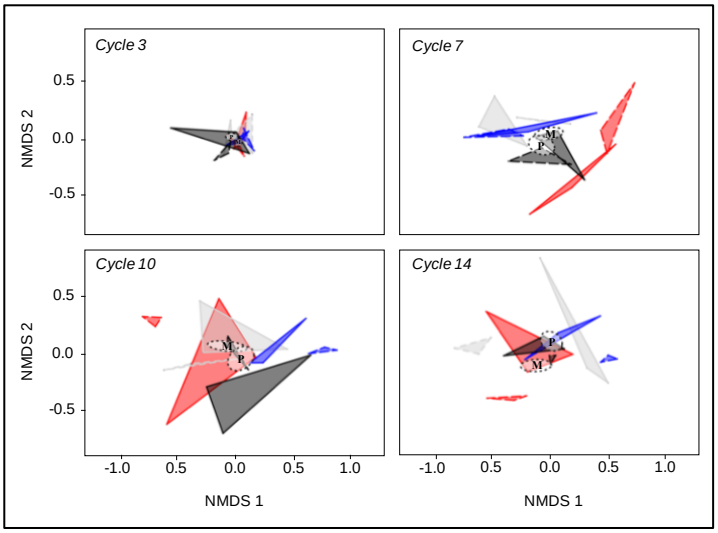

Figure 1.3 Non-metric multidimensional scaling of community structure over time. Polygons connect communities from the same selection treatment and reproduction method (three communities were analyzed per treatment and cycle). Black, Random; gray, Low; blue, Stabilizing; red, High. Polygons with solid borders: propagule; polygons with dotted borders: migrant pool. For each reproduction method, the centroid position (average position of the communities for a given reproduction method, taking SE into account) is represented by a dotted circle.

Richness was similar for every treatment at cycle 3 (between 34 and 37 OTUs) and underwent a significant decline over time until reaching values

of 16 to 24 OTUs (Figure 1.4; $F = 41.6$; $df = 31$; $p < 2 \times 10^{-16}$). Interestingly, this decline occurred later in L lines whatever the reproduction method, with no significant difference of richness between cycles 3 and 7 unlike all other treatments (Figure 1.4). In addition, in the treatment combining a high biomass selection and a migrant pool reproduction method (HM), the sharp decrease in richness between cycles 3 and 7 was followed by an unexpected regular increase in richness from cycle 7–14. This significant increase exhibited a small variance which suggests that this was not a random effect, for example due to a contamination, but rather a reliable change in community structure with an increase in abundance of species initially present at a level below the detection threshold of our molecular method.

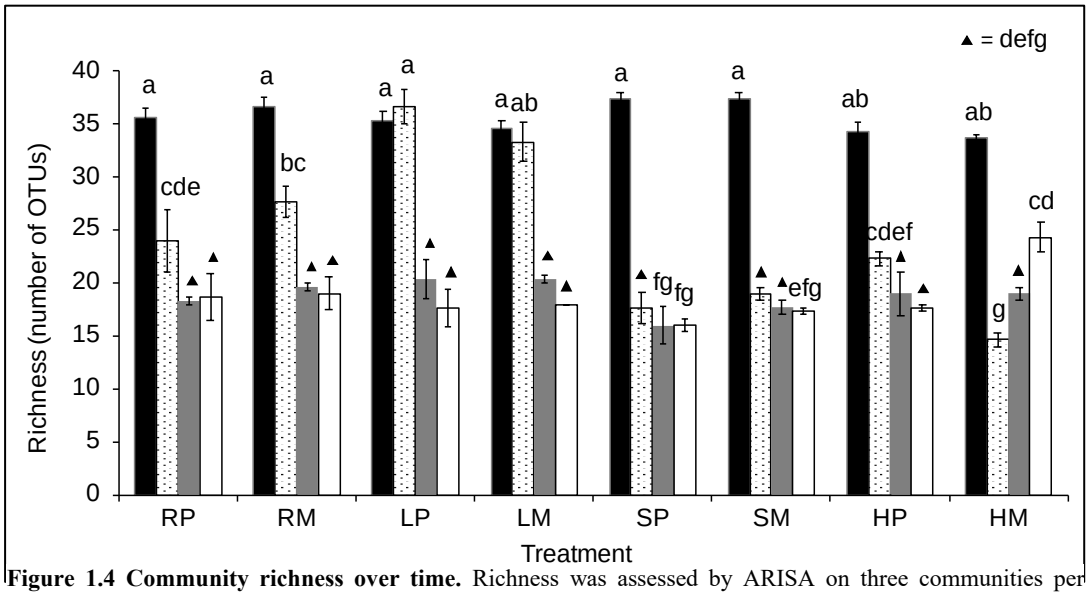

Figure 1.4 Community richness over time. Richness was assessed by ARISA on three communities per treatment and per cycle. Black, cycle 3; dotted, cycle 7; gray, cycle 10; white, cycle 14. Bars represent SE. Different letters represent significant differences (Tukey HSD test; $p < 0.05$). P, Propagule; M, Migrant pool; R, Random; L, Low; S, Stabilizing; H, High.

DISCUSSION

One of the main issues in conducting efficient artificial selection of microbial communities is to be able to preserve the entities responsible for the phenotype of interest (e.g., species, species-species interactions) from one cycle to the other (Arias-Sánchez et al. 2019). In this study, we experienced difficulties in reaching and fixing the targeted phenotypes. Indeed, only transient phases of divergence among the selected lines and between them and the randomly chosen lines were observed. This is in accordance with experiments by Swenson et al. (2000b). Very small initial differences in ecosystems, originating from variation in population size, or species composition, can be amplified by the complex dynamics of ecosystems through what is called the "butterfly effect" (Swenson et al. 2000b). More recently, an experiment demonstrated that the variance of the community property (CO_2 emission) and the heritability of this property declined along selection cycles, confirming the importance of the sampling effect in explaining differences in and collapse of the selected property (Blouin et al. 2015).

This points out a paradox in community selection: a minimal variance resulting from community dynamics is necessary for selection to act (variation principle), but if this variance occurs through the butterfly effect, then it could prevent any heritability between parents and their offspring (Penn 2003). This is consistent with our results which showed very low values of realized heritability. It indicated that only a small part of phenotypic variation, if any, was due to genetic additive variation which could explain our difficulties to maintain the effect of artificial selection over time. A modelling approach developed by Xie et al. (2019) suggested that artificial selection could be improved by lowering non-heritable variation (e.g., small variation in initial offspring community biomass). This could be done through the use of cell sorting instead of pipetting to create offspring communities and by extending the cycle duration to let the communities reach a stable state in which initial differences are compensated. It supposes that initial differences can be compensated contrary to what is expected under the butterfly effect hypothesis (the model developed by Xie et al. (2019) involved a two-species community whereas Swenson et al. (2000b) worked on complex microbial communities). Our experimental design probably induced too much non-heritable variation, which was a limitation in the parent/offspring resemblance and thus, in the selection treatment efficiency.

First of all, the effect of the selection treatments was confounded with a global decrease in biomass production at the beginning of the experiment which was probably due to a lack of adaptation and/or acclimatization of the microbial community (originated from the soil) to the experimental conditions despite the 48 h-cycle conducted in microplates before the start of the experiment. We cannot link this decrease in biomass production to a decrease in species richness, since in the L lines, the decrease in richness arose later (between cycles 7 and 10) than the decrease in biomass (from cycle 1 to 7). Interestingly, this initial decrease in biomass production tended to be shorter and less pronounced in H lines than in all other lines. Targeting a high biomass production, we probably selected the best-suited communities for the experimental conditions, at least at the beginning of the experiment.

The selection treatment influenced not only biomass production but also community structure. Even though we cannot assure that the observed changes in biomass production were not due to the increase in abundance of a unique microorganism, it is likely that the selection treatments have changed interaction patterns between members of the communities. Williams and Lenton (2007) proposed an ecosystem modelling approach to identify if interactions at the community level were necessary to explain some microbial ecosystem responses to artificial selection. They identified some cases in which the ecosystem response to selection was partly due to interactions between species, suggesting that these interactions can be under artificial selection pressure. The percentage of cases involving multiple species and interactions among them was higher in low lines than in high lines. Williams and Lenton argued that converging to a target (a fixed level of an abiotic factor in their study) is more difficult than diverging from it (which can be more easily achieved by a single microorganism). In our experiment, the targeted phenotypes did not correspond to fixed values so that, contrary to Williams and Lenton, our procedure did not involve converging to or diverging from a target. Despite this, our results showed

that selecting for low biomass production preserved community richness longer than selecting for high biomass production. As pointed out by Day et al. (2011), whether or not the response to artificial selection involves several interacting species depends on the selection target and the existing solutions to reach it. Thus, multispecies solutions may be easier to find selecting on a low biomass production.

Our results suggested that the reproduction method in an artificial selection experiment can be responsible for different responses to selection. The propagule method was predicted to be more reliable but more detrimental to species richness than the migrant pool method. It is difficult to draw an overall conclusion about whether one reproduction method is more reliable to preserve the property than the other, because only transient phases of divergence between these two methods were observed and because of the three-way interaction between the reproduction method, the selection treatment and the cycle. Contrary to our results, Williams and Lenton (2007) did not notice any difference between the propagule and the migrant pool methods in the ecosystem response to selection (*in silico* selection on a level of abiotic factors). However, the probability that the evolution of the ecosystem property can be due to interactions among species was higher with the propagule (4 and 38% of the cases in High and Low lines, respectively) than with the migrant pool method (0 and 25% of the cases in High and Low lines, respectively) (Williams and Lenton 2007). From this model, it can be concluded that the propagule method is likely to better conserve intra and inter-species interactions. In our experiment, it seemed that the migrant pool method was a better way of increasing biomass production considering H lines, but also S lines. This was in contradiction with the prediction that the best method would be the one that had the lower rate of reconfiguration of interactions network (i.e., propagule method) and suggested that it could depend on the targeted phenotype.

The migrant pool method is more often used than the propagule method in artificial selection experiments on microbial communities (Swenson et al. 2000a, b; Panke-Buisse et al. 2015; Blouin et al. 2015). This is mainly due to an expected decrease in richness stronger in the propagule than in the migrant pool method. Indeed, each sampling event at the origin of one community could be responsible for the loss of different species. With a regular pooling of several communities with different compositions, the experimenter can expect to prevent a decrease in richness. When the selection procedure is repeated several times as in our experiment, the migrant pool method was indeed more favorable to a recovery of specific richness (likely not previously detected with our molecular method) at least in the lines selected for a high biomass production. This reproduction method was also responsible for a higher level of variation than the propagule method, as indicated by the highest difference in parent-offspring biomass production and the highest divergence of community structure according to the selection treatment than with the propagule method. In our experimental conditions, it thus appeared that the variation brought by the migrant pool method was more favorable to artificial selection of high biomass lines than the propagule method.

In conclusion, preserving a microbial community phenotype over selection events is a key issue of artificial selection efficiency. Whether to mix several communities in selection procedures or not, or

the number of communities to mix, are questions that need to be asked before conducting an artificial selection experiment. The reproduction method is of importance as it can play a role on community structure and diversity and influence the targeted phenotype.

In the work presented in Chapter 1, we highlighted a decrease in community richness over the artificial selection procedure analogously to what happen at the individual level regarding the genetic diversity when a species is submitted to selection. Based on the idea that this decrease in richness could be linked to a decrease in phenotypic variation and thus to a decrease in the response to selection, we wanted to test whether starting the procedure from a richer community could help increasing artificial selection efficiency. The results are presented in Chapter 2 and will soon be submitted for publication.

CHAPTER 2: COMMUNITY DIVERSITY DETERMINES THE EVOLUTION OF SYNTHETIC BACTERIAL COMMUNITIES UNDER ARTIFICIAL SELECTION

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Abstract

Artificial selection can be conducted at the community level in the laboratory through a differential propagation of the communities according to their level of expression of a targeted function (i.e. community phenotype). Working with communities instead of individuals as selection units brings in additional sources of variation in the considered phenotype that can arise through changes in community structure and influence the outcome of the artificial selection. These sources of variation could even be increased by manipulating species diversity. In this study, we wanted to assess the effect of manipulating initial community richness on artificial selection efficiency, defined as the change in the targeted function over time as compared to a control treatment without artificial selection. We applied artificial selection for a high productivity on synthetic bacterial communities varying for their initial richness level (from one to 16 strains). Our results showed that, overall, the communities that were artificially selected were 16% more productive than the control communities. Community richness positively influenced community productivity and metabolic capacities but its influence on productivity changes over time was not linear. Our results suggested that community richness could influence artificial selection efficiency, but a convergence of the community composition might have limited the effect of diversity on artificial selection efficiency. We propose that applying artificial selection on communities varying for their diversity could allow finding communities differing for their level of expression of a function but also for their responsiveness to artificial selection, provided that their initial composition is different enough.

Key-words: artificial selection efficiency, community diversity, community evolution, synthetic bacterial communities

INTRODUCTION

In 1989, in the framework of the levels-of-selection theory (or multi-level selection), Wilson and Sober supported the idea that natural selection at the community level could occur *in natura.* In the two last decades, several attempts have been made to enhance or reduce a property or a function performed by a microbial community through artificial selection at the community level in the laboratory. The general principle is to *i*) grow replicates of a microbial community, *ii*) assess the performance of these communities regarding the targeted function, *iii*) propagate the best performing community(ies). This approach has been used to study the degradation of a toxic compound (Swenson et al. 2000a), the modification of the pH of an aquatic medium (Swenson et al. 2000b), CO_2 emissions (Blouin et al. 2015), chitinase activity (Wright et al. 2019), productivity (Raynaud et al. 2019), the hydrolysis of starch (Chang et al. 2020) and the growth promotion of a bacterial strain (Chang et al. 2020). All of the studies involving artificial selection of microbial communities showed that the engineering of complex microbial communities is not straightforward. In particular, improvements of the targeted function are often unstable (Swenson et al. 2000b; Raynaud et al. 2019; Wright et al. 2019) and registered at the beginning of the procedure only (Chang et al. 2020). This might be related to a decrease in community phenotypic variance and heritability over time (Blouin et al. 2015) but also to changes in community structure, due to the succession of species for example (Wright et al. 2019), that could limit artificial selection efficiency.

Artificial selection can be applied without any *a priori* knowledge of community composition or functioning but, assessing community diversity during an artificial selection experiment can allow a better understanding of community evolutionary dynamics in this context. It is well-known that the components of the diversity of a community (e.g. community richness, composition, evenness) can have an influence on many functions such as productivity or stability (Hooper et al. 2005). Several studies, conducted on bacterial communities and manipulating community richness (up to 72 species in Bell et al. (2005)), experimentally tested for effects of community diversity on the community respiration rate (Bell et al. 2005) or productivity (Gravel et al. 2011; Fetzer et al. 2015). These studies highlighted a positive and saturating relationship between the increase in community richness and the increase in the level of the measured function. Two main categories of mechanism can underlie a diversity-function relationship (Loreau et al. 2001): complementarity effects (i.e. the function is due to a combination of species through niche partitioning or facilitation between species) and selection effects (i.e. the function is due to a dominant species). Increasing community richness increases the probability of these mechanisms to occur (Loreau and Hector 2001) and thus to observe an increase in the studied function.

Beyond the effect of community diversity on the initial level of a function, increasing community diversity could also influence community response to selection. The term "evolution" is sometimes restricted to genetic changes over generations (Barraclough 2015). But, when it comes to community evolution, additional sources of variations can be involved in the community evolutionary response (Penn 2003; Williams and Lenton 2007), provided that they can be transmitted to the next "generation of communities" (i.e. that they are heritable; Goodnight 2000). Indeed, the community phenotype can result from allelic composition and intragenomic interactions (i.e. epistasis), population composition and intraspecific interactions, and from species composition and interspecific interactions. All these sources of variations in community response to artificial selection depend on community diversity. Selecting at the community level while increasing species richness, and thus the sources of variations, may increase the probability to observe extreme values for the targeted function among the fixed number of communities under selection. This increasing number of species should thus increase the selection differential (S) in the breeder equation ($R = h^2 \times S$, with R the response to selection and h^2 the heritability; Lush 1937). As a consequence, the response to selection (R) should be higher when there are many, as compared with few species, provided that the phenotype is reliably transmitted between parent and offspring communities (i.e. $h^2 > 0$).

In this study, we wanted to explore the link between the diversity of a community and the efficiency of artificial selection. Previous artificial selection experiments were mainly conducted on complex natural microbial communities (retrieved from soil or plant leaves for example) that were then grown under laboratory conditions. Some studies assessed the changes in microbial community diversity over the course of the experiment (Raynaud et al. 2019; Jacquiod et al. 2021) but community diversity was not intentionally manipulated. We designed an experiment that combined the approaches developed in artificial selection of microbial communities and in biodiversity-ecosystem functioning experiments. An artificial selection procedure was applied on synthetic bacterial communities including five richness levels from one to 16 strains. We defined artificial selection efficiency as the change in the targeted phenotype – a high productivity – over time as compared to a control treatment without artificial selection. We hypothesized that increasing the diversity of the selected communities could be responsible for a larger range of variation in productivity, providing more opportunities for selection to act, and thus enhancing the efficiency of artificial selection.

MATERIALS AND METHODS

Bacterial strains

Eighteen bacterial strains were used in this experiment. They were chosen based on the screening of 38 laboratory strains for their ability to grow in the chosen experimental conditions (detailed below). Based on the growth curves of the 38 strains (assessed by Bioscreen, Oy Growth Curves Ab Ltd, Finland), we excluded the strains that showed an absence of growth, a slow growth or

a decline, as well as strains that had a longer lag phase or a faster growth than the others. This was done to avoid the dominance of one or few strains from the very beginning of the experiment in communities due to too large differences in growth ability in our culture conditions. The 18 chosen strains belonged to three phyla, six classes and 13 genera (Table 2.1).

Table 2.1 Bacterial strains used in the experiment. Note that *Aminobacter aminovorans* was previously known as *Chelatobacter heintzii*.

Strain	Phylum	Class
Alcaligenes eutrophus JMP131	Proteobacteria	b-proteobacteria
Agrobacterium sp. 9023	Proteobacteria	a-proteobacteria
Aminobacter aminovorans SR38	Proteobacteria	a-proteobacteria
Arthrobacter sp.	Actinobacteria	Actinobacteria
Arthrobacter sp. BS2	Actinobacteria	Actinobacteria
Cupriavidus necator JMP134	Proteobacteria	b-proteobacteria
Dyadobacter fermentans DSM 18053	Bacteroidetes	Flavobacteria
Escherichia coli K12	Proteobacteria	g-proteobacteria
Escherichia coli WA803	Proteobacteria	g-proteobacteria
Microbacterium sp. C448	Actinobacteria	Actinobacteria
Pseudomonas azelaica	Proteobacteria	g-proteobacteria
Pseudomonas knackmussii DSM 6978	Proteobacteria	g-proteobacteria
Pseudomonas sp. ADP3	Proteobacteria	g-proteobacteria
Pseudomonas sp. ADPe	Proteobacteria	g-proteobacteria
Pseudopedobacter saltans DSM 12145	Bacteroidetes	Sphingobacteria
Ralstonia sp.	Proteobacteria	b-proteobacteria
Sphingomonas wittichii RW1	Proteobacteria	a-proteobacteria
Variovorax sp. 38R	Proteobacteria	b-proteobacteria

Community construction

The 18 strains were grown under five levels of initial richness: 1, 2, 4, 8, 16 strains. All the monocultures were grown (i.e. n=18 for level 1), and six communities per remaining level of richness were established (i.e. n=6 for levels 2, 4, 8 and 16). Community composition (Table S2.1) was determined by randomly choosing 16 strains among 18 without replacement to construct the six communities of the richness level 16. We built the communities from the lower richness levels as subsets of the communities of the upper richness level. The first eight strains that were randomly assigned to the first community of richness level 16 composed the first community of level 8 and so on until creating six communities of eight strains. The same method was used for levels 4 and 2.

Growth conditions

The culture medium was a mix of 1:5 lysogeny broth (LB) and 1:5 tryptic soy broth (TSB), these media notably differ for their carbon sources which can allow for niche partitioning (Van den Bergh et al. 2018). Before the start of the experiment, each strain was grown on a Petri dish (1:5 LB+TSB with agar) by streaking, starting from monocultures stored in 30% glycerol at -80 °C. Then, one colony per strain was picked and placed in 42 ml of culture medium in a flask (28 °C, 110 rpm, 24

h). The optical density (OD) was assessed at 600 nm (BioPhotometer 6131, Eppendorf, Germany) and each suspension was diluted to a final OD of 0.002. The diluted suspensions were used to inoculate monocultures and to build the communities by adding an equivalent volume of each required suspension according to the composition of the community. The monocultures and communities were grown in sterile 2 ml deep-well plates (Porvair Sciences, UK) filled with 1 ml of culture medium. Each monoculture (n=18) and community (n=24) was replicated 11 times i.e. 11 wells of a plate were inoculated with the same suspension. It resulted in six plates in total over which each level of richness was represented and an extra suspension was added as a control to track for possible "plate effects". Temperature was kept to 28°C and there was no shaking in order to allow for possible spatial niche partitioning (Van den Bergh et al. 2018).

Experimental evolution

A transfer into a new plate and fresh medium occurred every 84 h for 20 weeks resulting in 40 artificial selection cycles. The phenotype targeted by artificial selection was a high productivity which was assessed by OD measurement. Each 84 h, the content of the wells was homogenised by pipetting up and down and 200 µL of suspension were transferred into a microplate-reader compatible plate (Fisherbrand 96-Well plates, Fisher Scientific, USA). The OD was measured at 600 nm (Infinite M200 PRO, Tecan, Switzerland) and the 200 µL of suspension were discarded. The artificial selection treatment (AS) occurred through *i*) the identification of the well among ten showing the highest OD, *ii*) the sampling of 20 µL of the corresponding suspension and *iii*) the inoculation of 980 µL of fresh medium with these 20 µL of suspension. The two latter steps were repeated until the ten wells of the new plate were inoculated. As previously mentioned, there were 11 wells for each monoculture or community; while ten wells were dedicated to the artificial selection, the remaining well was used as a control without artificial selection. 20 µL of suspension were sampled from this well and inoculated into 980 µL of fresh medium whatever the OD of the suspension (No artificial Selection, NS). This treatment corresponded to a controlled natural selection (Conner 2003) in which environmental conditions were imposed but the communities were allowed to reproduce without artificial selection. At each transfer event, the suspensions that were used to inoculate the new plate (i.e. the suspension from the selected wells in AS and the suspension from the control wells) were stored in 30% glycerol at -80°C.

Post-selection

After the end of the experimental evolution, we revived the monocultures and communities from cycle 0 (i.e. initial inocula, hereafter called "ancestors") and 40 (i.e. cultures after 40 selection cycles, hereafter called "evolved under AS") from glycerol stocks. The control cultures (evolved under

NS) were also included. The aim was to assess the phenotype of ancestors and evolved monocultures and communities at the same time in one experiment to corroborate what was observed in the artificial selection experiment. The monocultures and communities were first grown in 20 ml of culture medium in flasks (28°C, 110 rpm, 24 h for the evolved under AS and NS and 48 h for the ancestors as 24 h were not enough for them to reach sufficient OD). The optical density was assessed at 600 nm (Infinite M200 PRO, Tecan, Switzerland), each suspension was diluted in culture medium to a final OD of equivalent 0.002 in BioPhotometer 6131 and allowed to grow in triplicate for 84 h in the growth conditions of the experimental evolution (i.e. deep-well plates, 28°C, no shaking, 1 ml of 1:5 LB+TSB). The OD was measured at 600 nm after 84 h of growth as previously described (see "Experimental evolution"). Three bacterial strains were grown on each plate (six in total) as controls for a possible "plate effect".

Description of the growth dynamics

To go further in the phenotypic description of the ancestor and evolved monocultures and communities, we used the same protocol as previously described (culture in flask at 110 rpm, 28 °C, for either 24 or 48 h, OD measurement, dilution to a final OD of 0.002) and inoculated triplicate of the suspensions into microplates that were suitable for the detailed analysis of growth kinetics (sterile plates honeycomb, Thermo Scientific, USA). The growth conditions were: 200 µL of 1:5 LB+TSB, 28 °C, 15 s of shaking occurring 5 s before each OD measurement, one measurement every 30 min for 84 h (Bioscreen, Oy Growth Curves Ab Ltd, Finland). Three bacterial cultures were grown on each plate (seven in total) as controls for a possible "plate effect".

Metabolism

We assessed the metabolic capabilities of the ancestor and evolved monocultures and communities (under AS and NS) using EcoPlates (Biolog, USA). 31 carbon (and nitrogen) sources belonging to six categories (amino acids, amines, carbohydrates, carboxylic acids, phenolic compounds and polymers ; Montserrat Sala et al. 2010) were tested. In the same way as the post-selection experiment and the growth dynamic description experiment, ancestors and evolved (under AS and NS) monocultures and communities were revived and grown in flasks (110 rpm, 28°C, either 24h or 48h). Then, OD was assessed and the suspensions were diluted in 0.9% NaCl solution to reach a final OD of 0.2 (tests were conducted before the start of the experiment and showed that cell washing gave similar results to those obtained with a dilution approach indicating that a remaining amount of culture medium in the suspension did not changed the results). EcoPlates were inoculated with 120 µL of diluted suspension (one plate per sample, each substrate was repeated three times per plate) and placed at 28 °C without shaking. When a substrate was used by the bacteria, a tetrazolium dye was reduced which produced a purple coloration which was assessed by OD measurement at 590 nm (Infinite M200 PRO,

Tecan, Switzerland). A first measurement was done four hours after the inoculation and then twice a day for four days.

Community composition analysis

To assess for changes in community composition over the experiment, we performed 16S rRNA gene and *gyrB* sequencing on communities from selection cycles 1, 14, 27 and 40 for both AS and NS. In order to track for the presence of contaminants, 16S rRNA gene and *gyrB* sequencing was performed on monocultures from selection cycles 1 and 40 (see Appendix 2.1 for the details on DNA extractions, PCR and bioinformatics analyses).

Statistical analyses

We first ran preliminary analyses to check for the presence of contaminants in the samples (i.e. strains that were not included in the initial species composition) and to determine at which step of the experimentations they occurred as it could have or not an influence on the results and more particularly on the validation of our main hypothesis (Appendix 2.2 and see Appendix 2.3 for the detail of the OD profiles over time including the contaminated samples). It resulted in the removal of 16.7% of the samples in the experimental evolution dataset and 14.3% of the samples in the growth dynamics, metabolism and post-selection datasets. The smallest sample size (n) occurred for the artificial selection treatment at richness levels 2 and 4 for which n was equal to 4 (instead of 6). The following analyses were conducted on the resulting datasets.

The data from the experimental evolution procedure were analysed with the following linear mixed model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma * l + (\alpha\beta)_{ij} + (\alpha\gamma)_i * l + (\beta\gamma)_j * l + (\alpha\beta\gamma)_j * l + I_k + E_{ijkl}$$

Y_{ijkl} is the OD of the individual (i.e. monoculture or community) of identity *k*, of initial richness level *i*, under selection treatment *j*, at cycle *l*. μ is the intercept. α_i is the effect of the initial richness level (qualitative: 1, 2, 4, 8, 16), β_j is the effect of the selection treatment (qualitative: AS, NS). $\gamma * l$ is the effect the selection cycle (quantitative: 1 to 40). The interaction effects between *i*) the initial richness level and the selection treatment $(\alpha\beta)_{ij}$; *ii*) the initial richness level and the selection cycle $(\alpha\gamma)_i * l$; *iii*) the selection treatment and the selection cycle $(\beta\gamma)_j * l$; *iv*) the initial richness level, the selection treatment and the selection cycle $(\alpha\beta\gamma)_j * l$ were also included in the model. I_k is the random effect of the individual and E_{ijkl} is the residual error. An autoregression structure of order 1 (AR1) was included to correct for temporal autocorrelation in the data. We expected that an increase in OD over the selection cycles will be *i*) stronger in AS than NS (i.e. significant effect of $(\beta\gamma)_j * l$); *ii*) stronger in communities with high initial richness than in the ones with low richness (i.e. significant effect of $(\alpha\gamma)_i * l$); and that the overall gain in OD will be *iii*) stronger at high richness level in AS than in NS (i.e. significant effect

of $(\alpha\beta)_{ij}$). Our main hypothesis regarding an increase in selection efficiency with the increase in richness would be verified *iv*) if the increase in OD over the course of the experiment is stronger in AS than NS when richness increases (i.e. significant effect of the three-way interaction $(\alpha\beta\gamma)_j * l$). The analysis was done on the selected wells only in order to balance the dataset between AS and NS (one OD value per individual, cycle and selection treatment). The OD values were $\log_{10}$ transformed in order to meet the criteria for normality and homoscedasticity.

We detected a plate effect in the post-selection experiment: the OD of the control strains of two of the plates was lower than the observed OD for the other plates. We calculated the difference in OD and add this value to the measured OD of the two plates. Note that this correction did not significantly influence the results of the analysis. In order to compare what was observed during the experimental evolution and what was obtained from the post selection experiment, the data were analysed with the following linear mixed model:

$$Y_{ikmn} = \mu + \alpha_i + \delta_m + \tau_n + (\alpha\delta)_{im} + (\alpha\tau)_{in} + (\delta\tau)_{mn} + (\alpha\delta\tau)_{imn} + I_k + E_{ikmn}$$

Y_{ikmn} is the OD of the individual of identity *k*, of initial richness level *i*, of history *m*, in dataset *n*. μ is the intercept, α_i is the effect of the initial richness level (qualitative: 1, 2, 4, 8, 16), δ_m is the effect of the history (qualitative: ancestor, evolved under AS, evolved under NS), τ_n is the effect the dataset (qualitative: experimental evolution, post-selection). The interaction effects between *i)* the initial richness level and the history $(\alpha\delta)_{im}$; *ii)* the initial richness level and the dataset $(\alpha\tau)_{in}$; *iii)* the history and the dataset $(\delta\tau)_{mn}$; *iv)* the initial richness level, the history and the dataset $(\alpha\delta\tau)_{imn}$ were also included in the model. I_k is the random effect of the individual and E_{ikmn} is the residual error.

The description of the growth dynamics allowed producing growth curves for each of the individual of the experiment under the three evolutionary histories (ancestor, evolved under AS, evolved under NS). We described the growth curves with segmented regressions which allowed getting the slopes of the different growth phases (four slopes) and the time of transition from one phase to the other (three breakpoints). From the growth curves, we also extracted the OD at 3.5 days (i.e. the targeted phenotype), the maximum OD and the time to reach the maximum OD. The obtained values for the three replicates of one individual were averaged. The data were analysed with the following linear model:

$$Y_{ikm} = \mu + \alpha_i + \delta_m + (\alpha\delta)_{im} + I_k + E_{ikm}$$

Y_{ikm} is the growth parameter of the individual of identity *k*, of initial richness level *i*, of history *m*. μ is the intercept, α_i is the effect of the initial richness level, δ_m is the effect of the history, $(\alpha\delta)_{im}$ is the effect of the interaction between the initial richness level and the history. I_k is the random effect of the individual and E_{ikmn} is the residual error. A principal component analysis (PCA) including the ten growth parameters was performed and Euclidean distances between ancestors and evolved under AS and NS were computed based on the coordinates given by the PCA.

The same linear model as for the growth dynamic description experiment was used to analyse the number of metabolized substrates by the ancestors and evolved monocultures and communities (see Appendix 2.4 for details on the distinction between metabolized and non-metabolized substrates). The level of substrate use (i.e. the maximum OD reached on the different substrates) was analysed with the following linear mixed model:

$$Y_{ikmop} = \mu + \alpha_i + \delta_m + \varphi_o + (\alpha\delta)_{im} + (\alpha\varphi)_{io} + (\delta\varphi)_{mo} + (\alpha\delta\varphi)_{imo} + I_k + S_p + E_{ikmop}$$

Y_{ikmop} is the OD of the individual of identity *k*, of initial richness level *i*, of history *m*, for substrate category *o* and substrate *p*. μ is the intercept, α_i is the effect of the initial richness level, δ_m is the effect of the history. φ_o is the effect of the substrate category. The interaction effects between *i)* the initial richness level and the history $(\alpha\delta)_{im}$; *ii)* the initial richness level and the substrate category $(\alpha\varphi)_{io}$; *iii)* the history and the substrate category $(\delta\varphi)_{mo}$; *iv)* the initial richness level, the history and the substrate category $(\alpha\delta\varphi)_{imo}$ were also included in the model. I_k is the random effect of the individual, S_p is the random effect of the substrate and E_{ikmnop} is the residual error. A PCA was conducted with the 31 substrates as variables.

All the analyses were performed with R version 3.6.3 with the following packages: nlme (Pinheiro et al. 2021) and lmerTest (Kuznetsova et al. 2017) for linear mixed models, car for type II analyses of variance (Fox and Weisberg 2019), emmeans for slope calculation (Lenth 2021), mclust for mixture models (Scrucca et al. 2016), segmented for segmented regressions (Muggeo 2008), FactoMineR for PCA (Lê et al. 2008).

RESULTS

Artificial selection and initial richness level effects on mean OD

All selection cycles together, the OD in AS was significantly higher than this observed for NS (selection treatment: χ^2=89; $p_{df=1}$<2.2x10^{-16}; Table 2.2): AS produced a gain in OD of 0.11, i.e. +16.4% as compared to NS. However, considering population mean in AS rather than the selected individuals only, the gain in OD in AS as compared to NS was +0.036, i.e. +5.3% (χ^2=15; $p_{df=1}$=1.1x10^{-4}). There was also a significant effect of the initial richness level on OD (χ^2=23, $p_{df=4}$=1.5x10^{-4}; Table 2.2). All selection cycles together, the OD tended to increase with the increase in the initial richness level (from 0.56±0.28 to 0.89±0.12) with significant differences between monocultures and levels 8 and 16.

OD of ancestors and evolved monocultures and communities

The OD of the monocultures and communities at the end of the experiment (i.e. evolved under AS or NS) differed from the OD of the monocultures and communities at the beginning of the experiment (i.e. ancestors). The OD of the ancestors was lower than the one of the evolved under NS which was lower than the one of the evolved under AS (0.58±0.23, 0.71±0.27 and 0.80±0.24

respectively; χ^2=83; $p_{df=2}$<2.2x10^{-16}; Table S2.2). Thus, the artificially selected monocultures and communities were more productive than the ancestors and than the evolved under NS. This result did not depend on the initial richness level (initial richness level*history: χ^2=3.0; $p_{df=8}$=0.93; Table S2.2) and was consistent when considering either the OD retrieved from the experimental evolution or the OD measured in the post-selection experiment (Figure 2.1a and b; no effect of the dataset: χ^2=1.2; $p_{df=1}$=0.28; Table S2.2). However, when OD was measured in different growth conditions than those of the experimental evolution (i.e. in the system used to assess growth dynamics), the effect of the evolution depended on the dataset (χ^2=329; $p_{df=4}$<2.2x10^{-16}) and the evolved under AS and NS showed a significantly lower OD than the ancestors (respectively 0.97±0.13, 0.95±0.16 and 1.1±0.22; Figure 2.1c) indicating that the abiotic environment influenced the expression of the phenotype under selection.

Table 2.2 Deviance table of the covariance analysis (ANCOVA) of the optical density (OD) through experimental evolution. The effect of the selection cycle (from 1 to 40), the initial richness level (1, 2, 4, 8 ,16), the selection treatment (artificial selection, no artificial selection) and their interactions on OD were estimated with a linear mixed model including the identity of the selection unit as a random effect factor and an autoregression structure. The conditional R^2 is presented (i.e. variance explained by both fixed and random effect factors; the marginal R^2 – fixed effect factors only – was 0.33).

	Df	Chi squared	p
Selection cycle	1	10.4	**1.24x10^{-3}**
Initial richness level	4	22.6	**1.53x10^{-4}**
Selection treatment	1	89.0	**<2.2x10^{-16}**
Selection cycle * Initial richness level	4	13.9	**7.52x10^{-3}**
Selection cycle * Selection treatment	1	0.734	0.391
Initial richness level * Selection treatment	4	9.09	0.059
Selection cycle * Initial richness level * Selection treatment	4	1.41	0.842
		R^2=0.80	

Artificial selection and initial richness level effects on OD change over time

There was no significant effect of the selection treatment on OD change over time (selection cycle*selection treatment: χ^2=0.73; $p_{df=1}$=0.39; Table 2.2). It indicated that, all richness levels together, the slope of the OD over the selection cycles was not different between AS and NS. On the contrary, the OD change over time was influenced by the initial richness level (selection cycle*initial richness level: χ^2=14; $p_{df=4}$=7.5x10^{-3}; Table 2.2): OD tended to increase over time for the lowest (monocultures) and highest richness levels (eight and 16 strains) whereas it tended to decrease for intermediate richness levels (two and four strains; Figure 2.2).

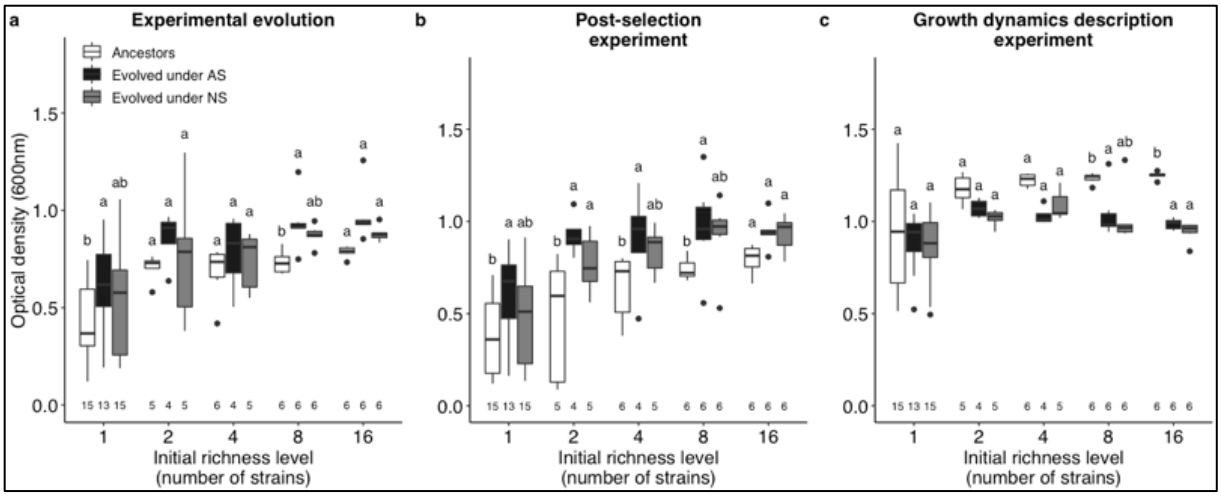

Figure 2.1 Optical density (OD) after 3.5 days of growth of ancestors and evolved monocultures and communities under artificial selection (AS) and no artificial selection (NS) depending on the initial richness level. a) OD measured during the experimental evolution experiment. The values of the ancestor corresponded to the values measured at cycle 1 (mean of AS and NS). The values of the evolved under AS and NS corresponded to the values measured at cycle 40. **b)** OD measured in the post-selection experiment (i.e. in the same experimental conditions). **c)** OD measured in the growth dynamics description experiment (i.e. in different experimental conditions). Each box represents the first quartile, the median and the third quartile for a given treatment, the end of the bars shows the minimal and maximal values within 1.5 times the interquartile range. The points outside of the boxes represent outliers. Sample sizes are given on the bottom of the graphs. Different letters represent significant differences between the levels of evolution within a richness level. White: ancestors; black: evolved under artificial selection; grey: evolved under no artificial selection.

Artificial selection efficiency regarding the initial richness level

The outcome of artificial selection (AS) as compared to no artificial selection (NS) did not differ between the initial richness levels (selection cycle*selection treatment*initial richness level: χ^2=1.4; $p_{df=4}$=0.84; Table 2.2; Figure 2.2). It indicated that AS was not significantly effective whatever the initial richness level. However, there is still evidence for a possible influence of AS on OD change over time as compared to NS. Indeed, the slope of the OD over the selection cycles tended to be higher in AS than in NS in four richness levels over five (Figure 2.2f). Also, the differences in the slopes between AS and NS tended to be influenced by the initial richness level. Among the richness levels that showed an increase in OD over time, the highest differences in slopes between AS and NS occurred for level 8, where the slope in AS significantly differed from zero contrary to the slope in NS ($4.4x10^{-3}$ and $2.4x10^{-3}$ respectively), and for monocultures. On the contrary, the smallest difference occurred with an initial richness of 16 strains where both slopes were very similar ($3.0x10^{-3}$ and $2.7x10^{-3}$ for AS and NS respectively; Figure 2.2f). It suggested that AS may have differentially influenced the increase in OD over time as compared to NS depending on the initial richness level. However, none of the richness levels responded enough to AS to observe significant differences. Interestingly, the correlation of the offspring-parent phenotype in AS was significantly higher in level 8 and monocultures than in level 16 (whereas it was not the case in NS; Figure S2.6), indicating a changing reliability of phenotype transmission with the change in the initial richness level.

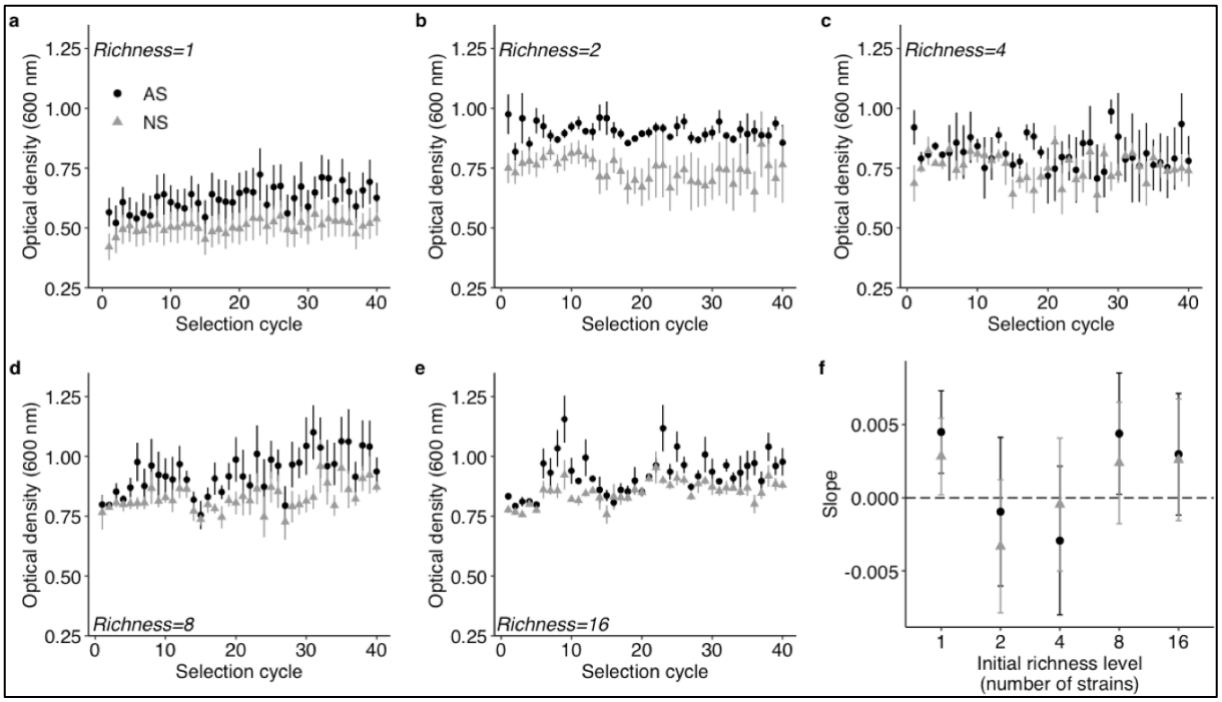

Figure 2.2 Changes in optical density (OD) over experimental evolution under artificial selection (AS) and no artificial selection (NS) depending on the initial richness level. a to e) The mean OD of the parents of the next population of selection units is represented by black circles for AS and grey triangles for NS for each initial richness level. Bars represent SE. Richness 1: n=13 in AS and 15 in NS (n=14 at cycle 33). Richness 2 and 4: n=4 in AS and 5 in NS. Richness 8 and 16: n=6 in AS and NS. The OD at cycle 0 was equal to 0.002 for all the treatments. **f)** The mean slopes of the regression lines predicted by a linear mixed model are presented in black circles for AS and grey triangles for NS for each initial richness level. Bars represent 95% CI.

Artificial selection efficiency within the initial richness levels

Considering the detail of the response of OD through time for each individual or community within a richness level, we noticed that the variability of the response tended to decrease with the increase in initial richness (standard deviation of the mean slope in AS and NS together of $1.0x10^{-2}$ and $1.6x10^{-3}$ for monocultures and level 16 respectively; Figure S2.7). It indicated that the changes in OD over time were more similar between richer communities than between less rich ones or monocultures, where the responses to evolution were more contrasted. Furthermore, there was a high variability in the difference in slope between AS and NS within a richness level, especially at low richness levels (i.e. in monocultures and richness level 2). In monocultures, the two highest differences in slopes between AS and NS occurred for the two *Arthrobacter* strains ($2.0x10^{-2}$ and $1.1x10^{-2}$ for *Arthrobacter* sp. BS2 and *Arthrobacter* sp. respectively). The two lowest differences between AS and NS occurred for two *Pseudomonas* strains ($-2.4x10^{-4}$ and $8.0x10^{-4}$ for *Pseudomonas* sp. ADPe and *Pseudomonas knackmussii* DSM 6978 respectively; Figure S2.7a). Thus, certain strains, and maybe genera, seemed to be more responsive to AS than others.

Growth dynamics of ancestors and evolved monocultures and communities

Overall, the growth parameters of the ancestors and evolved differed more in communities than in monocultures (grouping of the ancestors on Figure 2.3b but not on 2.3a). However, considering the difference in growth parameters between ancestors and evolved individual by individual (i.e. looking at the distance between the ancestor and the corresponding evolved monoculture or community), there was no difference between monocultures and communities (mean Euclidean distance of 2.6±2.1 and 2.9±1.1 respectively). It highlighted the variability of the response in monocultures in which certain strains showed strong differences in growth parameters between ancestors and evolved whereas other showed no changes (Figure S2.8a and b). All richness levels together, the growth parameters tended to differ more between ancestors and evolved (Euclidean distance of 3.0±1.6 between ancestor and evolved under AS and of 3.3±1.6 between ancestor and evolved under NS) than between evolved under AS and evolved under NS (1.9±1.2). Indeed, the growth parameters changed in the same direction between evolved under AS and evolved under NS. There was an increase in the slope of the exponential growth phase as compared to the ancestors, which was significant for evolved under AS only (Figure S2.9a; Table S2.3), and a decrease in the time to reach the maximum OD (3,263 min±926, 3,404 min±986 and 4,416 min±534 for evolved under AS, evolved under NS and ancestors respectively; Figure S2.6b; Table S2.3). The difference in growth parameters between evolved under AS and evolved under NS tended to be lower in monocultures as compared to communities (Euclidean distance of 0.98±0.72 and 2.3±1.1 respectively), indicating a possible difference in the potential to phenotypic change under AS.

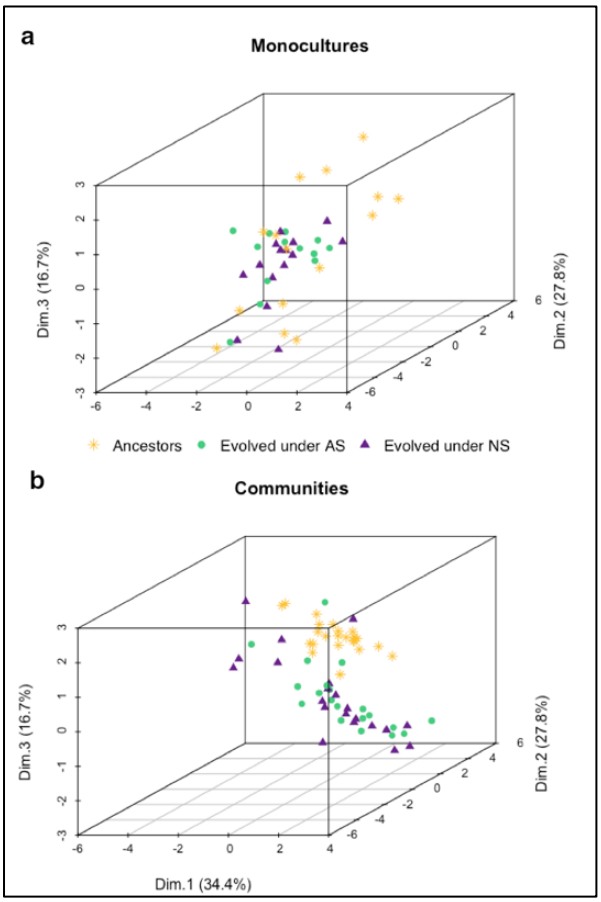

Figure 2.3 Principal component analysis of the growth parameters of ancestors and evolved a) monocultures and b) communities. The ten growth parameters included in the analysis were retrieved from the description of growth curves obtained by repeated optical density (OD) measurements over 3.5 days. Those parameters were: the four slopes of the different growth phases, the three times associated to the transition from one phase to the other, the OD at 3.5 days, the maximum OD and the time to reach the maximum OD. The results obtained for monocultures and communities are presented separately for readability but were obtained from a unique analysis. Star: ancestors; circle: evolved under artificial selection; triangle: evolved under no artificial selection.

Metabolism of ancestors and evolved monocultures and communities

The number of metabolized substrates increased with the increase of the initial richness level (it differed significantly between monocultures and the other richness levels; χ^2=64; $p_{df=4}$=5.0x10^{-13}; Figure 2.4). It highlighted the existence of substrate use complementarity between the strains of the experiment. There was neither an effect of the history on the number of metabolized substrates (χ^2=5.7; $p_{df=2}$=0.06) nor an effect of the interaction between the history and the initial community richness (χ^2=13; $p_{df=8}$=0.11). It indicated that metabolic profiles were stable throughout the experimental evolution.

The OD reached on the 31 considered substrates was influenced by the initial richness level (χ^2=43; $p_{df=4}$=1.2x10^{-8}; Table S2.4) and tended to be lower at lower richness levels (Figure 2.5a). There was an effect of the interaction between the initial richness level and the history (χ^2=39; $p_{df=8}$=6.1x10^{-6}; Table S2.4). On the one side, the OD of the evolved tended to be higher than this of the ancestors for monocultures and richness levels 4 and 8. On the other side, the OD of the communities evolved under AS was lower than this of the communities evolved under NS at level 4 and lower than this of the ancestors at level 16 (Figure 2.5b). Thus, there was a trend to a gain or a loss in metabolic capabilities throughout evolution which depended on the initial richness level. The effect of the initial richness level on OD depended on the substrate category (χ^2=127; $p_{df=20}$<2.2x10^{-16}; Table S2.4). Whereas the maximum OD tended to be achieved at level 4 for the phenolic compounds, the amines, the polymers and the amino acids, the OD tended to increase with the increase in richness for carbohydrates (maximum OD at level 8) and carboxylic acids (maximum OD at level 16; Figure 2.5c). It indicated that complementarity between the different strains occurred for certain substrates only.

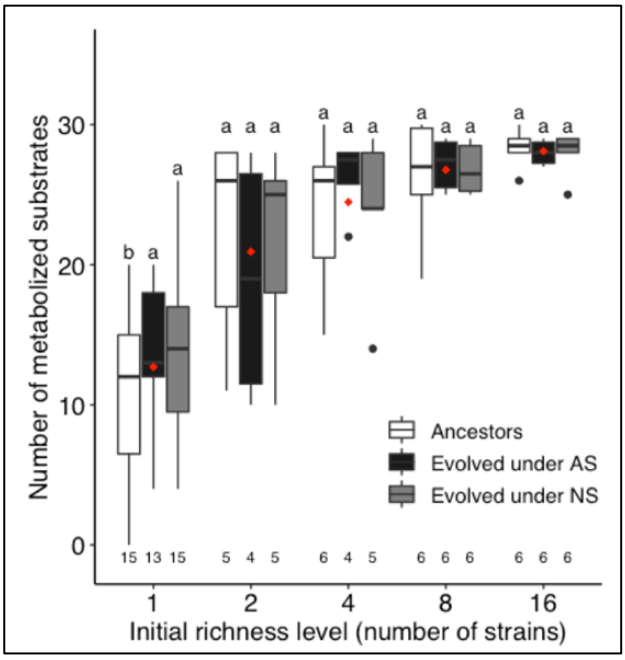

Figure 2.4 Number of metabolized substrates by ancestors and evolved monocultures and communities depending on the initial richness level. 31 substrates were tested in total. White: ancestors; black: evolved under artificial selection; grey: evolved under no artificial selection. Red diamonds represent the mean value for a given initial richness level. Each box represents the first quartile, the median and the third quartile for a given treatment, the end of the bars shows the minimal and maximal values within 1.5 times the interquartile range. The points outside of the boxes represent outliers. Sample sizes are given on the bottom of the graph. Different letters represent significant differences between the histories within a richness level.

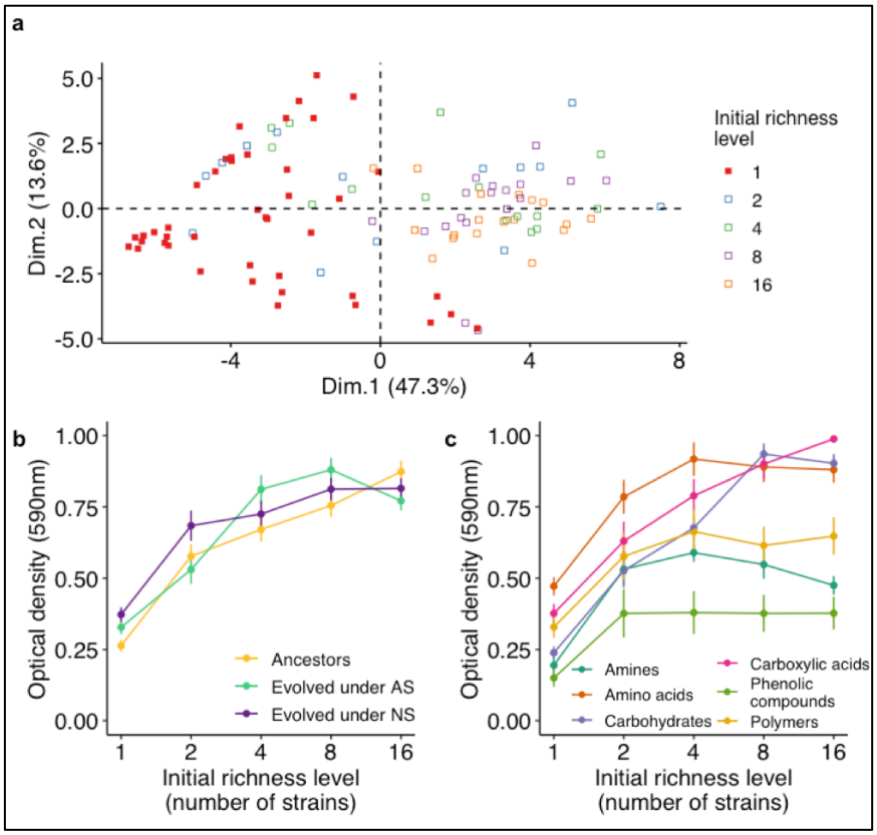

Figure 2.5 Substrate use depending on the initial richness level. a) Principal component analysis of the optical density measured on 31 carbon substrates for monocultures and communities. Ancestors, evolved under artificial selection and evolved under no artificial selection are all represented on the graph without distinction. The more a point is on the right of the graph, the more the corresponding monoculture or community reached a high OD on the tested substrates. **b)** Mean OD reached on the 31 tested substrates depending on the initial richness level and the history. Yellow: ancestors; green: evolved under artificial selection; violet: evolved under no artificial selection. **c)** Mean OD reached on each substrate category depending on the initial richness level. Bars represent SE. From lowest to highest value at level 1: phenolic compounds, amines, carbohydrates, polymers, carboxylic acids, amino acids.

DISCUSSION

Our results showed that artificial selection had an effect on the mean productivity of the bacterial communities and that community richness influenced both the mean productivity and productivity change over time (Table 2.2, Figure 2.2). Contrary to what was expected, there was no effect of the artificial selection on productivity change over time i.e. no increase in the artificially selected function. Previous studies showed significant changes over time in the selected function as compared to control treatments (Swenson et al. 2000a; Blouin et al. 2015; Chang et al. 2020). It is quite common however that artificial selection produces effects on the mean of a function rather than on the slope of function change versus time (Swenson et al. 2000b; Raynaud et al. 2019; Chang et al. 2020). The difficulty to observe a global trend in the change in a function under artificial selection is probably due to a lack of heritability, associated with the absence of stability in community structure (Chang et al. 2020; Jacquiod et al. 2021). A model developed by Xie et al. (2019) highlighted that the phenotypic variation in a community function is mainly due to non-heritable determinants, such as variation due to

pipetting (when inoculating for the creation of a new generation) or function measurement noise for example. In accordance, in our experiment, the mean difference in OD between AS and NS was of 0.11 when considering the selected parents of the next cycle whereas it dropped to 0.036 when considering the population mean, revealing an important part of un-transmitted phenotypic variation. The absence of change in the slope could also be due to natural selection preventing artificial selection to be effective. Indeed, in artificial selection of communities, natural selection is also at stake within a selection unit, as observed in the NS treatment. In AS treatment, within-unit natural selection may overwhelm between-unit artificial selection, making the latter inefficient, as suggested in Wilson and Sober (1989) and Arora et al. (2020).

Species diversity could have multiple effects in artificial selection experiments. In our study, the initial richness level of the community influenced the selected function (productivity) and other potentially related ecosystem functions (i.e. growth dynamics, metabolic profile and level of substrate use, Figures 2.4 and 2.5) as often observed in diversity-functioning experiments (Bell et al. 2005; Gravel et al. 2011; Fetzer et al. 2015). Sequencing data indicated that there was dominance in the communities from the beginning of the experiment (Figure S2.10). Indeed, when the initial composition of a community included both *Escherichia coli* and *Pseudomonas* sp. ADP (ADP3 or ADPe) strains, almost no other species than these two was detectable. Similar outcomes were observed in previous studies. In Goldford et al. (2018), despite the various origins of the twelve studied communities and their high initial richness level (from 110 to 1,290 exact sequence variants (ESV)), all of the communities converged to the same composition at the family level, i.e. Enterobacteriaceae and Pseudomonadaceae (from 4 to 17 ESV) after 12 serial transfers. This family-level composition was also observed in the study of Scheuerl et al., (2020) in all of the 64 studied communities after a five-month experimental evolution. Enterobacteriaceae and Pseudomonadaceae strains retrieved from the evolved communities in Goldford et al. (2018) were all able to grow on the metabolic by-products of all other strains of the community, indicating that cross-feeding was at stake in these communities which may also have occurred in our experiment. Based on the sequencing data, the *Escherichia-Pseudomonas* co-dominance structure occurred twice at level 2, three times at level 4, five times at level 8 and six times at level 16 (in both selection treatments). Thus, the observed positive diversity-functioning relationship could be due to the increased probability of selecting the cross-feeding community members by increasing the initial richness level (i.e. a sampling effect of the complementarity effect).

The effect of species richness in an artificial selection experiment can also occur through an interaction with the evolutionary dynamics or with the selection treatments. In our study, the initial richness level did not significantly influence the effect of the selection treatment (initial richness level*selection treatment, $p_{df=4}=0.059$) as the mean productivity was always (and similarly) higher in AS than in NS whatever the community richness. However, community richness influenced the evolutionary dynamics (initial richness level*selection cycle, $p_{df=4}=7.52\text{x}10^{-3}$, Table 2.2). The sign of the effect depended on the initial richness level but also on the considered community within a richness

level, it suggested an influence of community composition on the community evolutionary trajectory. Thus, as presented in the literature, our results suggest that there is an interplay between community ecology and community evolution (Johnson and Stinchcombe 2007; O'Brien et al. 2013) and indicate that the effect of community diversity could change with the timescale at which community function is considered. Species richness could also affect the way artificial selection influences the evolutionary dynamics (i.e. the efficiency of artificial selection, identified as the three-way interaction between the initial richness level, the selection cycle and the selection treatment in the Materials and Methods section). We hypothesized that artificial selection efficiency would increase with the initial richness level through an increase in the sources of variations (species composition, intra and interspecific interactions…) and hence an increase in the existing solutions to reach the targeted phenotype. However, there was no significant difference in the slopes of the OD response versus time between AS and NS depending on community richness in a linear model (selection cycle*initial richness level*selection treatment, $p_{df=4}$=0.842, Table 2.2). Nevertheless, there was still evidence that the initial richness level could influence artificial selection efficiency as the difference in OD change over time between AS and NS tended to be non-linearly affected by an increase in community richness (Figure 2.2f). Moreover, we noticed that the correlation between parent and offspring OD depended on the initial richness level and that it also responded idiosyncratically to an increase in richness (Figure S2.6). Previous modelling approaches highlighted that in artificial selection of communities, a fine balance between variation and heritability must be achieved (Penn 2003). Based on our results, we suggest that the search for this equilibrium could occur through the modulation of community diversity. But, in addition to the initial species richness, divergence between replicates within a richness level has to be considered to understand the effect of the initial diversity on the efficiency of artificial selection.

Increasing the initial richness level decreased the between-(meta)community variation within a richness level. This especially came with the design of our experiment but could also occur when working on natural communities. In our study, we built the different communities from an initial pool of 18 strains. As a consequence, whereas none of the communities of level 2 included strains in common, seven strains were found in the six communities of level 16 (and at least one *Escherichia* and *Pseudomonas* strain as discussed above). It is well-known that community composition has an effect on community functioning as, for a given richness level, a panel of community phenotypes can be observed depending on community composition (Bell et al. 2005; Fetzer et al. 2015). Between-community differences in composition could also be potential levers for artificial selection. In a recent study, Sánchez et al. (2021) proposed that an efficient directed evolution of microbial community would occur through a good exploration of the "ecological structure-function landscape". In order to explore more solutions to reach the targeted community phenotype (the "function" component of the landscape), multiple communities varying for their composition (the "structure" component of the landscape) have to be considered. In this idea, the first step of a directed evolution experiment would be to create a library of communities varying for their composition (Sánchez et al. 2021). In the light of our results,

we suggest that the differences in community composition in the initial pool of selection units must be sharp enough (e.g. family-level differences) to avoid resemblance in community dynamics that would reduce the exploration of multiple evolutionary trajectories. A first solution could be to start from an initial pool of species several times higher than the number of species in the highest richness level (e.g. 64 instead of 18 species to build six replicates of the 16-species level that deeply differ in their composition). Another way to promote sufficient compositional variability is the maintenance of multiple lineages over the experiment (Blouin et al. 2015; Jacquiod et al. 2021). In a recent study, Chang et al. (2020) started from a pool of 12 communities which were replicated seven or eight times each for a total of 92 communities. A selection cycle occurred through the selection and propagation of the 23 best performing communities among the 92 and, after six selection cycles, all of the communities stemmed from an unique parental community (Chang et al. 2020). The single lineage increased the probability that the gain in the targeted function under AS was due to the elimination of the less performing communities but not to an increase in the function itself. Applying artificial selection within several independent lineages would prevent the results to be due to ecological sorting (i.e. a simple identification of the best performing communities in an initial pool) and enhance the probability of finding communities that are responsive to the selection.

In the present study, we showed that the diversity of the communities could play a role in the artificial selection procedures. Community richness had an effect on the selected property and influenced the community evolutionary dynamics. Also, we found evidence that it could impact the efficiency of artificial selection, but the trade-off between increasing richness and maintaining variability in composition makes the effect of the initial richness non-linear. Indeed, one of the limitations that can occur when increasing initial community richness from a limited pool of species is the convergence in community composition that may reduce between-community variations for artificial selection to act upon. Once this limitation is avoided, we suggest that applying artificial selection on community varying for their diversity could allow exploring multiple variability/heritability balances. Protocol optimization is still needed for artificial selection of microbial communities to be efficient and, multiple lines of improvement have already been highlighted by recent modelling approaches and experimental studies. Further studies will be needed to disentangle the links between community ecological dynamics and community evolutionary trajectory, which will open the way for effective microbial community and microbiome engineering.

In chapter 2, we aimed at characterizing the evolved communities as compared to the ancestral ones to be able to understand the changes that could occur in microbial communities under the effect of evolution. To go further, we wanted to assess whether the interspecific interactions evolved during our selection procedure or not, to determine the need of considering microbial communities as selection units, since interspecific interactions are an emergent property at the community level. The results are presented in Chapter 3 and will soon be submitted for publication.

CHAPTER 3: THE CENTRAL ROLE OF THE INTERSPECIFIC INTERACTIONS IN THE EVOLUTION *IN* AND *OF* THE MICROBIAL COMMUNITIES

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#These authors supervised the study.

Abstract

The existence of interactions between the species of a community can give rise to emergent properties so that the phenotype of a community can be different from the sum of the phenotypes of the species composing this community. An evolution of a community phenotype can occur through an evolution in the species composing the community but also of the interspecific interactions. In this study, we aimed at investigating how widespread was the evolution of the interspecific interactions in the evolutionary response of a community. Following a five-month experimental evolution of bacterial communities, we investigated the role of the interspecific interactions in the evolutionary responses of eight two-species communities regarding productivity. We found evidence for an evolution of the interactions in half of the studied communities. This evolution gave rise to a change in community productivity that exceeded the change expected from the individual responses by 15%, but not always in the direction of a gain of community productivity. Even when the interactions did not evolve themselves, we found that they influenced the evolutionary responses of the bacterial strains within the communities which further affected community response. By changing the abiotic environment, we found evidence that the evolution within a community often promoted an adaptation of the bacterial strains to the abiotic environment, especially for the dominant strain in a community. Overall, this study suggested that the evolution of the interspecific interactions was frequent and that it could increase community response to evolution. We propose that the existence of an evolution of the interspecific interactions justifies the consideration of the community as a unit of selection.

Key-words: interspecific interactions, experimental evolution, synthetic bacterial communities, productivity, abiotic environment

INTRODUCTION

What makes a community is the existence of interactions between the individuals and species (Liautaud et al. 2019; Whitham et al. 2020). The interactions give rise to emergent properties at the community-level i.e. properties that are not predictable from the sum of the properties of the component species (Guo and Boedicker 2016; Madsen et al. 2018). It is thus relevant to consider the phenotype of a community as a whole, especially since it is increasingly recognized that community phenotype can respond to the evolution (Whitham et al. 2006). For community phenotype to evolve, the communities have to be: discrete, different from one another, able to reproduce and there must be a resemblance between the offspring communities and the parents (Doulcier et al. 2020). Specific ecological conditions can promote the fulfilling of these criteria which is referred to as ecological scaffolding (Black et al. 2020; Doulcier et al. 2020). These specific conditions can be reproduced under laboratory conditions especially when working on microbial communities as they can be easily compartmentalized and sampled to be propagated (Doulcier et al. 2020).

The evolution of community phenotype can involve or not an evolution of the interspecific interactions. From a theoretical standpoint, it is accepted that microbial community evolution can occur through genetic changes in the community members (e.g. through mutations, horizontal gene transfer, gene loss; Barraclough 2015; Gorter et al. 2020). Depending on the authors, changes in the relative abundances of the species within a community are also considered as part of the evolution of the community (Penn and Harvey 2004; Goodnight 2011) or not (Barraclough 2015; Gorter et al. 2020). In the latter case they are referred to as “ecological sorting” (Fiegna et al. 2015a). Furthermore, as well as the interactions are involved in the establishment of community phenotype, there is also evidence that they can contribute to the evolution of community phenotype. This has been investigated in the field of artificial selection at the community level through modelling (Williams and Lenton 2007) and experimental approaches (Goodnight 1990). Both approaches highlighted that genetic changes in the species within a community are not always sufficient to explain the observed response of the community to selection and suggested that the interspecific interactions can be involved in community evolution.

In parallel, other studies aiming at understanding the evolutionary dynamics of microbial communities, especially in response to environmental changes, provided detailed assessments of the evolution of interspecific interactions in synthetic communities. Hansen et al. (2007) showed that, in a two-species bacterial community, a mutation in one of the two strains induced a shift from a commensal interaction to a more exploitative one. This shift in the interaction occurred after five days of experimental evolution and gave rise to an enhanced community productivity. It indicates that the interactions can evolve through the evolution of one of the community members. Another way for the

interactions to evolve is through the evolution of multiple species in a community. In 2012, Lawrence et al. showed that, in a four-species bacterial community, changes in resource use in the four species when experimentally evolved in polyculture reduced the occurrence of negative interspecific interactions. It was associated with a higher productivity at the community level than this of a community which was built from the four species evolved in isolation. Changes in the interactions through the evolution of several species can also result from co-evolution, i.e. reciprocal adaptive changes in two populations or species (Janzen 1980). Coevolution is different from evolution in response to the presence of a species as it involves adaptation and counter-adaptation in the interacting species. In experimental conditions, coevolution can be evidenced either by tracking the evolutionary changes in each of the cocultured species or by comparing the evolutionary responses in treatments where coevolution is allowed or not respectively (Brockhurst and Koskella 2013).

An additional level of complexity emerges from the fact that the evolution of the interspecific interactions can depend on the abiotic environment. In the study of Hansen et al. (2007), the mutation in one strain that caused a shift in the interaction was conditional to the presence of the second strain but also to the growth in a structured environment which allowed the formation of biofilm. The influence of the environment was also highlighted in a study in which 25 different bacterial communities were experimentally evolved for 60 generations in three environments (Fiegna et al. 2015a). The authors discussed the evolution of the interactions by comparing the community responses with the cumulative responses of the corresponding monocultures; they showed that the change in community productivity through experimental evolution could be explained by the changes in the monocultures in two environments over three (i.e. by additive evolution). On the contrary, in the third environment, the change in community productivity could not be explained by additive evolution or ecological sorting which suggested an evolution of the interactions. This evolution gave rise to an increase in productivity in the more diverse communities as compared to the productivity of the corresponding ancestral communities (i.e. before experimental evolution). On the contrary, in the environments where no evolution of the interactions was detected, community productivity stayed similar or decreased during experimental evolution (Fiegna et al. 2015a). To go further, the abiotic environment can be modified by a species which can influence the evolution of other species in a community, this is referred to as niche construction (Matthews et al. 2014). In 2014, Andrade-Domínguez et al. showed that the pairwise interaction between a bacteria and a yeast shifted from commensalism to amensalism and then to antagonism when the environment started to be changed by the excretion of a bacterial growth inhibitor by the yeast. Resistance evolved in the bacterial population which lowered the fitness of the yeast. Thus, eco-evolutionary feedbacks are also involved in the evolution of the interactions and of the communities.

There are many studies that illustrate well the evolution of the interspecific interactions, thus, as pointed out by Gorter et al. (2020) the question is not anymore whether the interactions can evolve but how important is the evolution of the interactions in the communities. In this study, we aimed at

providing an insight into the prevalence of the evolution of the interactions in the evolution of community phenotype. Following a five-month experimental evolution of synthetic bacterial communities, we re-isolated eight pairs of strains that evolved in different communities. We assessed community and community member phenotypes by measuring the optical density as a proxy of productivity. We compared them with the ancestral phenotypes and the phenotypes obtained by assembling the same strains evolved in isolation to discuss the evolution of the interactions. We hypothesized that: *i)* the interspecific interactions played a role in the evolution of community phenotype (i.e. the phenotype of the evolved community would be different from this of a community reconstructed from strains that evolved in isolation); *ii)* this role occurred through an evolution of the interactions themselves (i.e. the evolutionary response of the community is not predictable from the evolutionary responses of the community members); *iii)* the evolution of the community phenotype depended on the environment. To verify this third hypothesis, we assessed the phenotype of the evolved strains and communities in a second environment in order to discuss the adaptation to the abiotic conditions of the environment of the experimental evolution.

MATERIALS AND METHODS

Origin of the studied communities

The eight two-strain communities studied in this experiment stemmed from an experimental evolution procedure in which bacterial strains and communities were grown for five months with a serial transfer each 3.5 days (see Chapter 2). This experiment involved 18 laboratory strains that were used to create communities differing for their initial richness levels (2, 4, 8 or 16 strains, 6 different communities per level, Chapter 2). During the experimental evolution, the strains and communities were grown in sterile 2 ml deep-well plates (Porvair Sciences, UK) filled with 1 ml of a mix of 1:5 lysogeny broth (LB) and 1:5 tryptic soy broth (TSB) and placed at 28°C without shaking. An optical density (OD) measurement was performed at each serial transfer (as a proxy of productivity) and the transfer occurred following two treatments: artificial selection (where the transferred replicate was the one with the highest OD among ten) and no artificial selection (where the replicate was transferred whatever its OD). The strains and communities were stored at -80°C in 30% glycerol before the experimental evolution (ancestors) and after the experimental evolution (evolved strains and communities). In a first step of isolation, all of the communities of richness level 2 (six), both under artificial selection and no artificial selection, were considered for being analysed in the present study. In a second step, all of the communities of richness level 4 (six) either under artificial selection or under no artificial selection were also considered to complete the experimental design. The pairs of strains that were finally included in the experiment are presented in Table 3.1 and responded to the following criteria: successful isolation of the strains from the evolved community and availability of the corresponding strains evolved in isolation.

Isolation of the strains from the evolved communities

To isolate the strains that evolved in communities, we revived the evolved communities from the glycerol stock by growing them on Petri dishes (1:5 LB+TSB with agar) by streaking. After 72h of growth at 28°C, we picked the colonies of differing morphologies and placed them on new separated Petri dishes by streaking. After a new cycle of growth, one colony per plate was picked, placed in 200 µl of 0.9% NaCl and 100 µl of this suspension were plated on a Petri dish with glass beads. At this step, 2 µl of suspension were used to perform a PCR for the identification of the strains (see below). After a new cycle of growth, several colonies were picked on each plate and put in 20 ml of medium in a flask (48h, 120 rpm). 800 µl of suspension were then stored at -80°C in 800 µl of 30% glycerol. As these isolation steps required four growth cycles during which evolution could act, we also performed these four growth cycles in the same conditions for the corresponding ancestral and evolved in isolation strains.

Table 3.1 Two-strain communities studied in the experiment. Some of the pairs of strains evolved in the absence of other strains (i.e. in two-strain native communities), whereas other pairs evolved in the presence of other strains (i.e. in four-strain native communities), this is specified into the column "Initial richness level of the native community". Some of the native communities evolved under artificial selection whereas others evolved under "no artificial selection" (i.e. natural selection only), this is specified in the column "Selection regime applied to the native community". In each community, strain 1 is the most productive of the two strains (highest OD) and strain 2 is the least productive one (lowest OD).

Community identifier	Strains	Initial richness level of the native community	Selection regime applied to the native community
Community A	1 *Variovorax* sp. 38R 2 *Pseudopedobacter saltens* DSM12145	2 strains	No artificial selection
Community B	1 *Variovorax* sp. 38R 2 *Pseudopedobacter saltens* DSM12145	4 strains	No artificial selection
Community C	1 *Pseudomonas knackmussii* DSM6978 2 *Variovorax* sp. *38R*	4 strains	No artificial selection
Community D	1 *Pseudomonas* sp. ADPe 2 *Escherichia coli* WA803	2 strains	No artificial selection
Community E	1 *Pseudomonas knackmussii* DSM6978 2 *Pseudopedobacter saltens* DSM12145	4 strains	No artificial selection
Community F	1 *Pseudomonas* sp. ADP3 2 *Escherichia coli* K12	4 strains	No artificial selection
Community G	1 *Escherichia coli* WA803 2 *Agrobacterium* sp. 9023	4 strains	Artificial selection
Community H	1 *Pseudomonas* sp. ADPe 2 *Escherichia coli* WA803	2 strains	Artificial selection

Identification of the strains

A PCR targeting 16S rRNA gene with the primers 27F/1492R (Miller et al. 2013) was performed for each strain isolated from the evolved communities. A digestion of the PCR products was then performed with the AluI restriction enzyme and followed by an electrophoresis for identification of the strains at the genus level. For the genera that were represented by several strains in our experiment (i.e. *Pseudomonas* and *Escherichia*), we performed further analyses for an identification at the strain level. We used data from *gyrB* sequencing at the community level to determine which *Pseudomonas* strain was present in the community and coupled it with analyses at the strain level for formal identification. The different strains were identified based on the presence or not of atzD gene (assessed by PCR) and the resistance or not to nalidixic acid and amoxicillin (assessed by growing the strains on Petri dishes containing a mix of the two antibiotics at a final concentration of 100 $\mu g.ml^{-1}$). *Escherichia coli* K12 and *Escherichia coli* WA803 were identified based on their ability to do or not lactose fermentation (which was assessed by growing the strains on Petri dishes on Drigalski agar medium).

Evolutionary history treatments

For each pair of strains (eight in total, hereafter identified as communities A to H; Table 3.1), eleven phenotypes were characterized by OD measurement in two different environments. Each of the two strains of a community was grown in its ancestral version (i.e. before experimental evolution), in its "evolved in isolation" version (i.e. after experimental evolution as an isolated strain) and in its "evolved in community" version. It resulted in six treatments (two strains and three evolutionary histories per strain; Figure 3.1a). Within each community, the most productive (highest OD at 3.5 days) of the two ancestral strains was referred to as "strain 1" and the least productive of the two strains was referred to as "strain 2". In addition to the assessment of the different strains' phenotypes, the community phenotype was also characterized (i.e. the OD of co-cultures). Each community was grown in its ancestral version (i.e. mix of the two ancestral

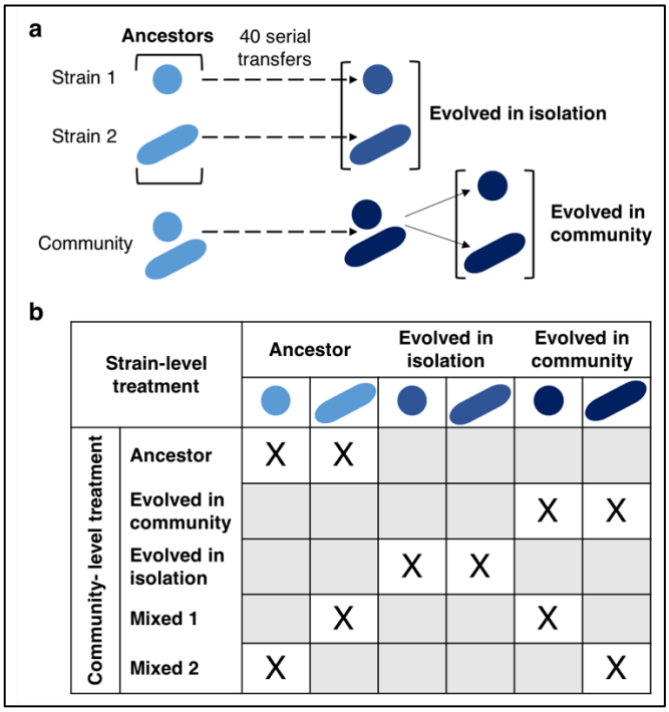

Figure 3.1 Experimental design. a) Each bacterial strain was previously experimentally evolved in isolation and as a member of a community (Chapter 2). At the end of this experimental evolution, the strains were isolated from the community in which they evolved. **b)** From the strains, different communities were built: ancestor (co-culture of two ancestral strains), evolved in community (co-culture of two strains that evolved together), evolved in isolation (co-culture of two strains that evolved in isolation), mixed 1 (co-culture of one ancestral strain and one strain evolved in community) and mixed 2 (co-culture of one ancestral strain and one strain evolved in community conversely to mixed 1).

strains), in its "evolved in isolation" version (i.e. mix of the two strains that evolved in isolation) and in its "evolved in community" version (i.e. mix of the two strains that evolved together in a community). Two communities mixing ancestral strains and strains evolved in community were also included: mixed community 1 (i.e. mix of strain 1 evolved in community and ancestral strain 2) and mixed community 2 (i.e. mix of strain 2 evolved in community and ancestral strain 1). It resulted in five treatments at the community level plus the six treatments at the strain level (Figure 3.1b).

Community construction, growth conditions and phenotype assessment

Before the start of the experiment, each strain was revived from the glycerol stock and grown in 20 ml of 1:5 LB+TSB in a flask (48h, 28°C, 110 rpm). The OD of the suspensions was measured (Infinite M200 PRO, Tecan, Switzerland) and the suspensions were diluted to a final OD of 0.002 in 1:5 LB+TSB. The eight two-strain communities were built by mixing an equivalent volume of each of the suspensions of the required strains. Then, two plates per community were inoculated with the suspensions at OD 0.002: a 2 ml deep-well plate (Porvair Sciences, UK; 1 ml of suspension per well, eight replicates per treatment) and a honeycomb plate (Thermo Scientific, USA; 400 µl of suspension per well, eight replicates per treatment). The growth conditions in deep-well plates were: 28°C, no shaking; the OD was measured after 3.5 days of growth by homogenising the well content, pipetting 200 µl of suspension and transferring it into a new plate for OD measurement at 600 nm (Infinite M200 PRO, Tecan, Switzerland). The growth conditions in honeycomb plates were: 28°C, 15 s of shaking 5 s before each OD measurement (600 nm), one measurement every 30 min. The growth conditions in deep-well plates corresponded to the growth conditions of the experimental evolution, hereafter we refer to these conditions as "environment 1". The growth conditions in honeycomb plates were different from these of the experimental evolution, hereafter we refer to these conditions as "environment 2".

Statistical analyses

The OD after 3.5 days of growth was analysed in two steps with two linear mixed models. The following model was used to analyse the effect of the evolution on strain and community phenotypes:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + I_l + E_{ijkl}$$

Y_{ijkl} is the OD of the biological entity *i* (three levels: strain 1, strain 2, community), of identity *l* (24 levels: strain or community identity), of evolutionary history *j* (three levels: ancestor, evolved in isolation, evolved in community), in environment *k* (two levels: environment 1, environment 2). μ is the intercept, α_i is the effect of the biological entity, β_j is the effect of the evolutionary history, γ_k is the effect of the environment. The interaction effects between *i)* the biological entity and the evolutionary history $(\alpha\beta)_{ij}$; *ii)* the biological entity and the environment $(\alpha\gamma)_{ik}$; *iii)* the evolutionary history and the environment $(\beta\gamma)_{jk}$; *iv)* the biological entity, the evolutionary history and the

environment $(\alpha\beta\gamma)_{ijk}$ were also included in the model. I_l is the random effect of the strain or community identity, E_{ijkl} is the residual error.

A second linear mixed model was built to analyse the effect of the evolutionary history of the community members on the community phenotype:

$$Y_{jkl} = \mu + \beta_j + \gamma_k + (\beta\gamma)_{jk} + I_l + E_{jkl}$$

Y_{jkl} is the OD of the community of identity *l* (8 levels: A to H), of evolutionary history *j* (five levels: ancestor, evolved in isolation, evolved in community, mixed 1, mixed 2), in environment *k* (two levels: environment 1, environment 2). μ is the intercept, β_j is the effect of the evolutionary history, γ_k is the effect of the environment, $(\beta\gamma)_{jk}$ is the effect of the interaction between the evolutionary history and the environment. I_l is the random effect of the community identity, E_{jkl} is the residual error.

To go into the details of the responses of and within each community, the OD after 3.5 days was then analysed with a linear model that included the identity of the individual as a fixed effect factor as well as the evolutionary history and the interaction between the identity and the evolutionary history. One model was built for the strains and one for the communities in both environments. The predictability of the community evolutionary response was analysed by comparing the observed difference in OD between the evolved community and the ancestral community to *i)* the observed difference in OD between strain 1 evolved in community and the ancestral strain 1, *ii)* the observed difference in OD between strain 2 evolved in community and the ancestral strain 2 and *iii)* the sum of the two previously mentioned differences (i.e. the sum of the evolutionary responses of strains 1 and 2). The mean responses and the corresponding 95% confidence intervals were obtained by bootstrapping (1,000 iterations of the calculation of the response from randomly sampled values with replacement).

All the analyses were performed with R software version 3.6.3 with lmerTest package for linear mixed models (Kuznetsova et al. 2017), car package for type II analyses of variance (Fox and Weisberg 2019) and emmeans package for pairwise comparisons (Lenth 2021).

RESULTS

The individual response is driven by the initial productivity in monoculture

The effect of the evolutionary history on OD depended on the biological entity, i.e. whether the considered phenotype was this of the community or of the community members, and it also depended on the environment (biological entity*history*environment: χ^2=48; $p_{df=4}$=1.0x10^{-9}; Table S3.1). Strain 1 and strain 2, the initially most and least productive strain respectively, responded differently to the evolution in environment 1. The OD of strain 1 when evolved in community tended to be higher than this of strain 1 as an ancestor and was higher than strain 1 evolved in isolation (respectively 0.677±0.177, 0.615±0.120, 0.548±0.224 UDO; Figure 3.2). On the contrary, strain 2 showed a higher

OD when evolved in isolation as compared to evolved in community (0.367±0.175 and 0.299±0.123 respectively).

Community response is driven by the most productive strain

In environment 1, the OD of the communities composed of strains that evolved together was not significantly different than the OD of the ancestral communities (respectively 0.649±0.178 and 0.630±0.129; Figure 3.2). But, it was higher than the OD of the communities in which the members evolved in isolation (0.467±0.146) suggesting that the evolution in community (i.e. co-culture) did not produce the same outcome than an evolution in isolation. The OD of the community with members evolved in community was similar to the OD of the communities that included a strain that evolved in community and an ancestral strain (mixed 1 and 2, Figure 3.3). It indicated that the presence of one evolved strain in the community was sufficient to express the "evolved" community phenotype.

The OD of the community was not different from the OD of strain 1 whatever the evolutionary history (respectively 0.611±0.162 and 0.617±0.183 on average; Figure 3.2). Also, the response of the community to the evolutionary history was similar to this of strain 1 (i.e. trend to increase in OD with an evolution in community as compared to the ancestor and trend to decrease in OD with an evolution in isolation; Figure 3.2). Thus, community phenotype seemed to be driven by strain 1.

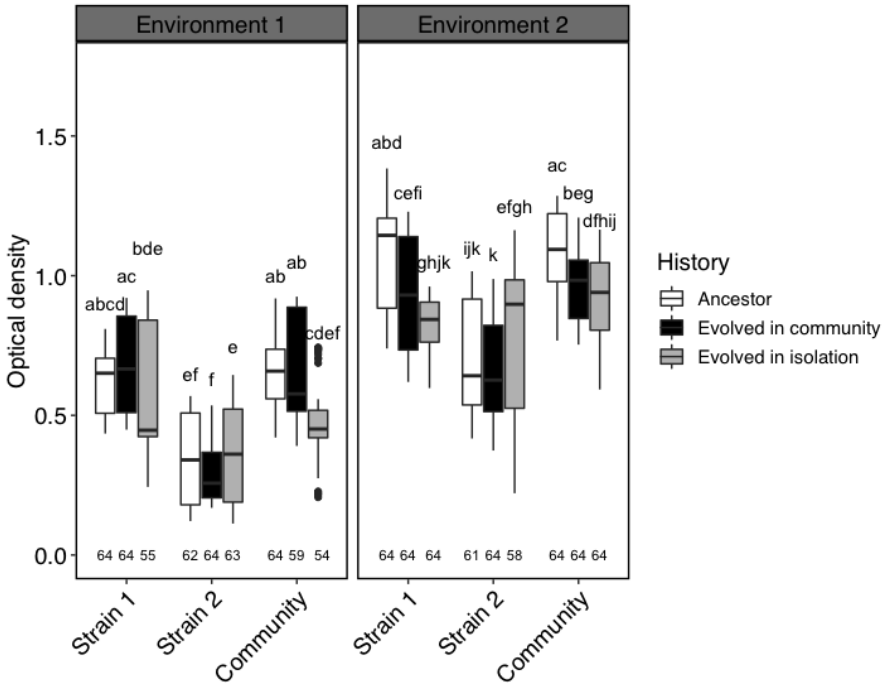

Figure 3.2 Optical density of the community and the community members depending on the evolutionary history and the environment. Environment 1: identical growth conditions to the experimental evolution; Environment 2: different growth conditions from the experimental evolution. Strain 1 is the most productive of the two strains in a given community (highest OD) and strain 2 is the least productive of the two strains (lowest OD). Community refers to the co-culture of strain 1 and strain 2. Different letters represent significant differences in OD within a given environment. Sample sizes are given on the bottom of the graphs.

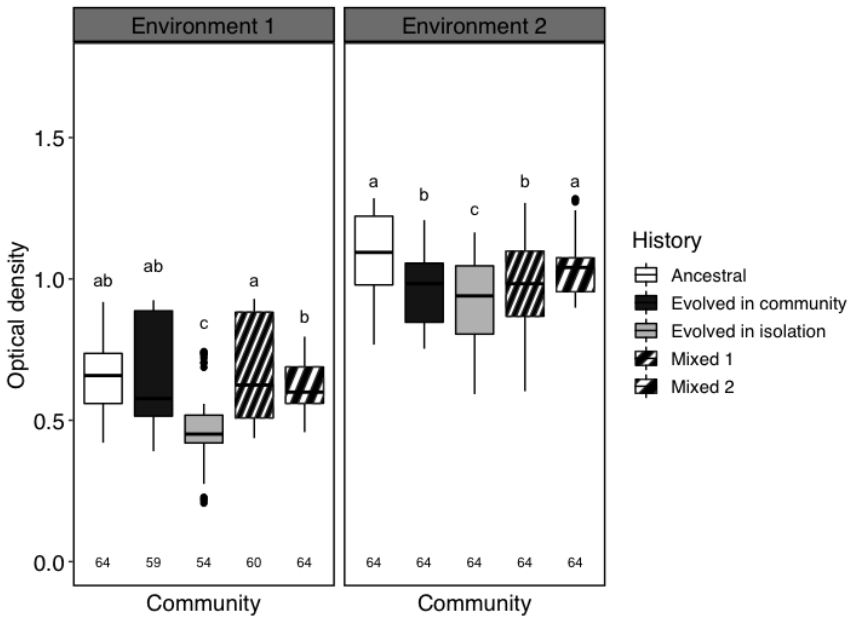

Figure 3.3 Optical density of the communities depending on the evolutionary history of their members and the environment. Environment 1: identical growth conditions to the experimental evolution; Environment 2: different growth conditions from the experimental evolution. Community refers to the co-culture of strain 1 and strain 2. In mixed community 1, the strain 1 evolved in community was grown with the ancestral strain 2. In mixed community 2, the ancestral strain 1 was grown with the strain 2 evolved in community. Different letters represent significant differences in OD within a given environment. Sample sizes are given on the bottom of the graphs.

Community response involves an evolution of the interactions in half of the cases

For all of the studied communities, there was a significant difference in OD between the evolved in community and evolved in isolation treatments (Figure 3.4) which highlighted the importance of the interactions in the evolution of community phenotype. This difference was in favour of the evolved in community treatment in seven of the eight communities (Figure 3.4). One evolved community showed no difference in OD as compared to the ancestral community (community G; Figure 3.4). Three communities showed differences in OD with the communities of all other evolutionary histories (communities A, C and F). It indicated that the only way to obtain the evolved community phenotype was through the presence of the two strains in their evolved in community version. The four remaining communities (B, D, E and H) showed no difference in OD as compared to at least the mixed community 1 (Figure 3.4) highlighting the role of strain 1 in the expression of the evolved community phenotype in these cases.

The evolutionary response of the community was predictable neither from the responses of the community members nor from the sum of their responses in four communities (A, C, F and H; Figure 3.5) which suggested that the evolutionary response involved an evolution of the interactions. In communities D and E, the community response was predictable from the response of strain 1 and in community B, it was predictable from the sum of the responses of the two strains (Figure 3.5). It suggested that the interactions did not evolve in these communities.

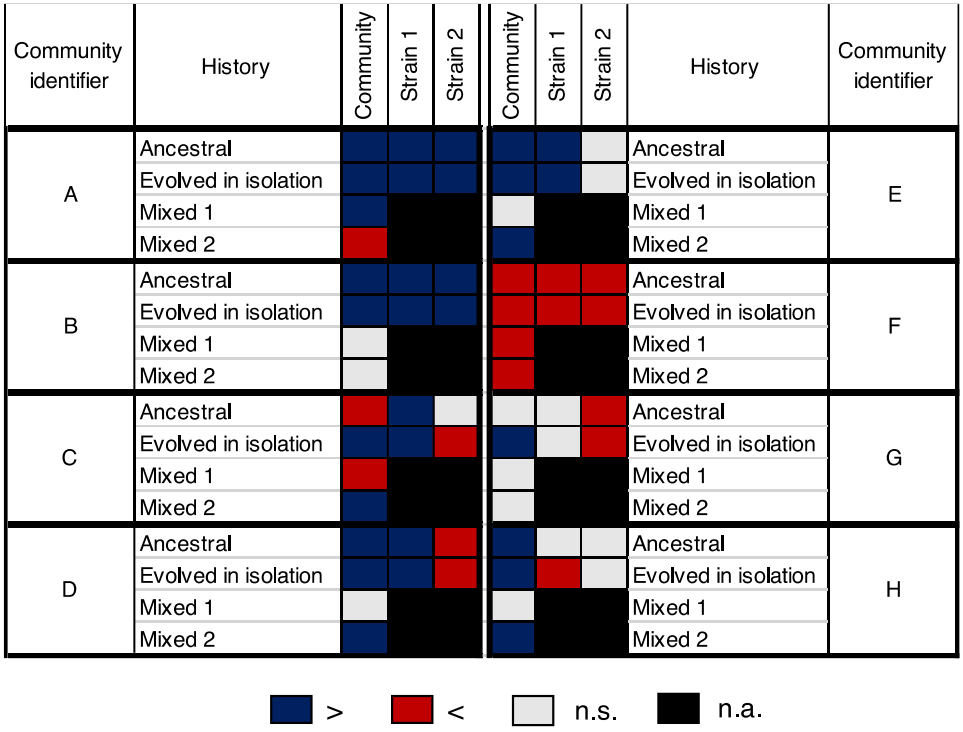

Figure 3.4 Effect of an evolution in community on optical density in environment 1 depending on the community. The OD of a community composed of strains that evolved together (in columns) is compared to the OD of a community including ancestral strains, strains evolved in isolation or one ancestral strain and one strain evolved in community (mixed 1 and mixed 2) (in lines). The OD of strains 1 and 2 evolved in community (in columns) is compared to the OD of the corresponding strain as an ancestor or evolved in isolation (in lines). Blue: significantly higher. Red: significantly lower. Light grey: no significant difference (α=0.05). Black: not applicable.

The robustness to an environmental change depends on the response

In environment 2, where the conditions differed from these of the experimental evolution, strain 2 showed a similar response to the evolutionary history than in environment 1 (Figure 3.2). On the contrary, the responses of strain 1 and of the community changed: the highest OD was observed for the ancestors followed by evolved in community and by evolved in isolation treatments. The expression of the "evolved phenotype" thus depended on the abiotic environment. As in environment 1, community phenotype and community response to the evolutionary history were similar to strain 1 (Figure 3.2). And, the OD of the mixed community 1 was similar to this of the community in which the strains evolved together (respectively 0.969±0.197 and 0.971±0.134) whereas mixed community 2 showed a higher OD that did not differ from this of the ancestral community (Figure 3.3). Thus, as in environment 1, the presence of one strain – here, strain 1 - in its evolved version was sufficient to observe the "evolved" community phenotype.

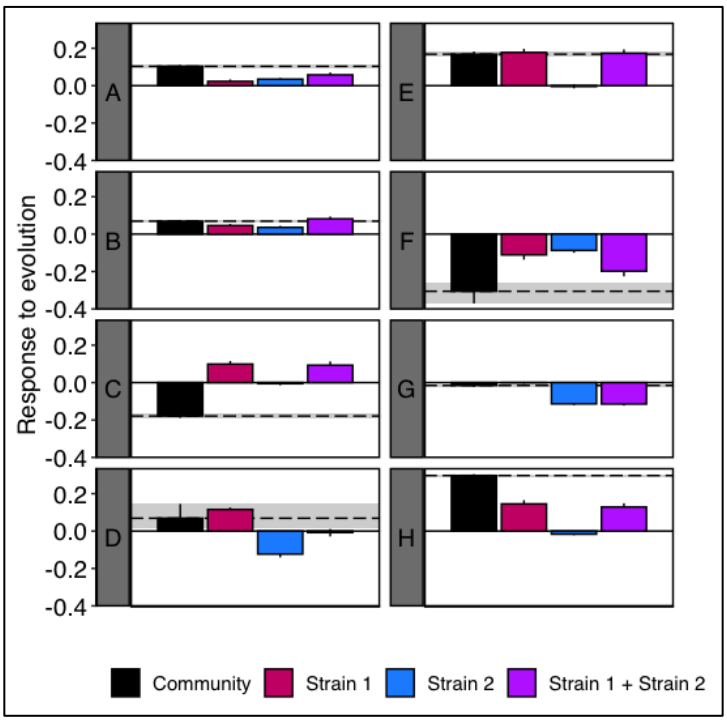

Figure 3.5 Predictability of the evolutionary response of the community. The observed evolutionary responses of the community, strain 1 and strain 2 in environment 1 were expressed as the difference in optical density as compared to the corresponding ancestor (i.e. ancestral community or ancestral strain 1 or ancestral strain 2 in environment 1). "Strain 1 + Strain 2" refers to the expected response to evolution that was obtained from summing the observed responses of strain 1 and strain 2. Bars represent 95% CI. On each graph, the black dashed line represents the mean value of the response of the community and the grey area represents the associated 95% CI. Means and 95% CI were obtained by bootstrapping.

Going into the details of the responses of the different communities, some of the changes in the strain and community phenotypes were consistently observed whatever the environment whereas others were not detectable or occurred in the opposite direction when the environment changed (Figure 3.6a). The phenotypic change in response to evolution in the evolved community (i.e. change in OD as compared to the ancestral community or the community with evolved in isolation members) was maintained in environment 2 for three communities over eight (A, C and F; Figure 3.6b). When a strain that evolved in community showed a significant increase in OD as compared to the ancestor, this pattern was always lost when the environment changed (Figure 3.6c and d). On the contrary, when a strain that evolved in community showed a significant decrease in OD as compared to the ancestor, this pattern was maintained in environment 2 in three cases over four. The changes in OD in the strain that evolved in community as compared to the corresponding strain evolved in isolation were maintained in environment 2 in nine cases over 13 (Figure 3.6c and d).

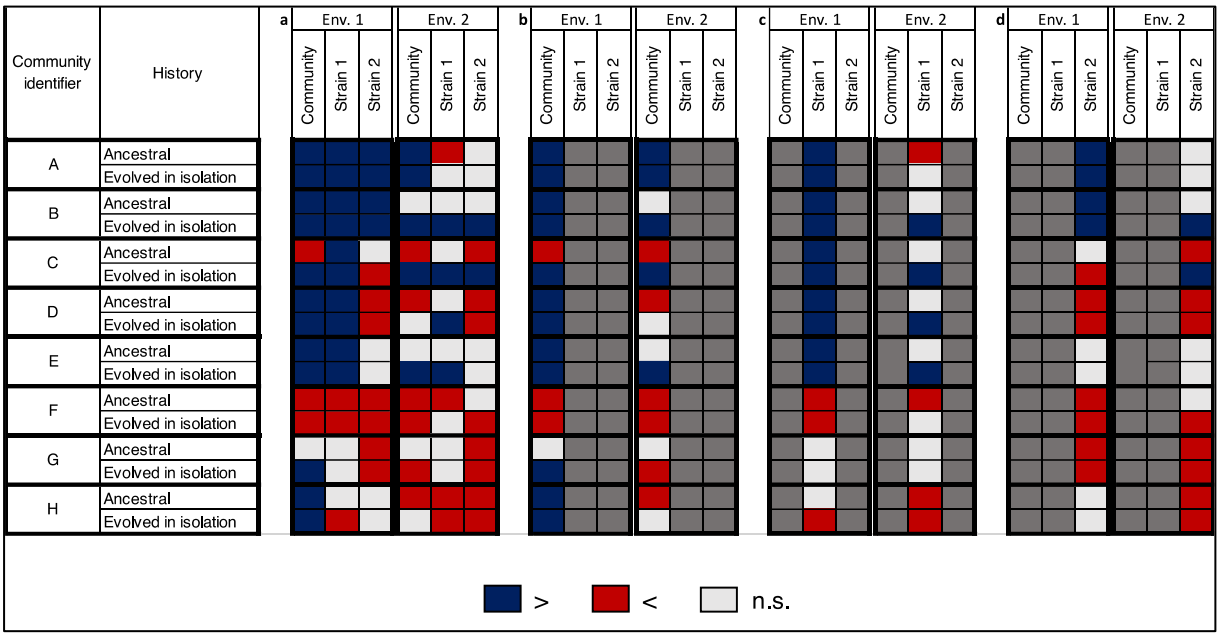

Figure 3.6 Effect of the environment on the expression of the evolved phenotype. The OD of a community composed of strains that evolved together was compared to the OD of a community including ancestral strains or strains evolved in isolation. The OD of strains 1 and 2 evolved in community is compared to the OD of the corresponding strains as ancestors or evolved in isolation. The results are presented for both environments. Environment 1: identical growth conditions to the experimental evolution; Environment 2: different growth conditions from the experimental evolution. Blue: significantly higher. Red: significantly lower. Light grey: no significant difference (α=0.05). The overall results are shown on panel a, and panels b, c, and d show the results of the community, strain 1 and strain 2 respectively. On those panels, only the comparisons of interest are shown, and the others are shaded in dark grey for readability.

DISCUSSION

We showed that the evolution of the strains in a community was influenced by the interspecific interactions. Indeed, an evolution in isolation never produced the same phenotype as an evolution in community (Figure 3.2). These results are in accordance with an increasing body of literature that highlights the effect of the biotic context, i.e. of the evolution within a community, on the evolutionary response of the community members. As an example, the occurrence and the maintenance of a mutation in a species can be conditional to the presence of another species (Hansen et al. 2007). To go further, several studies showed that the diversity of the community in which a species evolved influenced the evolutionary response of this species (Fiegna et al. 2015a; Jousset et al. 2016; Scheuerl et al. 2020). There is evidence that increasing community diversity can reduce the response of a species to evolution (Scheuerl et al. 2020) or the fitness of this species (Jousset et al. 2016). In Fiegna et al. (2015), the productivity of species that were isolated from the community in which they evolved decreased with the increase of the diversity of the community. Our experiment included two communities (A and B) that were identical in their composition (*Variovorax* sp. 38R and *Pseudopedobacter saltens* DSM12145) but that originated from two different communities: one community in which they evolved together only and one community in which they evolved with two other strains. In environment 1, there was no indication that this difference had an effect on the strain evolution. But, in environment 2,

Pseudopedobacter saltens DSM12145 had a much shorter lag phase when evolved in the community with *Variovorax* sp. 38R only (8.2 h 95% CI [7.9; 8.5]) than in all other conditions (32.2 h [32.0; 32.4] as an ancestor, 30.3 h [30.0; 30.6] when evolved in isolation, 22.9 h [22.7; 23.0] when evolved in the four-strain community; Figure S3.1). As pointed out by the study of Scheuerl et al. (2020), the evolutionary response of a strain would depend on the properties of the strain itself, on the properties of the community in which the strain evolves and on the interactions between these two components. Together, these studies highlight the importance of the biotic context on the evolutionary responses of the species.

The characterization of the community members on the basis of their productivity before experimental evolution allowed a good explanation of their responses to evolution, despite the fact that we grouped species from different genera under the entities "strain 1" and "strain 2" (model R^2=0.85; Table S3.1). Overall, we found that the most productive strain had a dominant role in explaining community phenotype and community response to evolution (Figure 3.2). It was probably highly linked to the fact that the studied community phenotype was productivity but, it also suggested that the most productive strain in monoculture was also the dominant strain in the community. From a theoretical standpoint, it is expected that the evolutionary rate of a species would be higher when its generation time is short and when its population size is high (Barraclough 2015), which are characteristics that can be associated to the dominant species in a community. In a recent study, Meroz et al. (2021) showed that the dominance of a bacterial species in a two-species community could be predicted from the initial growth rate and productivity of the species in monoculture. However, this was true at the beginning of the experiment but not at the end of the experimental evolution (after 400 bacterial generations). Together, these results suggest that the evolutionary rate of a species, and thus its importance in community evolution, could be predicted according to its frequency in the community but that the changes in this frequency over time would prevent the prediction of the responses at large timescales. In our study, we had evidence that the most productive strain as an ancestor dominated the evolved community, however, we did not investigate the relative abundances of the strains in the communities. This could be of interest as, even if we built the communities from an equivalent ratio of the two strains, we could expect differences in the final ratios after one cycle of growth between an ancestral and an evolved community under the effect of an evolution of the interactions for example.

We showed that the evolution of community phenotype, i.e. productivity, was dependent on the interspecific interactions. Indeed, as in the study of Lawrence et al. (2012), the phenotype of the evolved community could not be obtained by reconstructing a community from strains that evolved in isolation (Figures 3.2 and 3.4). We observed an effect of the interactions on community evolutionary response in all of the communities that showed an evolution in their phenotypes, i.e. seven among the eight (Figure 3.4, except G). However, this effect of the interactions depended on the studied community and occurred through three different ways. Community phenotype evolved through *i)* an evolutionary response of one strain conditionally to the presence of the second strain without evolution of the

interaction (communities D and E), *ii)* an evolutionary response of the two strains conditionally to their respective presence without evolution of the interaction (B), *iii)* an evolution of the interaction itself under the influence of one (H) or of the two strains (A, C and F; Figure 3.4 and 3.5). Thus, the evolution of the community phenotype involved an evolution of the interactions in more than half of the cases. It suggested that the implication of the evolution of the interactions in the evolution of community phenotype is not rare in experimental evolution of microbial communities. With a modelling approach, Williams and Lenton (2007) estimated that the responses of ecosystems to evolution under artificial selection would involve an evolution of the interspecific interactions in 4% of cases when targeting an increase in a property and in 38% of the cases when targeting a decrease in a property (this could be modulated by specific experimental choices). More recently, Fiegna et al. (2015a) showed that, the evolution of the productivity of beech tree bacterial communities was explained by ecological sorting at 0.35%, by additive evolution at 17.7% and by the evolution of the interspecific interactions at 14.3%. It is not straightforward to estimate the importance of the interspecific interactions in community evolutionary dynamics as their role seems to be highly dependent on the studied community but together, these results suggest that it is relevant to consider the evolution of the interactions when studying community dynamics, at least in laboratory experiments.

In the communities in which an evolution of the interspecific interactions was detected, the change in community productivity was higher than expected but the direction of this change was community-dependent. The response to evolution when the interactions evolved (i.e. in communities A, C, F and H) gave rise to a mean change in productivity of 34.6±12.9% i.e. +15.4±6.74% as compared to what was expected from the individual responses. However, in two communities over four (C and F) this change was negative (i.e. the productivity of the evolved community was lower than this of the ancestral community) and in one case it occurred whereas the sum of the individual responses was positive (C; Figure 3.5). In the other studies that reported an evolution of the interactions, the effect was to enhance community productivity (Hansen et al. 2007; Lawrence et al. 2012; Fiegna et al. 2015a). Furthermore, some authors registered a reduction of the negative interactions and the evolution of positive ones (Lawrence et al. 2012). In our study, we did not characterize the interactions, but we can hypothesize that different types of interactions led to different responses of the community phenotype to the evolution of the interactions.

The influence of the abiotic environment on the evolutionary responses of the communities and community members was community-dependent. For three of the four communities in which an evolution of the interactions was detected, the response to evolution was consistently observed in the two environments (communities A, C and F; Figure 3.6b) contrary to what was observed for the strains composing these communities (Figure 3.6c and d). A possible explanation would be that the evolutionary responses of the strains involved an adaptation to the abiotic component (so that the response is not consistently observed when changing the environment) but that the expression of the "evolved" interaction did not rely on the abiotic component or, as exposed by Hillesland and Stahl

(2010), relied on a condition that is found in the two environments. Previous studies have shown the importance of the resources on the outcome of the evolution of the interactions (Lawrence et al. 2012; Rivett et al. 2016). As the same culture medium was used in the two environments in our experiment, it could suggest that the evolution of the interactions implied modifications in resource sharing. Our results also suggested that the evolution in community often promoted an adaptation of the strains to the abiotic component, especially in strain 1 (Figure 3.6c and d). This is not expected since the theory predicts that there are trade-offs between the adaptation to the abiotic and to the biotic components (Lawrence et al. 2012; Barraclough 2015) and, that biotic forces are dominant over abiotic forces in driving species evolution (Red Queen hypothesis; Brockhurst et al. 2014). Thus, it is expected that strains that evolved in isolation would show a better adaptation to the abiotic environment than strains that evolved in community. It has been observed experimentally (Lawrence et al. 2012; Castledine et al. 2020) but seemed to be strain-dependent. In our study, interspecific interactions could have promoted the evolutionary responses to the abiotic conditions, which can occur through competition for example (Barraclough 2015). These results may be linked to the structure of the environment. Indeed, Gorter et al. (2020) proposed that in homogeneous environments, the evolution would act through the selection of traits that are directly beneficial for the carrier species. Thus, in some cases, the more efficient way for a strain to adapt to the presence of another strain could be through an adaptation to the abiotic conditions.

In this study, we aimed at investigating the importance of the evolution of the interactions in community evolution. There was evidence for an evolution of the interactions in half of the studied communities. Moreover, we highlighted that, even when they did not evolve themselves, the interactions influenced the evolution of both community phenotype and community members' phenotype. This shed light on the interplay between community ecology and community evolution which is further complexified by a dependence of the evolutionary response to the abiotic environment. Our results also suggested that the evolutionary response of a species as well as its role in the evolutionary response of the community could depend on its dominance in the community. The present study included eight communities, eight bacterial strains belonging to five genera and focused on pairwise interactions only. Further studies involving higher levels of community complexity are needed to investigate how widespread is the importance of interspecific interactions in community evolutionary dynamics. To conclude, our results suggested that the communities in which the interspecific interactions evolved were more likely to be independent on the abiotic environment to express the evolved community phenotype. This is of particular interest in the field of the artificial selection at the community level and its possible applications and it shed light on the possible relevance of considering the communities as selection units.

DISCUSSION AND FUTURE PROSPECTS

Through two artificial selection experiments at the community-level and one experimental study on the evolution of interspecific interactions, we brought to light several lines of improvement for artificial selection procedures and multiple links between community ecology and community evolution. We will discuss these two points in the following sections.

I. IMPROVING COMMUNITY-LEVEL ARTIFICIAL SELECTION

1. Avoiding the lack of resemblance between parents and offspring

In the two artificial selection experiments that we conducted (Chapter 1 and Chapter 2), we had difficulties to record a *constant* improvement in the targeted function over time. Along with what has been highlighted in the literature, we can identify several factors that limited our ability to apply an efficient selection. First of all, as often in artificial selection experiments at the community level, we observed erratic variations in the mean value of the targeted function from one selection cycle to the other. It means that the parental phenotype is not reliably re-established by the offspring communities. This absence of resemblance can result from intrinsic characteristics of the communities and/or from extrinsic factors linked to the experimental conditions. Indeed, a phenotype is the result of genetic factors and of the environment (and of the covariation and the interaction between the two; Visscher et al. 2008). What is heritable (in the broad-sense) is the fraction of the phenotypic variance that is due to the variance in the genetic factors (Visscher et al. 2008). In other words, the phenotypic variation among the potential parents (i.e. the selection units) has to be due to the species composing the community (their genes, their relative abundances and their interactions; Goodnight 2000) for it to be transmitted. If the phenotypic variation is due to extrinsic factors, for example to spatial ***variations in the environmental conditions***, the best performing unit would be this that have the most favourable conditions so that the variation is not heritable (Figure 4.1a). Such a lack of heritability could also occur if the variation in the phenotype is due to a ***measurement error*** (Xie et al. 2019). Variations in the environmental conditions would not necessarily affect the heritable determinants of the targeted phenotype. However, they can still affect the parent/offspring resemblance as illustrated in Figure 4.1b and create ***experimental noise***, this could be the case of temporal variations between the selection cycles for example. This experimental noise, sometimes called “batch effect”, can prevent us to register a global trend (= “signal” of the response to artificial selection). One way to detect a trend despite the noise is to increase the timescale at which the study is conducted as for the assessment of global change in temperatures for example (the ratio signal to noise increases with the increase of the studied timescale; Santer et al. 2011). It is more likely that an influence of the artificial selection, if there is one, will be registered when the number of cycles is large enough for the experimental noise not to be a problem. If the heritability in the parents is high (i.e. between-parent variation in the phenotype is due

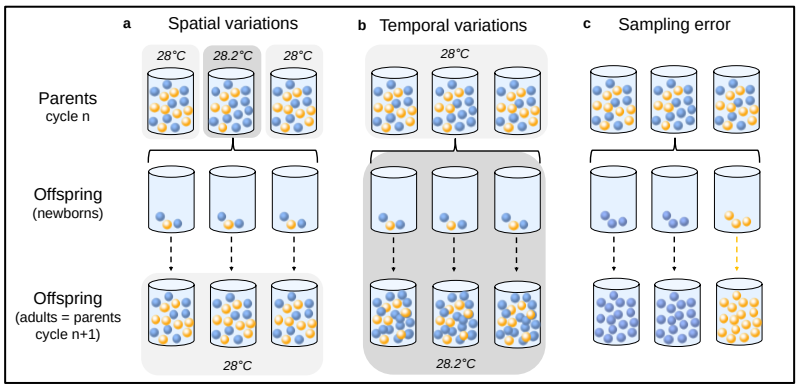

Figure 4.1 Schematic representation of extrinsic factors that can affect the community parent/offspring resemblance. The selection units are two-species communities. Different species are represented by different sphere colours. In this example, the targeted phenotype requires the presence of the two species and is allowed by a ratio biased in favour of the blue one so that the parental unit that is chosen to reproduce (in the middle) is the one that has more blue spheres than yellow ones. **a)** A difference in temperature between the selection units of a given generation is responsible for the expression of the targeted phenotype so that it is not heritable. **b)** The targeted phenotype is expressed by the offspring but at a different level due to temporal variations in the environmental conditions between the selection cycles. **c)** A sampling error induces the loss of one of the species so that the targeted phenotype is lost. Different colours of arrows represent different community dynamics.

to heritable determinants) and if the experimental conditions are stable, the offspring should resemble their parents, unless the determinants of the phenotype are not reliably transmitted by the experimenter. The reproduction of the parental communities involves a sampling step so that a ***sampling error*** can modify the composition of the offspring communities as compared to their parents (e.g. through the loss of a species; Figure 4.1c). This issue could be addressed through the use of an accurate method of sampling and inoculation i.e. pipetting could be replaced by cell sorting (Xie et al. 2019).

Beyond the issues brought by extrinsic factors, a lack of resemblance between the offspring communities and the parental ones could be the result of the community ecological and evolutionary dynamics (= intrinsic factors). Indeed, the need for stability in the microbial community composition and structure has been evidenced by several authors (Penn 2003; Penn and Harvey 2004; Jacquiod et al. 2021; Chang et al. 2021). According to Chang et al. (2021), the communities have to be "generationally stable" for the offspring to resemble their parents. It means that the ecological succession has to be identical from one cycle to the other so that the new generation of communities would have the same final composition than the parental community. A modelling approach has shown that this could be promoted by conducting several cycles of growth without selection prior to the beginning of the selection process in itself Chang et al. (2021). It has been confirmed experimentally by Jacquiod et al. (2021) who registered a stabilization of the bacterial community structure five cycles after the beginning of their experiment. Even when the stabilization phase is conducted, intrinsic factors related to the community functioning can disturb the offspring/parent resemblance. First, Swenson et al. (2000b) have suggested that a lack of resemblance between the offspring and parents could occur through the ***butterfly effect***. Indeed, complex interactions in the communities could lead to the amplification of very small initial differences among the offspring communities (Figure 4.2a). These small differences are thought to arise through sampling errors (Swenson et al. 2000b) so that, preventing them would also limit the occurrence of the butterfly effect. Beyond the sensitivity of the community

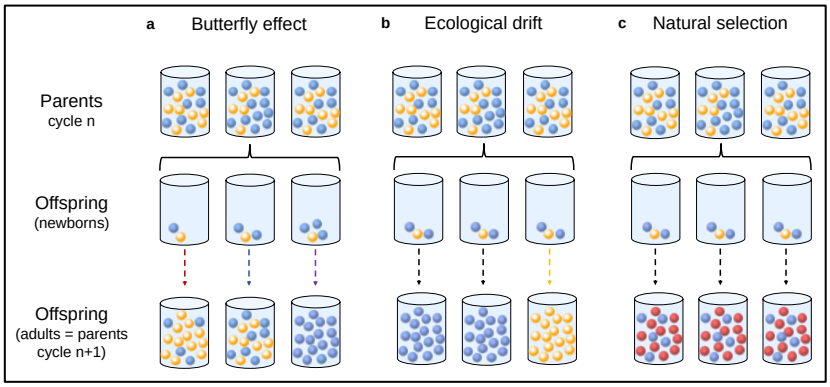

Figure 4.2 Schematic representation of intrinsic factors that can affect the community parent/offspring resemblance. The selection units are two-species communities. Different species are represented by different sphere colours. In this example, the targeted phenotype requires the presence of the two species and is allowed by a ratio biased in favour of the blue one so that the parental unit that is chosen to reproduce (in the middle) is the one that has more blue spheres than yellow ones. **a)** Small differences in the newborn communities are amplified by the complex dynamics of the community. **b)** The small size of the newborn community makes it highly sensitive to ecological drift and one species is stochastically lost. **c)** A mutation occurs in the "yellow species" and is naturally selected for at the individual level as the "red mutant" performs better than the individuals of the "blue species". Different colours of arrows represent different community dynamics.

to small variations in the initial conditions, the sampling step when creating an offspring generation could also play a role whatever the accuracy of the sampling method. Indeed, as well as populations are subjected to genetic drift (i.e. stochastic changes in the frequencies of the alleles that are not under selection; Kimura 1983), communities are subjected to ***ecological drift*** i.e. the stochastic fluctuation of species frequencies (Gilbert and Levine 2017). The lower the community size, the higher the expected effect of drift. Thus, a low sampling size (and more generally a low community size) will be associated with a high probability of changes in the species frequencies and potentially of the loss of the parental phenotype (Figure 4.2b). ***Natural selection*** within the units during a selection cycle could also lead to a lack of resemblance between the offspring and the parental communities. Indeed, within each artificial selection cycle, there are many individual generations so that natural selection at the individual level can operate and potentially conflict with the community-level artificial selection (Arora et al. 2020; Figure 4.2c).

In our two experiments, it is likely that we experienced a combination of these sources of a lack of parent/offspring resemblance but with different relative strengths. First, we increased the number of cycles between the experiment of Chapter 1 and this of Chapter 2 so that the effect of the experimental noise was probably lower in the second study. Then, regarding the heritability, it is clear that the communities of the first experiment were not stable, at least in the first half of the experiment, as a global decrease in biomass was observed whatever the treatment. It did not occur in the second experiment in which the initial inocula were built from the assemblage of laboratory strains. It highlights the importance of conducting several growth cycles before the start of the selection procedure to let the community adapt to the conditions to avoid the control of community phenotype by transient and non-heritable community dynamics (Jacquiod et al. 2021; Chang et al. 2021). Considering the reliability of the transmission of the phenotype determinants and along with the results obtained from the literature (Chang et al. 2020), the propagule reproduction method can be expected to be suitable for a reliable transmission. Indeed, we reached good levels of correlation between offspring and parental phenotypes in the second experiment and showed that the offspring/parent difference in phenotype in the first

experiment was lower with the propagule than with the migrant pool method. However, a reliable transmission of community phenotype would not necessarily ensure the success of the artificial selection, especially if it affects the existence of phenotypic variation.

2. Promoting phenotypic variation

The paradox in the artificial selection experiments is that variation between offspring and parents can prevent the transmission of the phenotype of interest but, at the same time, there must be variation between the units for selection to act (Lewontin 1970). Indeed, to achieve an efficient artificial selection, there must be phenotypic variations between the communities composing the population of communities among which selection is applied (= the metacommunity). First, one possibility is to create the initial metacommunity from samples that have various origins and to apply selection without consideration of the origin of the best-performing communities (Chang et al. 2020). The advantage of this method is that the initial phenotypic variance among the communities of the metacommunity is high, which can promote a strong response to the artificial selection. However, Chang et al. (2020) showed that this variance can be quickly used up as, in their study, in a few selection cycles, the selected communities were found to all originate from the same initial sample. Finally, the process eliminated the worst performing communities rather than improving the targeted property in the best performing ones. These results also suggest that, when the communities in the metacommunity all stem from the same parent, the phenotypic variance would be too low for the selection to be effective. In this case, the variation can arise through sampling effect when an offspring generation is created or through differences that would occur during the growth of the offspring (e.g. due to micro-variations in the abiotic conditions, the occurrence of a mutation, differences in cell behaviour). In the first experiments of Swenson et al. (2000a,b), the metacommunity was composed of identical communities, i.e. all stemming from the same initial pool, such as in the study of Chang et al. (2020) regarding the selection for an increase in amylase activity. Chang et al. (2020) suggested that the lack of efficiency of the artificial selection in their experiment could be, at least in part, due to a lack of variance in the metacommunity. In other studies, the selection process was repeated over several lines (i.e. there were several metacommunities) that were composed of communities that initially stemmed from the same initial pool (Swenson et al. 2000a; Blouin et al. 2015; Jacquiod et al. 2021), as what we did in the experiment of Chapter 1. The experimental design presented in Chapter 2 was slightly different as there were indeed several lines in each treatment but the metacommunities of the different lines did not stem from the same initial pool (there were six different species assemblages for each treatment). We can ask whether the maintenance of several *different* lines can contribute to an improvement of the artificial selection efficiency or not. Extra-analyses drawn from the data of the experiment of Chapter 2 suggested that the creation and maintenance of several lines could allow finding lines within which the variation is high enough so that they could be responsive to the artificial selection (Figure S4.1). Thus, the inter-

line phenotypic variation is not correlated to the artificial selection efficiency (Figure S4.2a) but could positively influence the probability of observing intra-line variation which is supposed to promote the artificial selection efficiency (Figure S4.2b). The existence of differences in the levels of intra-line variation between lines suggested that the selectable variation was mainly brought by differences occurring during the growth of the communities (= intrinsic factors) rather than by extrinsic factors such as sampling error under which we could expect similar levels of variation between the different lines.

Rather than "waiting for" the occurrence of phenotypic variation in the metacommunity, it could also be possible to intentionally induce it as proposed by Sánchez et al. (2021). Indeed, once the best-performing community has been identified in the metacommunity, one could create variants of this community so that the variance is not used up through the experiment. This is referred to as directed evolution (Sánchez et al. 2021). A "variant" of a community could be obtained through changes in the genetic material of the species or by changes in the community composition for example. Genetic changes within a species could occur through horizontal gene transfer by adding plasmids or bacteriophages in the medium (Sánchez et al. 2021). Community composition could be modified by the addition of one or multiple species (at random or based on specific knowledge on this/these species) or by the removal of one or several species (e.g. through dilution, filtration, the use of antibiotics or bacteriophages). An environmental perturbation (e.g. heat or cold shock) could also lead to the modification of the composition of the community. A balance between the creation of selectable variation and the preservation of the determinants of the targeted phenotype must be found for the artificial selection to work. In a recent study, Romdhane et al. (2021) showed that the application of different perturbations (i.e. antibiotics, antimicrobial peptide, filtration, heat shock, oxidative stress) led to different levels of depletions of the OTUs but also to different degrees of dissimilarity as compared to a non-perturbated community. Thus, the choice of the perturbation could be one way to control the level of variation that the perturbation will introduce. Another possibility could be to play on the intensity of the perturbation; for example, the environment could be changed slightly but continuously rather than suddenly (Sánchez et al. 2021) for not to induce drastic changes.

3. Including the right controls

The choice of the control treatments is not straightforward in artificial selection experiments at the community level. Two types of control treatments are presented in artificial selection experiments on microbial communities depending on the considered study[2]. Most of time, the control treatment correspond to a random line in which the parent(s) of the next generation is(are) selected at random

[2] Other control treatments were also proposed for artificial selection of microbiomes (i.e. microbial communities associated to a host), they are presented in Mueller and Sachs 2015. They include: a *constant inoculation treatment*, where the transferred microbial community comes from a stock, i.e. is not allowed to evolve; a *null inoculation treatment*, where what is transferred to the host contains no microbe e.g. sterilised soil or water; a *solute inoculation treatment*, where the biotic components of the inoculant are removed but where the nutrients can be transferred.

(Blouin et al. 2015; Arora et al. 2020; Chang et al. 2020; Jacquiod et al. 2021), as in Chapter 1. Another possibility is to include a control in which there is no artificial selection (i.e. no selection at the group level), that is, the communities are all passaged from one cycle to the other (Wade 1976; Chang et al. 2021), as in Chapter 2. These two controls do not allow answering the same question. The communities of the artificial selection treatment and these of the no artificial selection treatment both experience the same environmental conditions, the successive passages from one cycle to the other (with the associated reproduction method) and the within community natural selection, so that the only difference between the two is the existence of a selection at the community level. Thus, the no artificial selection control addresses the question: are the results obtained from the artificial selection at the community level different from what is obtained from natural selection occurring *within* the communities? In the random selection treatment, the communities experience all the previously mentioned conditions (environment, cycles, within unit natural selection) but also a selection at the community level, i.e. a functioning in metacommunity with a bottleneck at the community-level, so that the only difference with the artificial selection treatment is the type of community level selection, i.e. directional or random. Thus, the random selection control addresses the question: is the effect of a directional selection *among the communities* different from the effect of a random selection? Ideally, both controls should be included but, along with the need for replication, it gives rise to a sharp increase in the experimental effort. In our first experiment (Chapter 1), we included a random selection treatment only so that we could discuss whether a random community-level selection gave the same results as a directional community selection. However, we could not conclude on the effect of the within-unit natural selection on the targeted property whereas there was evidence that its influence was significant as a lower artificial selection intensity (in the stabilising selection treatment) gave rise to larger variations in the targeted function. In our second experiment (Chapter 2), we included a no artificial selection treatment only so that we could discuss the effect of the natural selection on the variations in the targeted property over time. However, we cannot assure that what was observed under directional artificial selection would not have been obtained under random community selection. Thus, when including the two controls is not possible, the interpretations must be done carefully, and the choice of the control should be determined by the research question. If the interest is to discuss the effect of an experimental parameter (i.e. the number of parents to be selected, the reproduction method, the sample size) on the efficiency of group-level selection, then it would be more interesting to include the random control in which there a metacommunity dynamics. On the contrary, if the interest lies in demonstrating that group-level selection is needed to reach a level of a property then it would be more interesting to include the no artificial selection treatment to evidence that natural selection within the community alone cannot explain the results.

What is important too is the way the data from the control are presented. Indeed, as pointed out by Chang et al. (2020), it is common that the effect of the artificial selection treatment appears relatively to what is observed in the control treatment but not necessarily in absolute value. As an example, Chang

et al. (2020) registered a difference in the mean amylase activity between the directional selection treatment and the random control at the end of the experiment, however it was due to a decline in the function in the control rather than an increase in the treatment. It was discussed by the authors and easily visualized as both the data from the directional selection treatment and the random selection were presented on the graphs. However, when the data are shown as differences from the control, the interpretation is less straightforward regarding the efficiency of the artificial selection. As, finally, what is expected from these experiments is a change in the targeted function in the desired direction, it is important to show the global trend under artificial selection in absolute value so that the control is also the initial level of the targeted property before selection. If the artificial selection is used for an applicable purpose, this control could be sufficient as it would allow ensuring that the level of the desired property is higher in the selected community than initially. However, including a no artificial selection treatment could still have an interest because, if the same results can be obtained from an experimental evolution without selection, this would decrease the experimental effort needed to obtain the community of interest.

4. Summary: the optimal community-level artificial selection experiment

In this section, I will base on the knowledge brought by the literature and by our experiments to describe what would be the optimal experimental design to register a significant effect of an artificial selection at the community level on the increase in a property. A schematic representation is shown in Figure 4.3. First of all, ***the choice of the targeted phenotype*** is of importance as some functions are more likely achieved by a multi-species assemblage than by a species alone (Williams and Lenton 2007). Thus, in this thought-experiment, the targeted property could be the degradation of an environmental pollutant. In order to bring and maintain phenotypic variation between the communities, several metacommunities should be built from samples originating from ***different environments***. Considering the need for replication within each treatment and for the experiment not to be completely unrealistic, samples should be taken from three distinct environments (n=3 biological replicates). The composition of these three initial communities should be analysed through sequencing in order to ensure that they ***differ greatly in their family-level composition*** (see Chapter 2). If not, variation in community composition should be created at this step (e.g. through a dilution approach). Based on the recommendations of Mueller and Sachs (2015), ***six lines within each treatment*** would be required (here the treatment is the origin of the initial sample), that is to say six metacommunities per treatment. Each metacommunity should be composed of ***10 replicates of a community*** (Mueller and Sachs 2015) which is a good compromise between replication and experimental effort. This should be replicated three times (i.e. 3x6 metacommunities): one set of metacommunities for the ***directional artificial selection treatment*** (AS), one set for the ***random selection control*** (RS) and one set for the ***no artificial selection control*** (NS). This would end up with a total of 540 units, i.e. communities, per cycle (we had 462 units

per cycle in the experiment of the second chapter). The experiment should start with ***five cycles of growth without selection***, i.e. with the passage of all of the communities, for the communities to be stable (Chang et al. 2021). During this step, pipetting should be suitable and allow creating small variations between the communities while not preventing the communities to reach stability. A ***monitoring of community biomass*** throughout the experiment would allow assessing community ecological dynamics for a better understanding of the outcome of the procedure (Wright et al. 2019). Once the communities are stable, the selection process can start. The ***phenotyping*** of the communities regarding the targeted property should be done ***in triplicates*** for each community to reduce the impact of measurement error (Xie et al. 2019). Then, ***the best performing community among the ten*** of a metacommunity should be used to create ten offspring communities ("top-dog" strategy; Xie et al. 2019). This strategy has been shown to be more efficient than a strategy with a lower selection strength (i.e. "top-tier" strategy in which several parental communities would have been used to create the offspring) when non-heritable determinant of the phenotypic variation are controlled (Xie et al. 2019). To do so, the creation of the offspring should be done through ***cell-sorting*** rather than pipetting to avoid inducing quantitative differences between the offspring units i.e. each offspring community should be inoculated from the same number of cells (and ideally the same species relative abundances should be preserved; Xie et al. 2019). I propose that the reproduction method should be chosen in order to maximize the resemblance between offspring and parents, even if it can reduce the occurrence of phenotypic variation. Thus, the ***propagule method*** should be used. The parental community in the random control should be randomly selected and all of the communities should be passaged in the no artificial selection control. The parents of each generation should be stored for further analyses such as DNA sequencing. Based on the results of our experiments, the experiment should be conducted at least for ***20 cycles***. The growth conditions (e.g. the duration of a cycle, the amount of resources, the temperature) should be determined for the phenotyping and transfer steps to occur when the community is in the ***stationary phase*** of its growth (i.e. in the late successional stages when community composition is supposed to be less variable and community diversity (especially regarding evenness) is supposed to be higher; Ortiz-Álvarez et al. 2018). The choice of the environmental conditions could also be guided by the aim of promoting community diversity (see Chapter 2). Multiple species are more likely to coexist if niche complementarity is allowed; it could be promoted experimentally through the creation of a heterogeneous environment (e.g. no agitation of the medium) or through the inclusion of a high diversity of substrates.

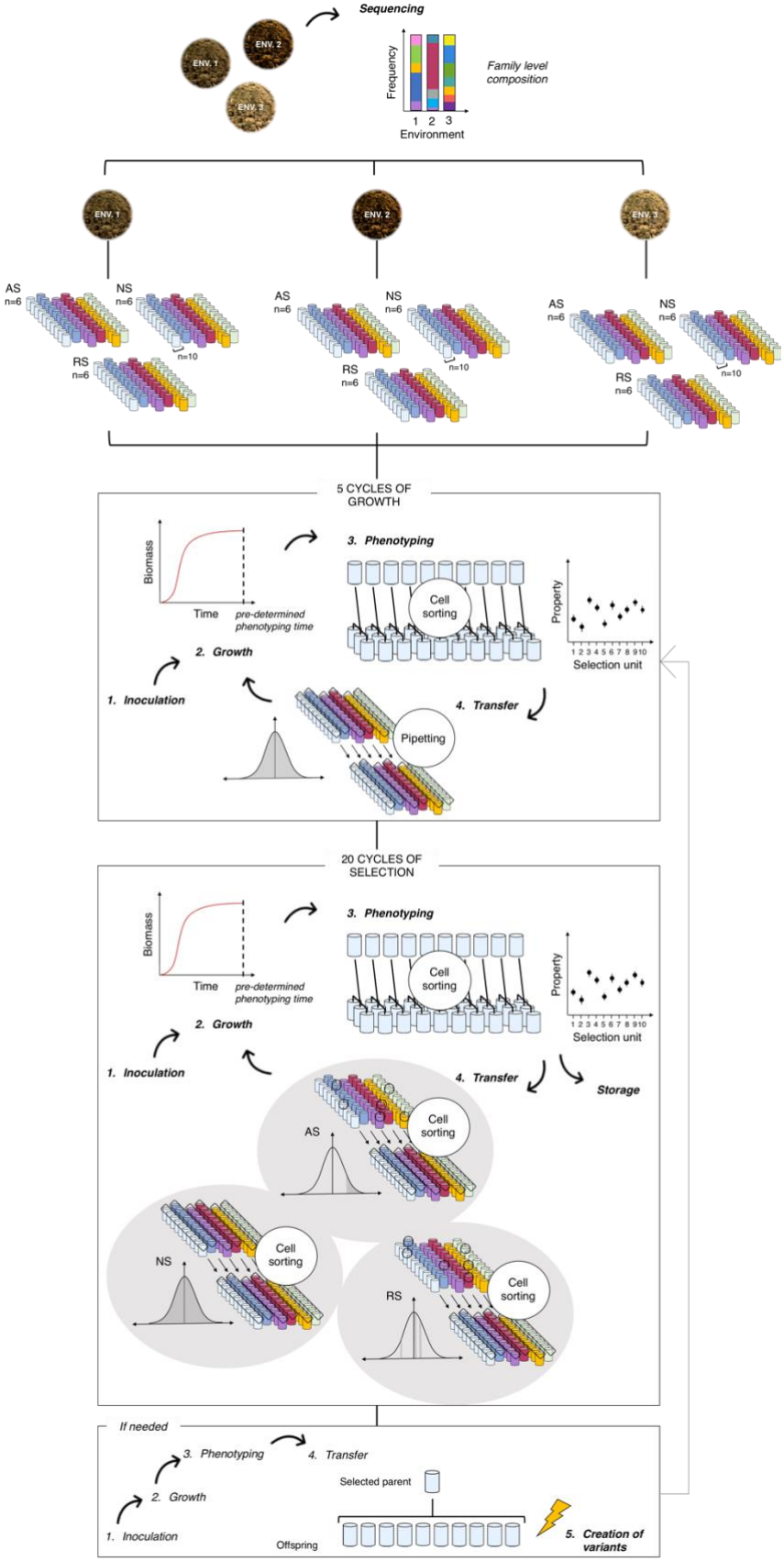

Figure 4.3 Schematic representation of the optimal community-level experiment. See section I.4 of the Discussion for a detailed explanation. Env.: environment.

Once the environmental conditions are determined, they should be as controlled as possible. Also, every steps of the experiment that could be automated should be (e.g. through the use of sterile pipetting robots) to avoid errors from the experimenter. Considering that the emphasis was on the parent/offspring resemblance when designing the reproduction step (i.e. use of a cell sorter and propagule reproduction method), it would be possible that the phenotypic variation between the communities would be too low for the selection to act. So, if the artificial selection is inefficient or if the response to selection saturates, a step of ***creation of phenotypic variation*** could be introduced (Sánchez et al. 2021). This step could occur through the application of a perturbation once the offspring communities are created from the selected parents so that, once the heritable determinants of the parental phenotype are transferred, the perturbation would potentially lead the offspring communities on different trajectories and thus create phenotypic variation. Including a step of creation of variation would require to re-conduct the community stabilization step (Sánchez et al. 2021).

II. UNDERSTANDING COMMUNITY ECO-EVOLUTIONARY DYNAMICS

1. Interplay between interspecific interactions and evolution

Synthetic microbial ecology allows working on simplified communities with the aim of understanding their functioning and the role of the interactions in the establishment of community-level properties (Großkopf and Soyer 2014; Dolinšek et al. 2016). The number of species included in synthetic communities (i.e. communities that are created artificially by co-culturing species[3]; Großkopf and Soyer 2014) is much lower than this of natural communities and, synthetic communities are grown under controlled environmental conditions. Despite this, it is clear that the study of synthetic communities can contribute greatly to the understanding of community ecology and evolution (Bengtsson-Palme 2020).

In our third study, we showed that the evolution in and of the communities was influenced by a shared evolutionary history of the species composing these communities in accordance with what was reported in the literature (Lawrence et al. 2012). It occurred in two ways: through an influence of the interspecific interactions on the evolution of community and community members' phenotype (effect of ecology on evolution) and through an evolution of the interspecific interactions themselves (effect of evolution on ecology). In the first case, the presence of a species influences the evolution of a second species so that the evolutionary response at the community level would be predictable from the evolutionary responses at the individual level. Furthermore, there is evidence that some of the characteristics of a species could allow predicting its evolutionary response when grown in a community and potentially its importance in the evolutionary response of the community. Indeed, the theory

[3] Note that several authors use "synthetic community" to refer to assemblages of microorganisms in which at least one strain is genetically modified (Canon et al. 2020).

predicts that the evolutionary rate of a species would depend on its generation time (the shorter the generation time, the faster the adaptation) and on its population size (the higher the population size, the higher the mutation occurrence; Barraclough 2015). Furthermore, it has been shown that these two parameters could allow predicting which species would dominate a simple community composed of two species (Meroz et al. 2021). So, in a two-species community, the dominant species (which is presumably the one that has the highest growth rate and/or productivity in isolation) is more likely to have a higher evolutionary response than the other species and, we suggest from our results (Chapter 3) that this response is likely to involve an adaptation to the abiotic conditions. Our results also suggested that, in turn, the second species is likely to adapt to the presence of the dominant species, i.e. to the biotic conditions. This kind of eco-evolutionary dynamics has also been evidenced in the study of Rodríguez-Verdugo and Ackermann (2021). They studied the evolution of a two-species bacterial community in a fluctuating environment. The experiment ran over 30 days with a transfer each day either in citrate or in benzyl alcohol in alternation. *Pseudomonas putida* had a much higher population size than *Acinetobacter johnsonii* in citrate and slightly lower in benzyl alcohol so that, overall, the population size of *P. putida* was higher (note however that this would not have been predictable from their relative performances in monoculture; Rodríguez-Verdugo et al. 2019). There were two outcomes of the co-culture: either the co-existence of the two species or the extinction of *A. johnsonii*. Through genome sequencing the authors showed that there were mutations in *P. putida*. Also, the growth of the mutants was higher than this of the ancestor both on citrate and benzyl alcohol. When the outcome was the co-existence, there were also mutations in *A. johnsonii* but the growth of the mutants did not increase as compared to this of the ancestor (Rodríguez-Verdugo and Ackermann 2021). These results suggested that *P. putida* adapted to the abiotic conditions whereas *A. johnsonii* adapted to the presence of *P. putida*. Thus, in a very simple community, we could expect that the dominant species will adapt to the abiotic conditions and that its presence would exert a selection pressure that will induce an adaptation of the non-dominant species. Further experiments will be needed to investigate the validity of this hypothesis.

When the interactions themselves respond to the evolution, the community evolutionary trajectory is difficult to predict, even in synthetic communities. Indeed, it has been shown that an evolution of the interactions can lead to an increase in community function (e.g. productivity, Hansen et al. 2007; Lawrence et al. 2012). But, on the contrary, in our experiment (Chapter 3), the evolution of the interactions led to a reduction of community productivity in two of the eight studied communities. Thus, the evolution of the interactions does not necessarily give rise to an increase in community functioning. However, our results suggested that an evolution of the interactions could be associated to a higher stability in community function which occurred through the reliable expression of the evolved community phenotype in two different environments. This has to be further investigated as it could notably depend on the type of interactions that are at stake. Indeed, the evolution of positive interactions could increase the interdependency between the species and, as a result, if a perturbation negatively

affects one species it could also affect the other species and thus lead to a lower stability of the community (Gorter et al. 2020). It is challenging to predict the effect of an evolution of the interactions on the community phenotype and, it would also be difficult to predict in which cases we can expect an evolution of the interactions as it may depend on multiple factors linked to the abiotic environment (Hansen et al. 2007; Fiegna et al. 2015a) or to the biotic environment. The study of pairwise interactions is a good starting point to shed light on the mechanisms that could influence community evolution, however, in complex communities it is expected that there would be diffuse (co)evolution rather than pairwise interaction evolution (Johnson and Stinchcombe 2007). Thus, the existence of higher-order interactions in complex communities would increase the complexity of the system and decrease the predictability of its functioning.

2. Interplay between community diversity and evolution

The diversity is one of the main focus of community ecology. Consistently to what is commonly observed in natural conditions (Balvanera et al. 2006), we registered an increase in community functioning with the increase in richness of our synthetic bacterial communities (Chapter 2). To go further, we also evidenced a link between the initial richness of the communities and their evolution. More specifically, the direction of the community productivity change over the experimental evolution was affected by the richness of the community. However, this effect of community ecology on community evolution was not linear and there were also great differences between the evolutionary trajectories of communities of the same initial richness but with differing compositions. We are aware of one other experimental study (Fiegna et al. 2015a) in which community diversity has been intentionally manipulated and in which the effect of community diversity on the evolution of a community trait has been assessed. In this study, increasing the initial community richness positively influenced the change in community productivity over time: there was no significant change in monoculture productivity through the evolution whereas productivity was significantly increased as compared to the ancestral communities in the initially richer communities (12 bacterial species; Fiegna et al. 2015a). Interestingly, the authors showed that an evolution of the interactions was involved in the evolution of community productivity and, that changes in the interactions occurred more in richer communities. However, this positive effect of community richness on the evolution of community productivity and the implication of an evolution of the interspecific interactions were observed in one of the three studied environments only (Fiegna et al. 2015a). Thus, as it could be expected, it is difficult to capture how community diversity would influence community evolution as it could depend on multiple factors such as the abiotic environment, the identity of the species, the interactions between the species and the evolution of these interactions. We think that the theoretical bases of the link between community diversity and community evolution could be thought in the framework of the adaptive landscapes. Trying to conceptualize how the diversity could influence the shape of the adaptive

landscapes and the way the communities would navigate these landscapes could provide several guidelines for the design of experimental studies.

If little is known on the effect of community diversity on community evolution as a whole, there are more predictions and more studies about the effect of community diversity on the evolution of the species within this community. First, theoretically, an increase in community richness could positively affect the evolutionary rate of a species as the presence of other species would exert additional selection pressures (Fiegna et al. 2015a; Barraclough 2015). A species would thus adapt to the biotic conditions (= Red Queen Hypothesis; Brockhurst et al. 2014) or to the abiotic conditions due to the presence of competitors for example. An increase in community diversity could also promote the evolution through horizontal gene transfer and thus have a positive effect on the evolutionary rate of the species (Barraclough 2015). On the contrary, we could expect a negative effect of community richness on the evolutionary rate of the species under the hypothesis that an increase in richness will lead to a decrease in the population size within each species (Lawrence et al. 2012). Another negative effect of the community richness could occur through the limitation of co-evolution (co-evolution is known to drive rapid evolution of the involved species; Brockhurst et al. 2003) due to the existence of a complex interaction network with species involved in interactions with multiple other species (Barraclough 2015). Fiegna et al. (2015b) showed that, overall, the effect of increasing community richness was to increase the evolution of species growth rate and productivity, but this effect was saturating or reversing at the highest richness levels (up to twelve bacterial species). It suggested that the evolution of a species would be promoted by the presence of few other species as compared to what would happen in isolation but, from a certain point, additional species could reduce the strength of the biotic interactions and lower the evolution rate (Fiegna et al. 2015b). This was also suggested by a recent study that highlighted an increased evolutionary capacity of the bacterial strains in the less diverse communities (complex communities that had the lowest Shannon index; Scheuerl et al. 2020). In this study, the evolutionary response of the strains depended on the identity of the strain and its initial adaptation to the environment, on the community in which it evolved (some communities were more "permissive" than others) and on the interactions between both strain and community properties. Thus, it is clear that there is a close and complex link between diversity and community and community members' evolution so that a shift in diversity (e.g. through the introduction or the loss of a species in a (natural) community) could have an impact over a long timescale.

3. The interactions between inter- and intraspecific diversity… and community evolution?

In a recent study on a plant community, we showed that the interspecific diversity interacted with the intraspecific diversity to shape the productivity of the community (Appendix 5). Indeed, while increasing the number of genotypes within a plant species led to a decrease of plant productivity in monoculture, increasing the number of genotypes within a plant species in a two-species community

did not affect plant productivity. We highlighted that increasing interspecific diversity could change the interactions between the different genotypes within a species. In the other way, it has also been shown that an increase in intraspecific diversity could have an effect on the interspecific competition and on species dominance pattern in a plant community (Fridley and Grime 2010; Schöb et al. 2015). In our experiments on bacterial communities, it is highly probable that intraspecific diversity (i.e. diversity in the bacterial populations) played a role both in community functioning and evolution. In bacteria, the mechanisms of emergence and maintenance of diversity within a population are known (Rainey et al. 2000), however, less is known about the interactions between intra and interspecific diversity. One study on bacterial biofilms showed that, for the three studied bacterial strains, the frequency of variants (i.e. intraspecific diversity) was high when grown in monoculture and decreased with the increase of the community richness (Kelvin Lee et al. 2016). Accordingly, in the study presented in Chapter 3, we noticed the existence of two distinct sub-populations of a *Pseudomonas* strain when evolved in monoculture (two distinct colony morphologies of *Pseudomonas* sp. ADP3 were observed and this variation in phenotype was heritable), whereas we did not notice it when this strain was isolated from the four-species community in which it evolved, even if it would need further dedicated investigations to be confirmed. Conversely, another study showed that an increase in community diversity (up to eight bacterial strains), and more particularly of functional diversity, can reduce the fitness of a species and promote its diversification (Jousset et al. 2016). Thus, these studies suggest that, the interspecific interactions could affect the evolution within the species and promote or reduce intraspecific diversity. It could be interesting to investigate the effect of this interplay on the evolution of the interspecific interactions and of the communities.

4. Impacts of the deliberate introduction of microbial communities in the environment on the structure, functioning and evolution of native communities

In the previous sections, we discussed the close links between diversity, interspecific interactions and community functioning and evolution and we highlighted the complexity of the organization in community and the challenge that it represents to predict community dynamics. In this context it is relevant to ask what could be the impact of introducing a community obtained from artificial selection into an environment in which a microbial community is already established. Indeed, in its applied version, the purpose of the artificial selection of microbial communities or microbiome is to engineer a community for it to achieve a desirable function that has been targeted to meet a need (e.g. enhancing plant nutrition, degrading a pollutant). It means that, finally, the selected microbial community will be introduced in the environment in which there is a need for the targeted function to be performed. If this environment is an artificial and closed system such as a bioreactor (see examples in Sabra et al. 2010) the main focus will be the efficiency of the engineered community and the abiotic conditions that will promote it. However, if the environment is an open and natural system such as a

plant or a soil, we have to consider the native community and the possible impacts the inoculation could have on it. The joining of two communities that were previously separated is referred to as "community coalescence" (Rillig et al. 2015). It is expected that community coalescence will have different outcomes depending on: the environmental conditions, the mixing ratio of the communities, the interaction interface and the temporal dynamics of the encountering of the communities (Rillig et al. 2015). In the context of the inoculation of an engineered microbial community into a given environment, we could expect the endemic community to benefit from a "priority effect" (Rillig et al. 2015) so that the engineered community would not be able to invade the environment. There are experimental studies that confirm this expectation. Actually, the intentional introduction of microbes in the environment is already used, in particular in agroecosystems in which microbial inoculants are applied in order to enhance plant health and productivity (Berg 2009). What is often observed is that the inoculants – which are currently mostly composed of one or few bacterial or fungal species – often become quickly undetectable after their release in the soil (Mawarda et al. 2020). However, Mawarda et al. (2020) found that, in 86% of the reviewed studies (108 in total), the inoculation changed the structure of the native community of the soil. Furthermore, in 80% of the studies that assessed the long-term effects, there was no return to the initial community structure despite the disappearance of the inoculant (i.e. no resilience). This is a major concern as we can expect that a change in community structure will further influence community evolution and functioning as well as the resulting ecosystem processes. Furthermore, there is evidence that the effect of the inoculation can be context-dependent and thus have unexpected outcomes. As an example, some microorganisms that are known to establish mutualistic associations with plants (e.g. mycorrhizal fungi) can switch to a pathogenic lifestyle depending on the abiotic environment (Jack et al. 2021). Contrasted outcomes of microbial inoculation have also been evidenced in medicine. Indeed, specific microbial community structures are found to be associated with certain diseases such as obesity, cardiovascular diseases or asthma (Cho and Blaser 2012) so that there is a considerable interest for the microbiome in human health. Fecal microbiota transplant (FMT, where stools are collected from a healthy donor to be transferred to a patient) has been used to treat the infections by *Clostridium difficile* and has shown positive results (Youngster et al. 2016), however severe side-effects have also been encountered due to the transmission of multi-drug resistant microbes during certain transfers (Madhusoodanan 2020). When FMT was used to treat ulcerative colitis, it has been shown that the success of the transplantation (i.e. the resemblance of the microbiota of the recipient to that of the donor) was dependent on the patient as well as the outcome of the treatment (either improvement or deterioration of the clinical conditions; Angelberger et al. 2013). From these fields in which microbial community introduction is already performed, we can see that there are critical concerns about the effect of the introduced community on the resident community and the related processes (Mawarda et al. 2020), the risk of invasion and the related consequences (Jack et al. 2021) and the possible unforeseeable effects (Angelberger et al. 2013; Madhusoodanan 2020). Thus, the next challenge regarding the engineering of microbial communities, including through artificial selection,

will be to include consideration of the possible effects on the biotic and abiotic environment in which it will be introduced. Several avenues have already been suggested such as the engineering of the community or of a sub-sample of the community that is native from the targeted environment rather than of species that are not endemic, or the promotion of the specificity of the engineered community to its host (e.g. a plant species; Jack et al. 2021).

CONCLUSION

The microorganisms play key roles in health as the members of the human microbiome, in agriculture as the members of the plant or animal microbiome, in the environment as pollutant degraders or biogeochemical cycle members, in industry as chemical producers or wastewater degraders… The organization in community allows the emergence of properties that are of particular interest regarding the importance of microorganisms in these multiple processes. In this context, artificial selection at the community level is a promising top-down approach that could allow engineering microbial communities for applied purposes. In this work, we highlighted several limitations that are still at stake before being able to apply an efficient community-level selection. Many lines of improvements have already been highlighted and there is an increasing body of literature in this field so that it may become possible to engineer microbial communities and microbiomes through this approach. This will raise ethical questions linked to the use of these communities with desired functions (e.g. should we inoculate them in natural conditions? in closed systems only? which degree of manipulation is acceptable?) and more particularly when it will come to microbiome manipulation. Beyond the possible applications, artificial selection experiments at the community level can contribute to the understanding of community dynamics. Indeed, the organization in community confers unique properties but also unique challenges when the aim is to understand community ecology and evolution. In this work, we illustrated the multiple interactions that take place between the ecology and the evolution of the communities and within the communities and provided evidence that communities could be considered as selection units. Given the complexity of community ecology and evolution, the study of simplified communities in the laboratory can contribute greatly to the understanding of community functioning in the broad-sense.

APPENDIX 1: SUPPLEMENTARY MATERIAL CHAPTER 1

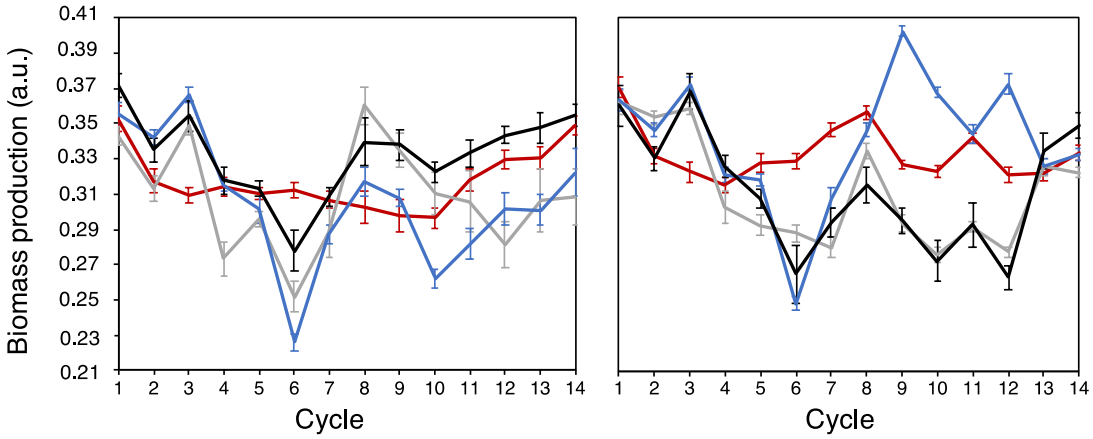

Figure S1.1 Biomass production over time. Left: Propagule method; right: Migrant pool method. Black: Random; grey: Low; blue: Stabilizing; red: High. One point is the average biomass production of 30 communities. Bars represent SE.

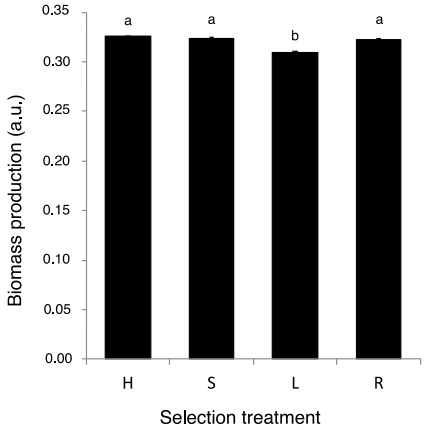

Figure S1.2 Biomass production all cycles together. Mean biomass production depending on the selection treatment (n = 840). H: High; S: Stabilizing; L: Low; R: Random. Bars represent SE. Different letters represent significant differences.

Table S1.1 ANOVA of the linear mixed model describing the response of biomass production to fixed effect factors: cycle (1 to 14), selection treatment (Random, Low, Stabilizing, High), reproduction method (Propagule, Migrant pool) and their interaction. Line is embedded in the model as a random effect factor. Marginal R^2=0.62 (variance explained by the fixed effect factors). Conditional R^2=0.76 (variance explained by fixed and random effect factors).

	df	Sum sq	Mean sq	F	p
Cycle	13	1.46	0.11	302.41	$< 2.2x10^{-16}$
Selection treatment	3	0.00	0.00	0.75	0.55
Reproduction method	1	0.00	0.00	0.95	0.36
Cycle x selection treatment	39	0.65	0.02	45.11	$< 2.2x10^{-16}$
Cycle x reproduction method	13	0.02	0.00	4.72	$3.6x10^{-8}$
Selection treatment x reproduction method	3	0.00	0.00	1.99	0.20
Cycle x selection treatment x reproduction method	39	0.53	0.01	36.85	$< 2.2x10^{-16}$

Table S1.2 Realized heritability (h^2) calculated as the slope of the linear regression of the cumulative selection response to the cumulative selection differential. A linear regression was conducted for each line independently. Taking into account correction for multiple testing (Bonferroni correction), h2 differs significantly from zero when $p<0.006$.

Line	h^2±SE	df	F	R^2	p
HM	0.02±0.06	11	0.16	0.00	0.70
HP1	0.15±0.06	11	5.75	0.28	0.04
HP2	0.09±0.09	11	1.20	0.02	0.30
HP3	0.09±0.05	11	3.26	0.16	0.10
LM	0.13±0.08	11	2.57	0.12	0.14
LP1	0.18±0.12	11	2.29	0.10	0.16
LP2	-0.03±0.12	11	0.06	0.00	0.82
LP3	-0.19±0.14	11	2.01	0.08	0.18

APPENDIX 2: SUPPLEMENTARY MATERIAL CHAPTER 2

Appendix 2.1 Community composition analysis

Molecular biology

In order to perform DNA extractions, monocultures and communities from cycles 1 (i.e. initial inocula after 84 h of growth) and 40 and communities from intermediate cycles 14 and 27 were revived from the glycerol stocks in the growth conditions of the experiment (i.e. deep-well plates, 28°C, no shaking, 84 h, the culture medium volume was increased to 1.8 ml following the instructions of the DNA extraction kit manufacturer; DNeasy UltraClean 96 Microbial Kit, Qiagen, Netherlands). After 84 h of growth, the plates were centrifuged for 24 min at 2,250 RCF, the supernatant was discarded, the plates were centrifuged again for 8 min at 2,250 RCF and the remaining supernatant was discarded. The obtained cell pellets were stored at -20°C. DNA extractions were then conducted following the kit manufacturer's instructions. The extracted DNA was quantified (Quant-iT dsDNA Assay Kit high sensitivity, Invitrogen, USA) and each sample was diluted to 0.5 $ng.\mu L^{-1}$. A mock community (mock 1) was created by adding an equivalent volume of extracted DNA (0.5 $ng.\mu L^{-1}$) of each of the 18 strains and included into the samples to be analysed as well as another mock community (mock 2) with other strains than those of the experimentation. We first performed 16S rRNA gene amplification (V3-V4 region) using the PCR primers Pro341F/Pro805R (carrying 5' tails for Illumina sequencing) at a final concentration of 0.25 µM for 1 ng of DNA. The cycling conditions were: 98 °C (3 min), 25 amplification cycles at 98 °C (30 s), 55 °C (30 s), 72 °C (30 s) and a final step at 72 °C (10 min). Then we performed a second PCR to allow the barcoding of the PCR products with NGS primers at a final concentration of 1 µM using the same cycling conditions but reducing the number of amplification cycles to eight. The obtained PCR products were normalized (SequalPrep Normalization Plate Kit, Applied Biosystems, USA), pooled and sequenced on Illumina MiSeq (2x250 bp; Genoscreen, France). We then performed *gyrB* gene (coding for the subunit B of DNA gyrase) amplification using PCR primers that were specifically designed for the 18 strains of the experiment: gyrB55F (5'-GTNMGHAARCGBCCNGS-3') and gyrB347R (5'-TGHARRCCRCCVGANAC-3') carrying the 5' tails for Illumina sequencing. We designed these primers to prevent nonspecific amplification of *parE* gene (coding for the subunit B of DNA topoisomerase IV) which shows high identity levels with *gyrB* as noticed by *in silico* testing of pre-existing *gyrB* primers. The primers were used in a final concentration of 2 µM for 1 ng of DNA. The cycling conditions were: 98°C (4min), 35 amplification cycles at 98 °C (45 s), 58 °C (1 min), 72 °C (1 min) and a final step at 72 °C (10 min). The obtained PCR products were purified (Pronex Size-Selective Purification System, Promega, USA) following the manufacturer's protocol to remove the unfixed PCR primers (approximate size cut-off of 250 base pairs) as they were degenerate. The next steps were identical to those of 16S rRNA gene: second PCR to attach the barcodes, normalization, pooling and Illumina MiSeq sequencing.

DNA database construction

As there is no available full length *gyrB* database, we built our own *gyrB* database. We isolated the forward and reverse sequences of isolated ancestral strains from the rest of the sequences obtained on the Illumina MiSeq run. We then assembled the forward and reverse sequences (PEAR; Zhang et al. 2014) and discarded unassembled sequences. Sequences smaller than 100 base pairs (bp) were discarded as well as chimeras (which were detected *de novo*). OTU clustering was done at the 94% identity level (VSEARCH; Rognes et al. 2016). An OTU table was built and the sequences of the dominant OTUs in each sample were aligned against GenBank database using BLAST (Altschul et al. 1990) to check for the strain and the gene identity before being added to the database. Sequences that did not correspond to *gyrB* were not included in the database except one sequence found for Arthrobacter sp. BS2 which showed 94% identity with a gene whose product is a topoisomerase IV (which is a product of *parE* gene) whereas there was no sequence that corresponded to *gyrB*. Each sequence that was included in the database was given an identifier and the corresponding taxonomy was reported in another file. We ran again the process starting from the same set of forward and reverse sequences, setting the minimal sequence length to 100 and adding a reference-based chimera check step with our database as reference. Once OTU clustering was done (94% identity) and the OTU table was built, the taxonomy was assigned using our reference database: each sequence was aligned against the reference sequences and the highest identity level was kept to assign the corresponding taxonomy provided that the alignment length was higher than 250 bp. The obtained sequences for each sample were added to the database (after checking for strain and gene identity) as well as sequences of the strains of the mock 2. There were four strains for which no sequence was retrieved with this procedure (*Aminobacter aminovorans* SR38, *Arthrobacter* sp, *Microbacterium* sp. C448, *Pseudopedobacter saltans* DSM 12145), *gyrB* sequences were collected from GenBank or personal data and added to the database. For sake of consistency, we followed the same procedure for the construction of the 16S rRNA gene database (minimal sequence length: 350 bp, OTU clustering at 94% identity, minimal alignment length: 350 bp).

Identity threshold for OTU clustering

Three replicates of two mock communities were sequenced and their analysis was used to choose the identity level threshold for OTU clustering by maximizing the number of detected strains (based on the taxonomy) while minimizing the number of OTUs. The first mock community (mock 1) was a combination of the 18 strains of the experiment belonging to 13 genera and 15 or 16 species. The second mock community (mock 2) was a mix of 39 strains (other than those of the experiment) belonging to 17 genera and 27 species. We tested identity level thresholds for OTU clustering ranging from 0.92 to 0.97. On *gyrB* sequences, the highest number of detected strains on mock 1 was 11 and was obtained with an identity level of 0.95; it corresponded to 56 OTUs (at 0.94 identity level we

detected 10 strains and 29 OTUs and at 0.96 we detected 9 strains and 98 OTUs). On mock 2, the 0.95 identity level allowed detecting 24 strains and 130 OTUs. On 16S sequences, 11 strains were detected in mock 1 whatever the identity level with an OTU number varying between 22 and 97. On mock 2, the highest number of detected strains was 25 and was obtained with both 0.94 and 0.95 identity levels but with 82 and 101 OTUs respectively. We thus chose the 0.94 threshold (which corresponded to 36 OTUs on mock 1 against 32 with the 0.95 threshold).

Bioinformatics analyses

The forward and reverse sequences obtained from Illumina MiSeq sequencing were assembled using PEAR (Zhang et al. 2014) and unassembled sequences were discarded as well as sequences smaller than 200 bp for *gyrB* and 350 bp for 16S rRNA gene. OTU picking was conducted against the reference databases that we constructed. First, chimeras were eliminated with *de novo* and reference based chimera detection, then, OTU clustering was done using VSEARCH (Rognes et al. 2016) at identity thresholds of 0.95 and 0.94 for *gyrB* and 16S respectively (based on the results we obtained on the mock communities). One representative sequence per cluster was kept based on the highest base pair number. The taxonomy was assigned to each sequence based on the highest identity level with the sequences of our reference database and with a condition on the length of the alignment (> 200 and >350 bp for *gyrB* and 16S respectively). An OTU table was then constructed. Based on the expected community composition, we noticed the presence of unexpected OTUs with very low counts in several samples. This could be due to critical mistags i.e. a sequencing barcode is associated with the wrong sequence and this can not be detected as it corresponds to an existing barcode combination (which is highly probable as we used almost all possible barcodes combinations; Esling et al. 2015). Very low counts of an unexpected OTU can also be due to cross-talk, i.e. a read is assigned to the wrong sample at the demultiplexing time. Contrary to critical mistags, this can be corrected *a posteriori*. We estimated *de novo* the cross-talk rate for each OTU using the UNCROSS2 algorithm (Edgar 2018). For each OTU count in each sample, this algorithm gives a score *t* which is close to 0 when there is no indication for cross-talk and close to 1 when cross-talk is detected. When *t* was higher than 0.1 (i.e. the threshold recommended by Edgar 2018), the corresponding count was set to 0. The main OTU was identified for each strain and the corresponding corrected counts were included in the final OTU tables. From these corrected OTU tables for 16S rRNA gene and *gyrB*, we built one presence/absence OTU table based on the condition that one strain was present if the corresponding OTU count was higher than 100 and zero for 16S and *gyrB* respectively. The final OTU tables contained only the counts of the strains coded as "present".

Appendix 2.2: Contaminant management

After the experimental evolution, the experimentation was conducted in the following order starting from the same glycerol stocks: growth profile study, metabolism study, DNA extractions and PCR, post-selection experiment. Therefore, when a contamination occurred in a sample during the experimental evolution, it was detectable in all of the datasets and we removed the corresponding sample from the analyses. When the indication for possible contamination involved only one of the datasets, we considered that it was not sufficient to remove the corresponding sample from the analyses (see Figure S2.1 for more details). When a sample was contaminated in a given selection treatment (i.e. AS or NS), we removed this sample from the datasets for the affected selection treatment and kept the corresponding sample in the other selection treatment. When both selection treatments were affected, we removed the samples and the corresponding ancestor in the datasets. It resulted in the removal of 16.7% of the samples in the experimental evolution dataset, distributed as follows: artificial selection: 21.4%; no artificial selection: 11.9%; richness 1: 22.2%; richness 2: 25%; richness 4: 25%; richness 8: 0%; richness 16: 0%. In the growth profile dataset, metabolism dataset and post-selection dataset, it resulted in the removal of 14.3% of the samples distributed as follows: artificial selection: 21.4%; no artificial selection: 11.9%; ancestors: 9.5%; richness 1: 20.4%; richness 2: 22.2%; richness 4: 16.7%; richness 8: 0%; richness 16: 0%.

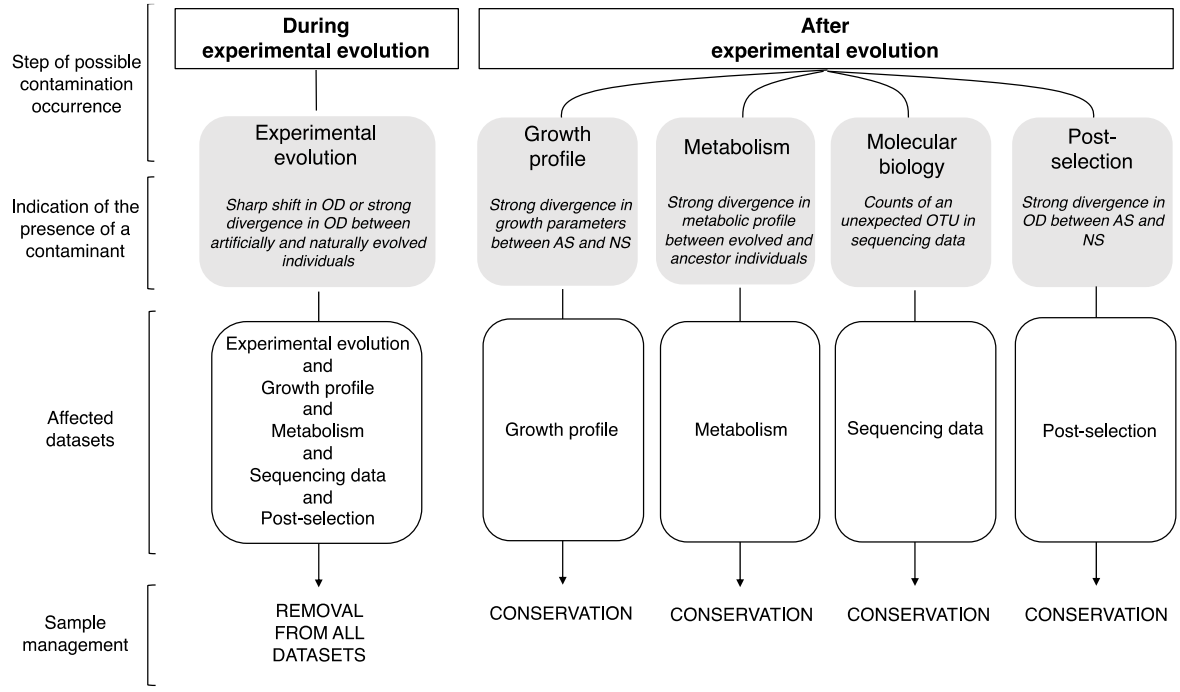

Figure S2.1 Contaminant management in the datasets. There were five different steps in the experimentation, each of which corresponding to a distinct dataset. The different steps are presented from left to right in the order in which they were conducted. The indication of the presence of a contaminant in a sample was defined as a deviation from the global trend observed for a given dataset (e.g. in metabolism dataset, the metabolic profile is overall very similar between ancestors and evolved individuals, a shift in metabolic profile thus suggested a possible contamination). If a contamination occurred during the experimental evolution, it affected all of the steps of the experimentation and thus, there were indications of the presence of a contaminant in all of the datasets. In this case, we considered that the amount of information was sufficient to remove the sample from the analyses with low error probability. On the contrary, when there was an indication of the presence of a

contaminant in only one of the steps (which did not occur in the experimental evolution dataset), we considered that the amount of information was not sufficient enough to remove the sample. Another possibility was the contamination of the glycerol stocks from which the different steps were conducted, in this case we would have indications for the presence of a contaminants in at least two steps in a row which was not observed. OD: optical density; OTU: operational taxonomic unit.

Appendix 2.3 Detail of the optical density profiles over the selection cycles

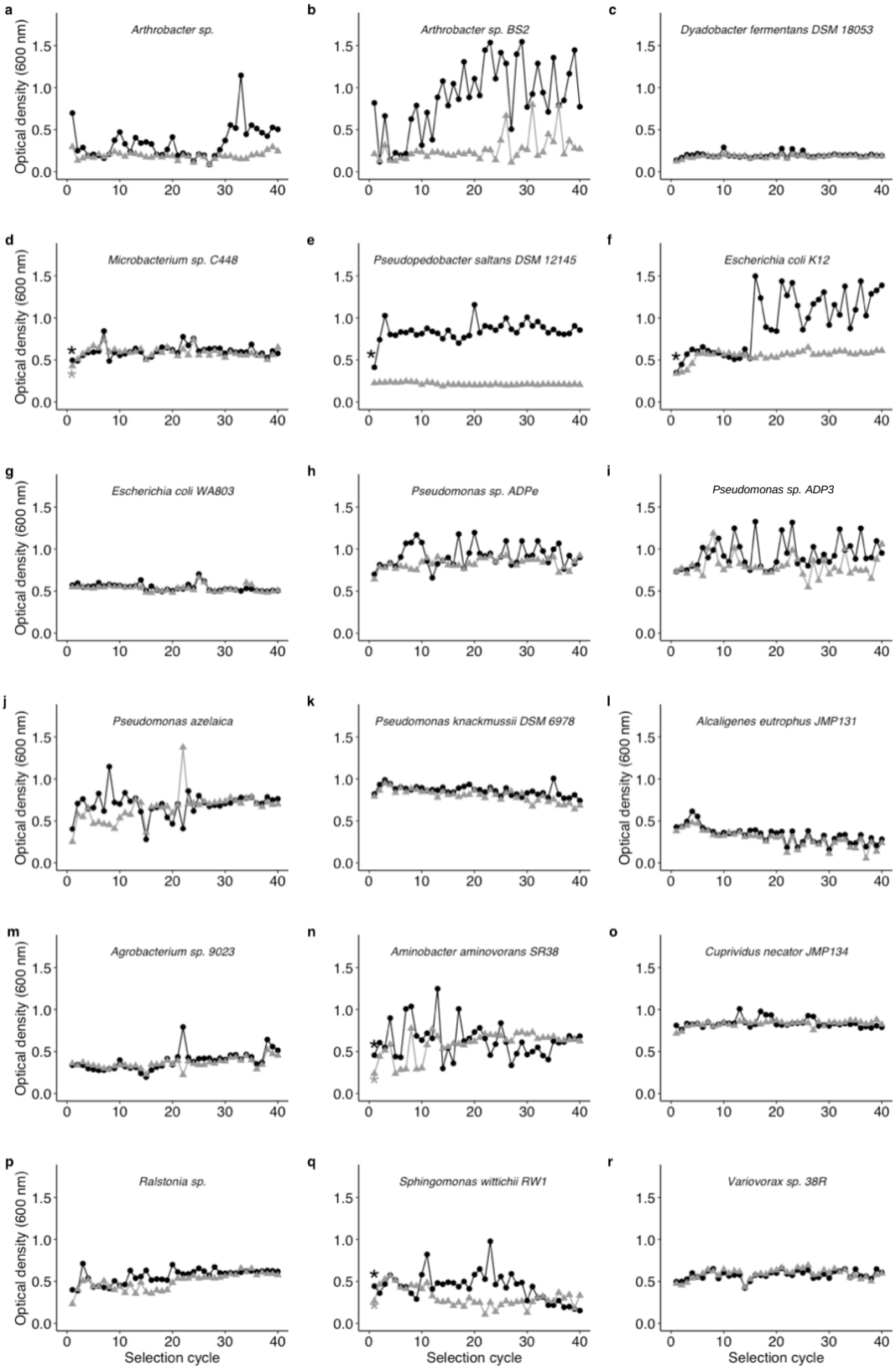

Figure S2.2 Changes in optical density (OD) over experimental evolution under artificial selection and no artificial selection depending on the strain. The OD of the parent of the next population of selection units is represented by a black circle for AS and a grey triangle for NS. The OD at cycle 0 was equal to 0.002 for all the treatments. The treatments in which a contamination was detected are identified by an asterisk (at cycle 1).

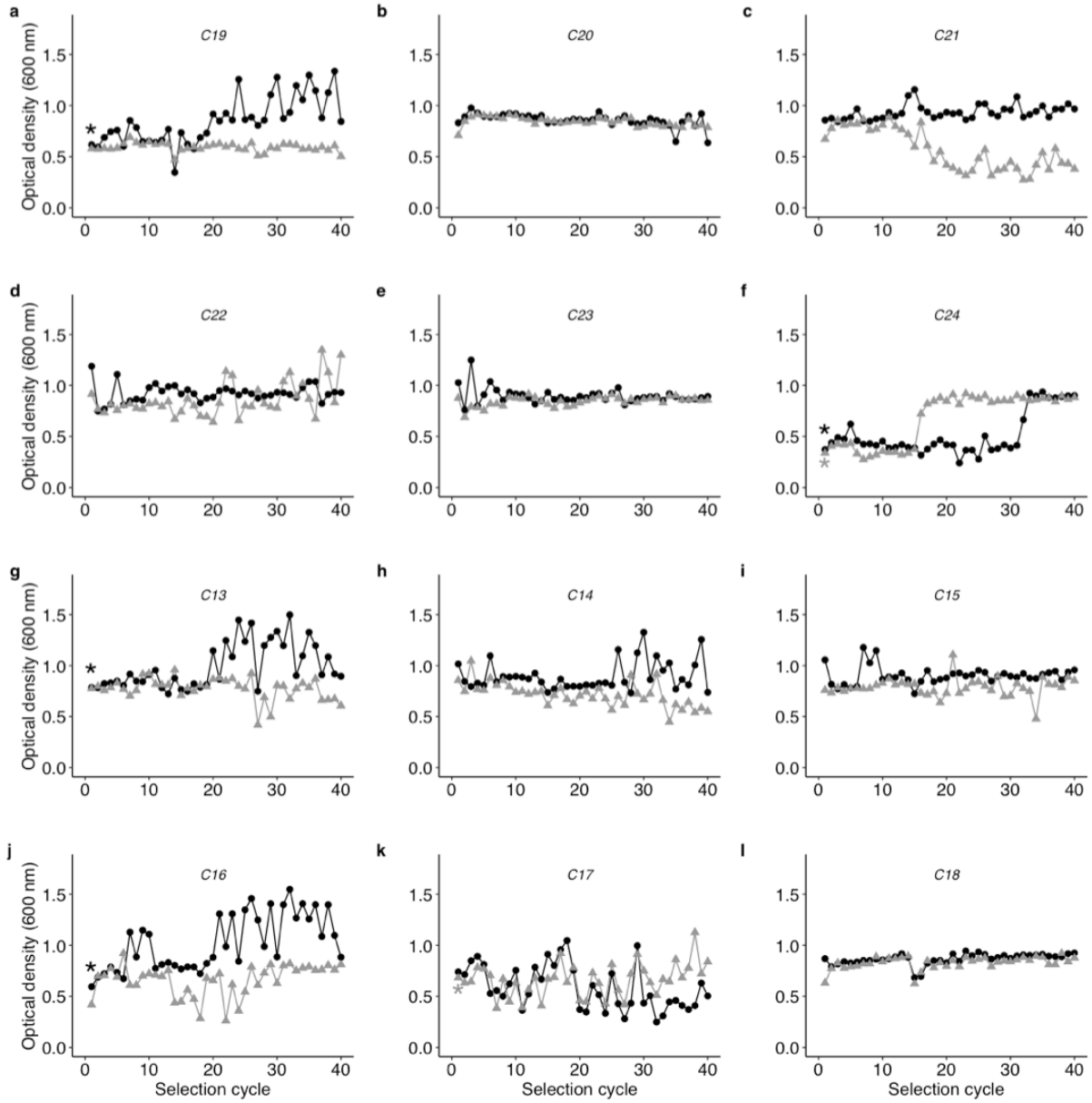

Figure S2.3 Changes in optical density (OD) over experimental evolution under artificial selection and no artificial selection in levels of richness 2 and 4. a to f) communities of richness level 2. **g to l)** communities of richness level 4. The OD of the parent of the next population of selection units is represented by a black circle for AS and a grey triangle for NS. The OD at cycle 0 was equal to 0.002 for all the treatments. The treatments in which a contamination was detected are identified by an asterisk (at cycle 1).

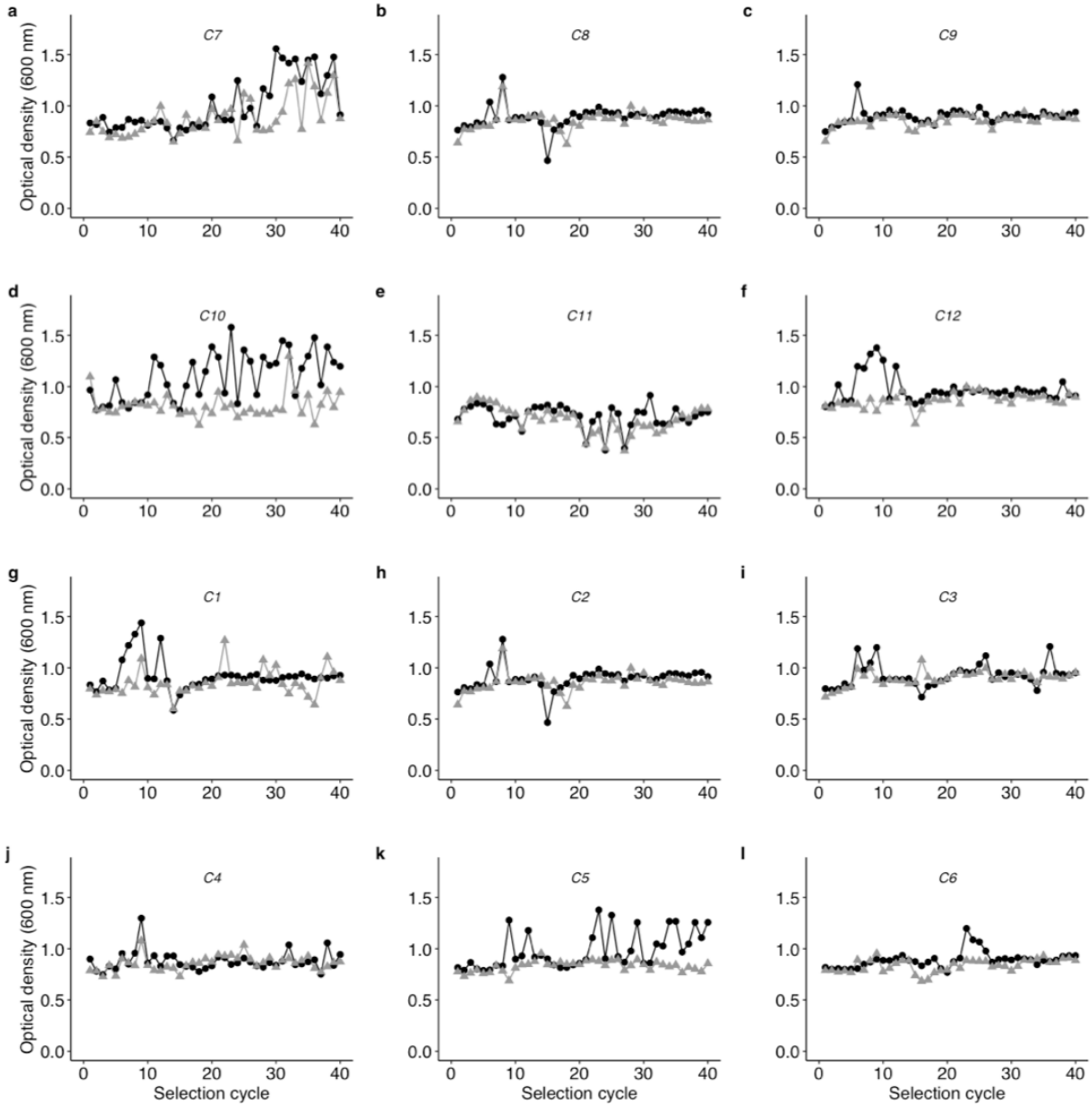

Figure S2.4 Changes in optical density (OD) over experimental evolution under artificial selection and no artificial selection in levels of richness 8 and 16. a to f) communities of richness level 8. **g to l)** communities of richness level 16. The OD of the parent of the next population of selection units is represented by a black circle for AS and a grey triangle for NS. The OD at cycle 0 was equal to 0.002 for all the treatments.

Appendix 2.4 Metabolism data analysis

To analyse the metabolism data, we first calculated the difference between the OD produced from one substrate and the mean OD of the control wells (containing water) for each individual at each studied time (negative values were set to zero). Then, for each substrate, we determined a threshold value from which the OD was sufficiently different from zero to consider the substrate as metabolized. To determine this threshold value, we identified two populations in the values differing from zero using a mixture model, these two populations corresponded to a first group of values of low variance and high density (non-metabolized) and to a second group of values with a higher variance and a lower density (metabolized). The OD of the sample separating the two populations was taken as the threshold value (Figure S2.5a). For six substrates over the 31, the values had to be divided into three populations in order to distinguish the high-density low-variance population (Figure S2.5b and c). A substrate was considered as metabolized if the measured OD overpassed the threshold value for this substrate at least for the two last measurements in time and for the three replicates. For the metabolized substrates, we extracted the maximum OD and then described the metabolization dynamic by linear regression or linear segmented regression. For each sample, we ran regressions with zero to two breakpoints and kept the slope estimations of the regression showing the highest R^2. In order to compare the slopes whatever the regression used, we kept the maximum slope value for each sample. The maximum slope and maximum OD were averaged for the three replicates of an individual. These two indicators were highly correlated (ρ=0.96, $p<2.2x10^{-16}$) therefore the analyses were conducted on the maximum OD only.

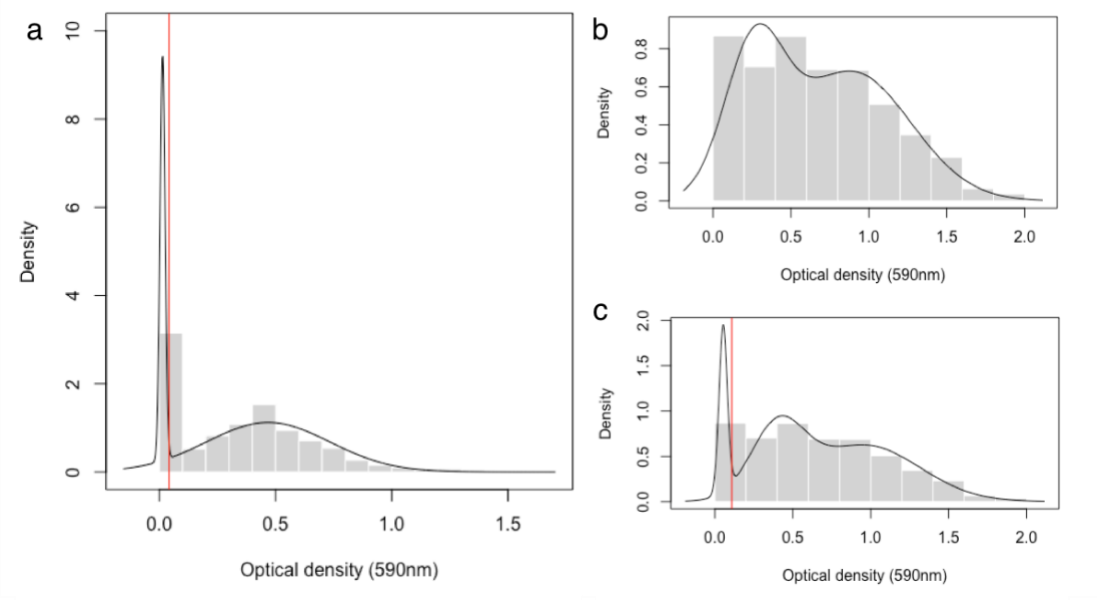

Figure S2.5 Threshold value determination for metabolism data analysis. For each of the 31 substrates studied in the experiment, we determined a threshold value of optical density (OD) from which we considered that a substrate was metabolized as compared to the OD measured on a control containing water. **a)** For β-methyl-D-glucoside, as for 24 other substrates, a mixture model with two gaussian distributions enable to distinguish between a first population of value of high density and low variance (i.e. samples in which the substrate was not metabolized) and a second population of low density and high variance (i.e. samples in which the substrate was metabolized). In these cases, the threshold value (represented by a vertical red line) was the OD of the sample separating the two populations. **b)** For pyruvic acid methyl ester, as for five other substrates, a mixture model with two gaussian distributions did not allow the identification the population of values of high density and low variance. **c)** In these cases, fitting a mixture model with three gaussian distributions was necessary. The threshold value was the OD of the sample separating the first two populations.

Appendix 2.5 Supplementary figures

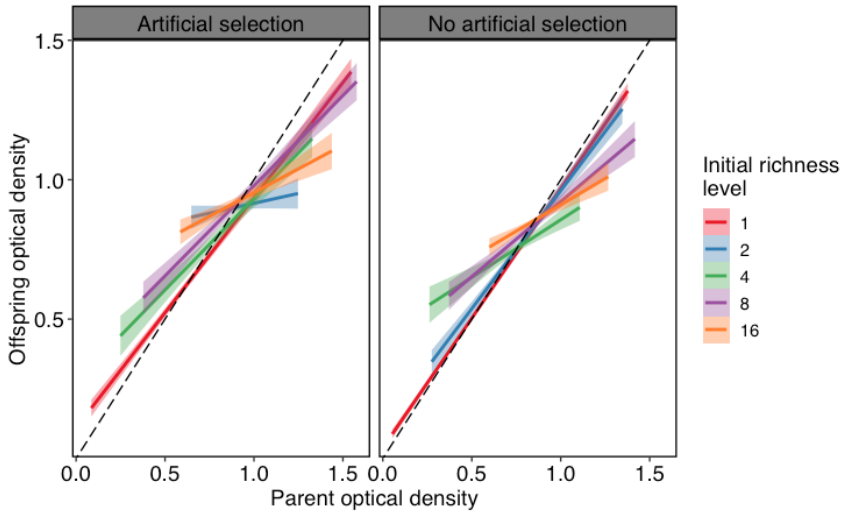

Figure S2.6 Correlation between offspring and parent phenotype. The optical density of the offspring (i.e. OD of the selected well at selection cycle n+1) is presented against the optical density of the parents (i.e. OD of the selected well at selection cycle n) for each initial richness level within each selection method (left: artificial selection, right: no artificial selection). The theoretical regression line for a correlation value of 1 is represented by a black dashed-line. There was a significant effect of the interaction parent optical density*initial richness level*selection method on the offspring optical density (χ^2=28; $p_{df=4}$=1.2x10^{-5}).

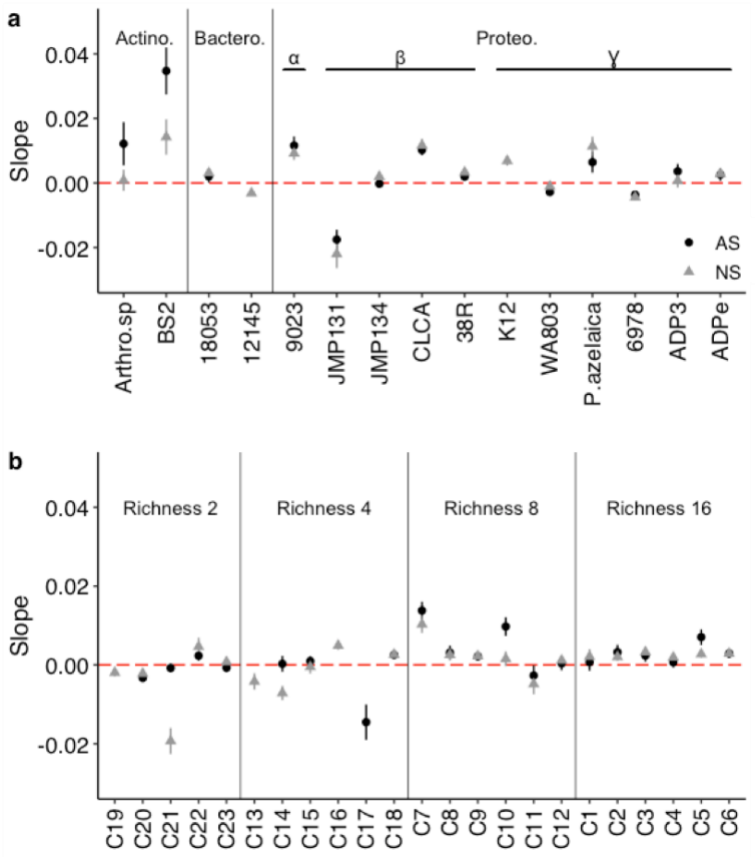

Figure S2.7 Changes in optical density (OD) over the experimental evolution under artificial selection (AS) and no artificial selection (NS) for a) each monoculture and b) community of the experiment. The slopes of the regression lines predicted by a linear mixed model (with the identity of the individual nested into the selection method as a random effect factor on the slope and the intercept) are presented in black circles for AS and grey triangles for NS for each initial richness level. Bars represent SE. Actino.: Actinobacteria, Bactero.: Bacteroidetes, Proteo.: Proteobacteria. α: α-Proteobacteria, β: β-Proteobacteria, ɣ: ɣ-Proteobacteria.

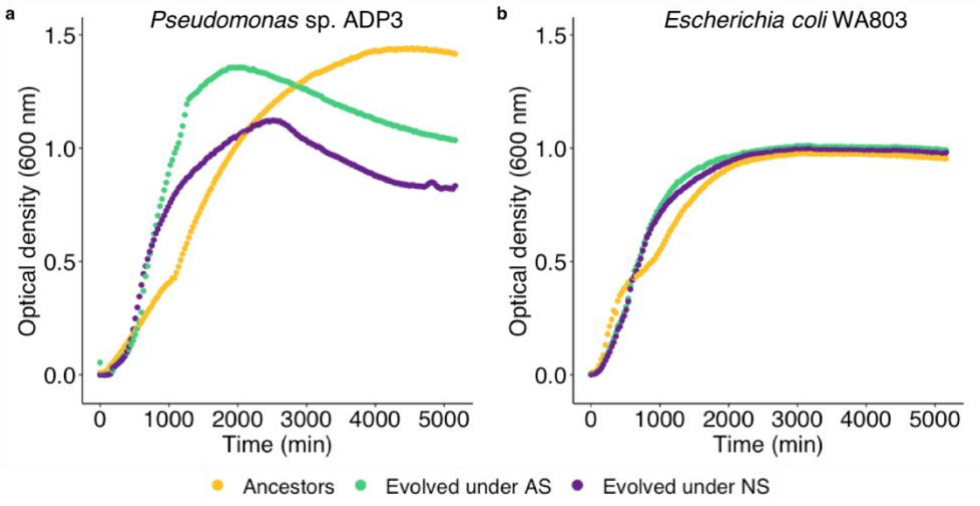

Figure S2.8 Growth curves of two monocultures depending on their evolutionary history. Each point corresponds to the mean optical density of three independent measurements. Yellow: ancestors, green: evolved under artificial selection, violet: evolved under no artificial selection.

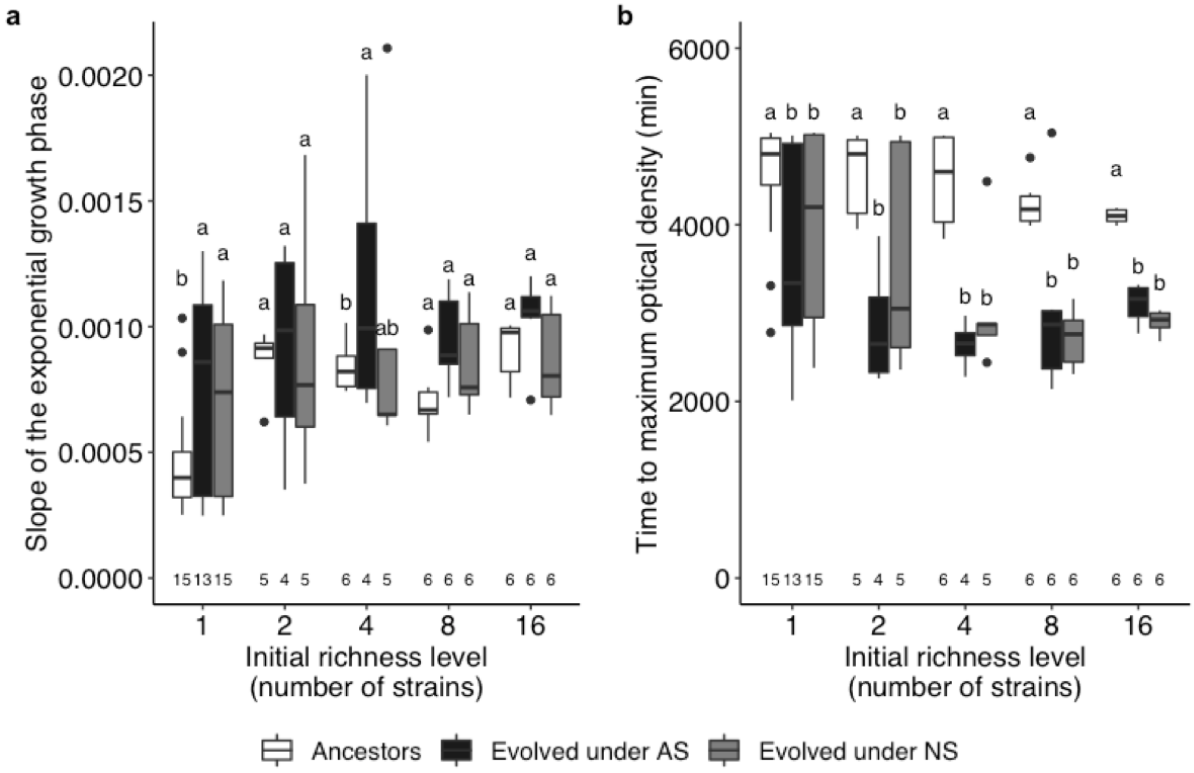

Figure S2.9 Growth parameters of the ancestors and evolved monocultures and communities under artificial and no artificial selection depending on the initial richness level. a) Slope of the exponential growth phase. **b)** Time to reach the maximum OD. Each box represents the first quartile, the median and the third quartile for a given treatment, the end of the bars shows the minimal and maximal values within 1.5 times the interquartile range. The points outside of the boxes represent outliers. Sample sizes are given on the bottom of the graphs. Different letters represent significant differences between the levels of history within a richness level. White: ancestors; black: evolved under artificial selection; grey: evolved under no artificial selection.

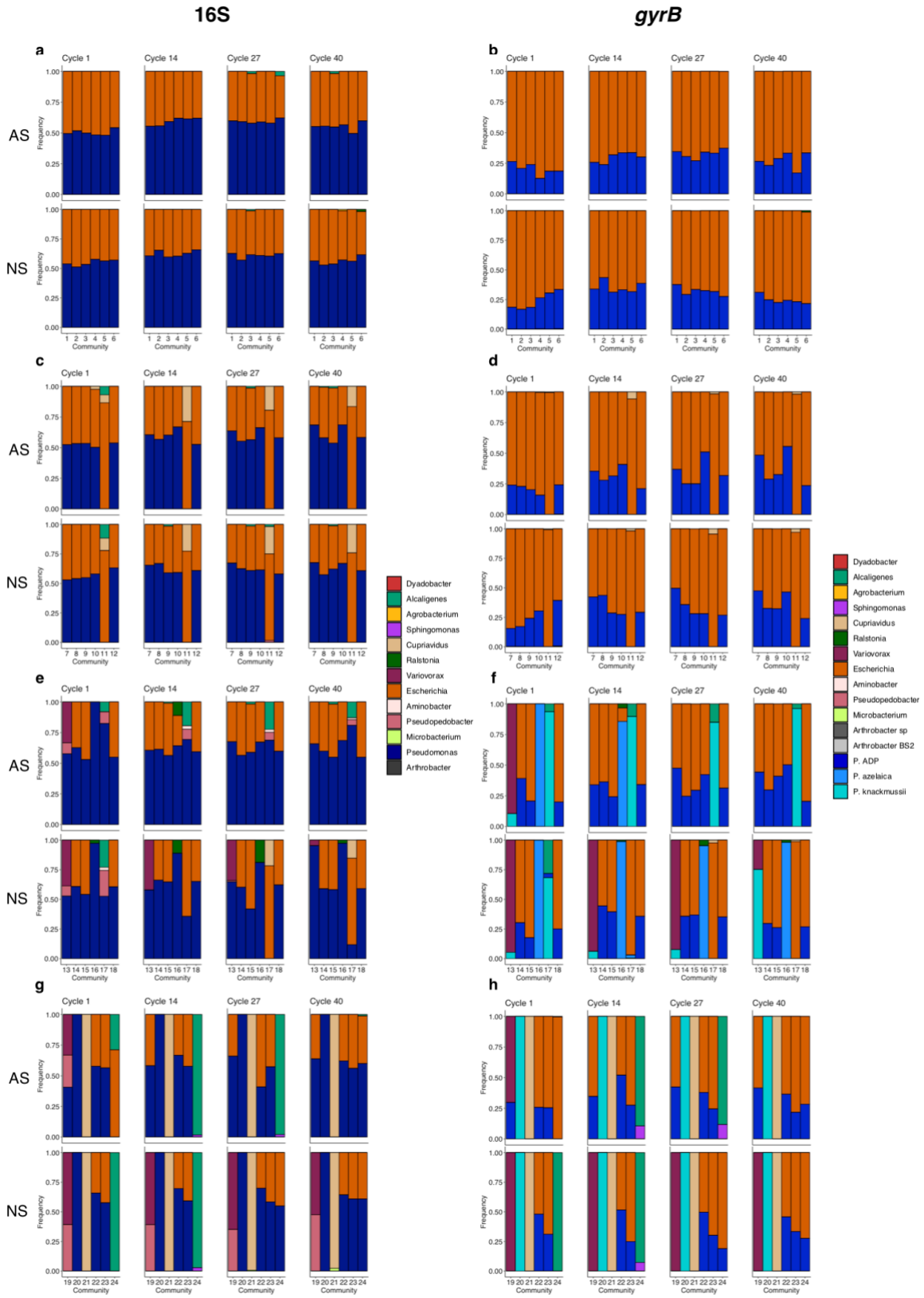

Figure S2.10 Community composition over the selection cycles. The composition of the communities was analyzed by 16S RNA gene sequencing (a, c, e, g) and by gyrB gene sequencing (b, d, f, h) at cycles 1, 14, 27 and 40 (from left to right) for communities under artificial selection (AS; on the top) and no artificial selection (NS, on the bottom). The data from 16S RNA gene sequencing provide information at the genus level whereas the data from gyrB gene sequencing enable to distinguish the different strains, except for the two E. coli strains and the two P. sp. ADP strains. The relative frequencies of the different strains in a community were obtained from the sequence counts (note that the 16S RNA gene is a multicopy gene whereas gyrB is a monocopy gene). The samples in which a contamination was detected are included in the figure.

Appendix 2.6 Supplementary tables

Table S2.1 Theoretical community composition at the beginning of the experiment. Six different communities per richness level (four levels) were built. The communities from level 16 were built at random from a pool of 18 strains and the communities from the lower levels of richness were subsets of the communities from the higher levels. The full names of the strains are given in Table 2.1.

Level 16	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**
	V. sp. 38R *P. saltans* DSM 12145 *A. aminovorans* SR38 *P. knackmussii* DSM 6978 *M.* sp. C448 *C. necator* JMP134 *P.* sp. ADP3 *E. coli* K12 *E. coli* WA803 *P.* sp. ADPe *A. eutrophus* JMP131 *S. wittichii* RW1 *R.* sp. *A.* sp. *A.* sp. BS2 *P. azelaica*	*A. aminovorans* SR38 *P. knackmussii* DSM 6978 *P. saltans* DSM 12145 *A. eutrophus* JMP131 *P.* sp. ADPe *E. coli* WA803 *S. wittichii* RW1 *A.* sp. 9023 *E. coli* K12 *C. necator* JMP134 *A.* sp. BS2 *M.* sp. C448 *D. fermentans* DSM 18053 *P.* sp. ADP3 *A.* sp. *V.* sp. 38R	*E. coli* WA803 *A.* sp. 9023 *A.* sp. BS2 *P. saltans* DSM 12145 *M.* sp. C448 *C. necator* JMP134 *A. eutrophus* JMP131 *S. wittichii* RW1 *P. azelaica* *P.* sp. ADPe *A.* sp. *D. fermentans* DSM 18053 *E. coli* K12 *R.* sp. *P. knackmussii* DSM 6978 *A. aminovorans* SR38	*D. fermentans* DSM 18053 *E. coli* K12 *P. saltans* DSM 12145 *V.* sp. 38R *A. eutrophus* JMP131 *M.* sp. C448 *P.* sp. ADPe *S. wittichii* RW1 *P. knackmussii* DSM 6978 *P.* sp. ADP3 *R.* sp. *A.* sp. 9023 *E. coli* WA803 *P. azelaica* *A.* sp. *A.* sp. BS2	*P.* sp. ADPe *A.* sp. 9023 *A.* sp. *S. wittichii* RW1 *V.* sp. 38R *P.* sp. ADP3 *P. knackmussii* DSM 6978 *C. necator* JMP134 *E. coli* WA803 *P. azelaica* *A. aminovorans* SR38 *R.* sp. *P. saltans* DSM 12145 *M.* sp. C448 *A. eutrophus* JMP131 *E. coli* K12	*A. aminovorans* SR38 *A.* sp. 9023 *A.* sp. BS2 *P.* sp. ADPe *M.* sp. C448 *V.* sp. 38R *D. fermentans* DSM 18053 *P. azelaica* *C. necator* JMP134 *P.* sp. ADP3 *P. knackmussii* DSM 6978 *R.* sp. *A. eutrophus* JMP131 *S. wittichii* RW1 *E. coli* K12 *P. saltans* DSM 12145
Level 8	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**
	V. sp. 38R *P. saltans* DSM 12145 *A. aminovorans* SR38 *P. knackmussii* DSM 6978 *M.* sp. C448 *C. necator* JMP134 *P.* sp. ADP3 *E. coli* K12	*E. coli* WA803 *P.* sp. ADPe *A. eutrophus* JMP131 *S. wittichii* RW1 *R.* sp. *A.* sp. *A.* sp. BS2 *P. azelaica*	*A. aminovorans* SR38 *P. knackmussii* DSM 6978 *P. saltans* DSM 12145 *A. eutrophus* JMP131 *P.* sp. ADPe *E. coli* WA803 *S. wittichii* RW1 *A.* sp. 9023	*E. coli* K12 *C. necator* JMP134 *A.* sp. BS2 *M.* sp. C448 *D. fermentans* DSM 18053 *P.* sp. ADP3 *A.* sp. *V.* sp. 38R	*E. coli* WA803 *A.* sp. 9023 *A.* sp. BS2 *P. saltans* DSM 12145 *M.* sp. C448 *C. necator* JMP134 *A. eutrophus* JMP131 *S. wittichii* RW1	*P. azelaica* *P.* sp. ADPe *A.* sp. *D. fermentans DSM 18053* *E. coli* K12 *R.* sp. *P. knackmussii* DSM 6978 *A. aminovorans* SR38
Level 4	**C13**	**C14**	**C15**	**C16**	**C17**	**C18**
	V. sp. 38R *P. saltans* DSM 12145 *A. aminovorans* SR38 *P. knackmussii* DSM 6978	*M.* sp. C448 *C. necator* JMP134 *P.* sp. ADP3 *E. coli* K12	*E. coli* WA803 *P.* sp. ADPe *A. eutrophus* JMP131 *S. wittichii* RW1	*R.* sp. *A.* sp. *A.* sp. BS2 *P. azelaica*	*A. aminovorans* SR38 *P. knackmussii* DSM 6978 *P. saltans* DSM 12145 *A. eutrophus* JMP131	*P.* sp. ADPe *E. coli* WA803 *S. wittichii* RW1 *A.* sp. 9023
Level 2	**C19**	**C20**	**C21**	**C22**	**C23**	**C24**
	V. sp. 38R *P. saltans* DSM12145	*A. aminovorans* SR38 *P. knackmussii* DSM 6978	*M.* sp. C448 *C. necator* JMP134	*P.* sp. ADP3 *E. coli* K12	*E. coli* WA803 *P.* sp. ADPe	*A. eutrophus* JMP131 *S. wittichii* RW1

Table S2.2 Deviance table of the analysis of variance (ANOVA) of the optical density (OD) of the ancestors and evolved monocultures and communities under artificial and no artificial selection depending on the initial richness level. The effect of the initial richness level (1, 2, 4, 8 ,16), the history (ancestors, evolved under artificial selection, evolved under no artificial selection), the dataset (experimental evolution, post-selection) and their interactions on OD were estimated with a linear mixed model including the identity of the selection unit as a random effect factor on the intercept. The conditional R^2 is presented (i.e. variance explained by both fixed and random effect factors; the marginal R^2 – fixed effect factors only – was 0.48).

	Df	Chi squared	p
Initial richness level	4	54.5	**4.07×10^{-11}**
History	2	82.6	**$< 2.2 \times 10^{-16}$**
Dataset	1	1.18	0.278
Initial richness level * History	8	3.01	0.934
Initial richness level* Dataset	4	1.45	0.836
History * Dataset	2	3.16	0.206
Initial richness level * History * Dataset	8	6.08	0.639
		R^2=0.75	

Table S2.3 Deviance tables of the analyses of variance (ANOVA) of the growth parameters of the ancestors and evolved monocultures and communities under artificial and no artificial selection depending on the initial richness level. The effect of the initial richness level (1, 2, 4, 8 ,16), the history (ancestors, evolved under artificial selection, evolved under no artificial selection) and their interaction on the slope of the exponential growth phase and the time to reach the maximum OD were estimated with a linear mixed model including the identity of the selection unit as a random effect factor on the intercept. The conditional R^2 are presented (i.e. variance explained by both fixed and random effect factors; the marginal R^2 – fixed effect factors only – were 0.24 and 0.40).

	Slope			Time to maximum OD		
	Df	Chi squared	p	Df	Chi squared	p
Initial richness level	4	12.0	**$1.76x10^{-2}$**	4	9.14	$5.77x10^{-2}$
History	2	16.3	**$2.88x10^{-4}$**	2	86.6	**$<2x10^{-16}$**
Initial richness level * History	8	5.01	0.756	8	13.2	0.107
		$R^2 = 0.56$			*$R^2=0.69$*	

Table S2.4 Deviance table of the analysis of variance (ANOVA) of the optical density (OD) of the ancestors and evolved monocultures and communities under artificial and no artificial selection depending on the initial richness level and the substrate category. The effect of the initial richness level (1, 2, 4, 8 ,16), the history (ancestors, evolved under artificial selection, evolved under no artificial selection), the substrate category amines, amino acids, carbohydrates, carboxylic acids, phenolic compounds, polymers) and their interactions on OD were estimated with a linear mixed model including the identity of the selection unit and the substrate as random effect factors on the intercept. The conditional R^2 is presented (i.e. variance explained by both fixed and random effect factors; the marginal R^2 – fixed effect factors only – was 0.20).

	Df	Chi squared	p
Initial richness level	4	42.7	**$1.21x10^{-8}$**
History	2	14.7	**$6.42x10^{-4}$**
Substrate category	5	4.17	0.526
Initial richness level * History	8	38.5	**$6.10x10^{-6}$**
Initial richness level * Substrate category	20	127	**$<2.2x10^{-16}$**
History * Substrate category	10	12.5	0.256
Initial richness level * History * Substrate category	40	29.2	0.896
		$R^2=0.58$	

APPENDIX 3: SUPPLEMENTARY MATERIAL CHAPTER 3

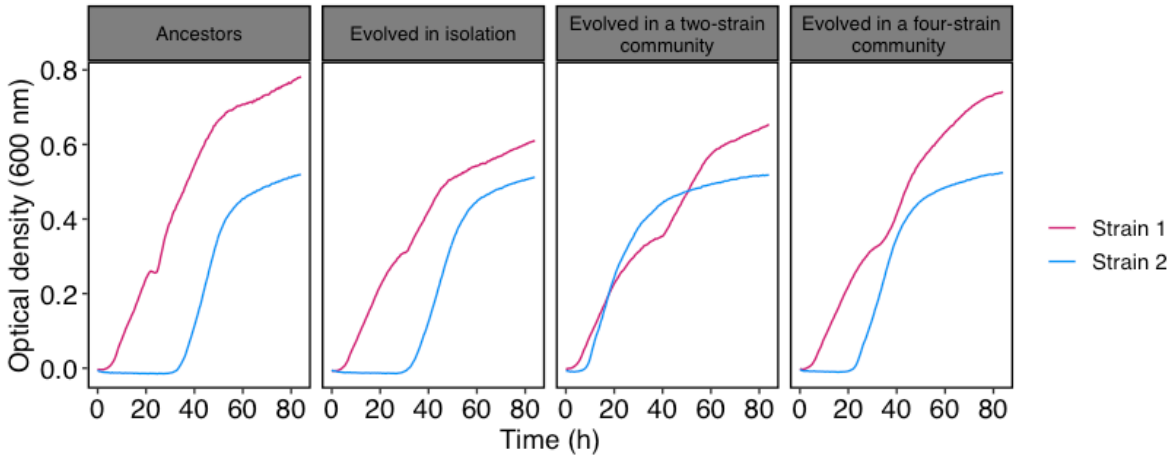

Figure S3.1 Growth curves of *Variovorax* sp. 38R (Strain 1) and *Pseudopedobacter saltens* DSM12145 (Strain 2) as monocultures in environment 2 depending on their evolutionary history. The optical density at 600 nm was assessed every 30 minutes for 84 hours. The curves represent the mean values obtained from eight replicates of each strain grown as a monoculture.

Table S3.1 Analysis of variance (ANOVA) of the optical density (OD) of the communities and community members. The effect of the biological entity (strain 1, strain 2, community), the history (ancestors, evolved in community, evolved in isolation), the environment (1, 2) and their interactions on OD were estimated with a linear mixed model including the identity of the strain or community as a random effect factor. The conditional R^2 is presented (i.e. variance explained by both fixed and random effect factors; the marginal R^2 – fixed effect factors only – was 0.63).

	Df	Chi squared	p
Biological entity	2	19.3	**6.38×10^{-5}**
History	2	104	**$< 2.2 \times 10^{-16}$**
Environment	1	2817	**$< 2.2 \times 10^{-16}$**
Biological entity * History	4	193	**$< 2.2 \times 10^{-16}$**
Biological entity * Environment	2	19.3	**6.32×10^{-5}**
History * Environment	2	46.5	**7.95×10^{-11}**
Biological entity * History * Environment	4	47.9	**1.01×10^{-9}**
		$R^2=0.85$	

APPENDIX 4: SUPPLEMENTARY MATERIAL DISCUSSION

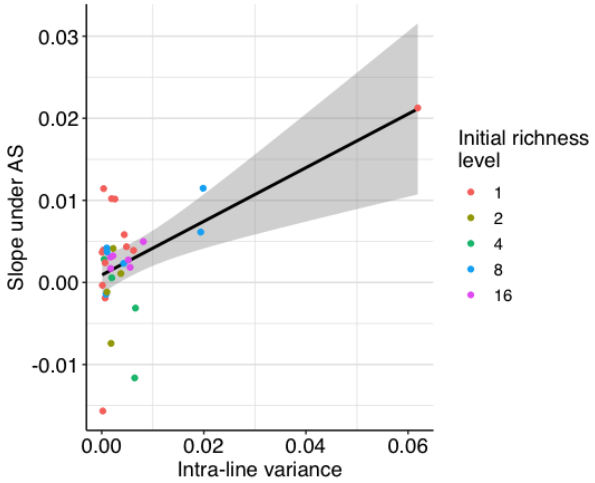

Figure S4.1 Effect of the phenotypic variance within lines on the artificial selection efficiency. In Chapter 2, we had five treatments (i.e. five levels of initial richness) in which we had between four and 13 lineages (i.e. metacommunities) once the contaminated lines were removed. Each line was composed of ten communities. The change in optical density (i.e. the property targeted by the artificial selection) through the selection cycles (= "slope under AS") was calculated for each line of the experiment (i.e. each metacommunity), to describe the artificial selection efficiency, as well as the intra-line variance in optical density. This figure shows the positive effect of the intra-line variance on the artificial selection efficiency ($y=0.33x+9.0x10^{-4}$, $p_{df=31}=8.7x10^{-4}$, $R^2=0.28$). This positive effect is respectively due to one and two lines of richness levels 1 and 8 that showed a much higher variance than the others. These lines have a high leverage value (i.e. a high influence on the regression) however there are not outliers (their Cook's distance is lower than 0.5 which means that the removal of one of them would not alter the results; Cook 1977).

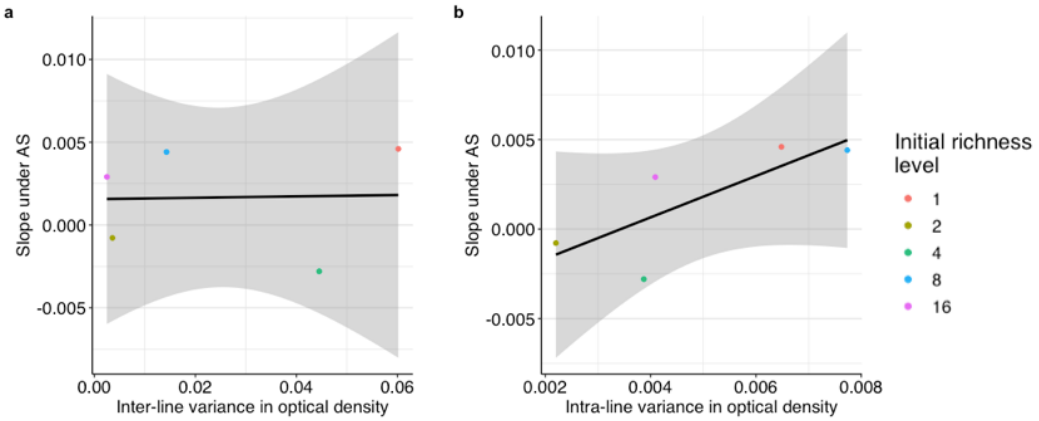

Figure S4.2 Effect of the phenotypic variance among and within lines on the artificial selection efficiency. For each of the five treatments of Chapter 2, we determined the change in optical density through the selection cycles (= "slope under AS") to describe the artificial selection efficiency. Then, for each treatment, we also characterized the variance in optical density between the different lines and within the different lines. Figure S4.2a shows the absence of effect of the inter-line variance on the efficiency of the artificial selection ($y=0.0041x+0.0016$, $p_{df=3}=0.96$, $R^2=0$). Figure S4.2b shows that there is a trend to a positive effect ($y=1.2x-0.0040$, $p_{df=3}=0.12$, $R^2=0.47$) of the intra-line variance on the efficiency of the artificial selection i.e. the treatments with the highest intra-line variance (richness levels 1 and 8) were also those with the highest slope under AS.

Between the experiments presented in chapters 1 and 2, we conducted an experiment to study the biodiversity-ecosystem functioning relationship. We wanted to assess the potential influence of soil microbes on the relationship between plant species diversity, plant genotypic diversity and productivity. The results of this study are presented below and have been published in Plant and Soil in July 2021 (https://doi.org/10.1007/s11104-021-05071-z).

APPENDIX 5: SOIL MICROBES DRIVE THE EFFECT OF PLANT SPECIES AND GENOTYPIC DIVERSITY INTERACTION ON PRODUCTIVITY.

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Abstract

The effect of plant species and genotypic diversity on productivity has been well documented but little is known about the contribution of the interaction between species and genotypic diversity. Since the influence of soil microorganisms on the plant diversity-productivity relationship is increasingly recognized, we investigated potential interactions between plant species diversity, plant genotypic diversity and soil microbes. We hypothesized that the non-additive effects of plant species and genotypic diversity on productivity could be microbe-driven.

We set up pea and wheat monocultures and mixtures in a growth chamber, varying for their levels of genotypic diversity under three different soil microbial contexts. We assessed plant shoot and root biomass production, soil mineral nitrogen content and described the soil bacterial communities.

We found that shoot biomass in mixtures involving both species and genotypic diversity was higher than expected considering the additive effects of plant species diversity and genotypic diversity. Rather than a synergy between two positive effects of species and genotypic diversity, we observed that species mixture compensated the negative effect of genotypic diversity. Regarding microbial influence, we found that the effect of plant species diversity, plant genotypic diversity and their interaction on productivity were all driven by the soil microbial community as no effect was observed in a pre-sterilised soil.

Our study suggests that the plant diversity-productivity relationship could be shaped by a three-way interaction between plant species diversity, plant genotypic diversity and soil microbes. Thus, plant-microbe and plant-plant interactions could be a determinant of the plant diversity-productivity relationship.

Keywords: diversity-productivity relationship, genotypic diversity, plant-microbe interactions, soil microbes, species diversity

INTRODUCTION

Plant species diversity is known to have positive effects on ecosystem functioning. In particular, a lot of studies highlighted a positive effect of species diversity on productivity (Tilman et al. 1996; Hooper and Vitousek 1997; Yachi and Loreau 1999; Hector et al. 1999) with evidence that functional group richness, and further, functional dissimilarity between groups (Heemsbergen et al. 2004), play a greater role than species richness per se (Grime 1997; Balvanera et al. 2006). The mechanisms that could explain a positive diversity-productivity relationship are often grouped under two main categories of effects: "complementarity effects" (i.e. resource partitioning and/or facilitation) and "sampling effect" (increase in the probability of including a highly productive species with the increase in the number of species in a community; Loreau et al. 2001; Cardinale et al. 2002). Complementarity effects, and more particularly complementarity in resource use, have received attention as they are potential levers for enhancing the use of available resources in soils and decreasing nutrient losses (Tilman et al. 1996). A recent study highlighted that peak overyielding in a five-species mixture (i.e. gain in productivity as compared to mean monoculture performance) co-occurred with an enhanced resource-use efficiency for water, nitrogen and light (resource use related to dry mass production; Mason et al. 2020).

The same mechanisms can be involved in positive effects of within-species genotypic diversity on productivity (Hughes et al. 2008). Indeed, although genotypic variability within a plant species is supposed to be lower than between species variability (Jung et al. 2010; Albert et al. 2011; Kichenin et al. 2013), many studies have shown a positive effect of increasing within-species genotypic diversity on productivity (Johnson et al. 2005; Crutsinger 2006; Cook-Patton et al. 2011). Cook-Patton et al. (2011) showed that increasing the genotypic diversity in an herbaceous species (from one to eight genotypes) gave rise to a similar increase in productivity (about 17%) than increasing species diversity (from one to eight old-field species excluding the previously studied herbaceous species). But, in another study, Prieto et al. (2015) showed that increasing the species diversity (from one to five grassland species) increased productivity, whereas increasing the genotypic diversity within each species of the mixture (from one to ten genotypes per species) did not influence plant productivity.

To go further, there is evidence that species and genotypic diversity can interact and lead to modifications in competition and dominance patterns among species (Fridley and Grime 2010; Crawford and Rudgers 2012; Schöb et al. 2015). As an example, Fridley and Grime (2010) showed with neighbourhood modelling that an increase in genotypic diversity in grassland communities lead to a reduction in interspecific competition which tended to increase community effective richness (i.e. a higher number of species was maintained over the experiment, with a dependence on soil depth). In this

case, this change in community structure was not large enough to induce a change in productivity. But, in other studies, it has been shown that increasing genotypic diversity within a plant species can either increase the benefit of species diversity on productivity (Crawford and Rudgers 2012) or decrease it (Schöb et al. 2015). Thus, understanding the interaction between species and genotypic diversity is mandatory to understand the plant diversity-productivity relationship.

Soil microorganisms can also greatly influence plant primary productivity, through direct (mutualists and pathogens) or indirect effects (free-living microorganisms through their influence on resource availability; Van der Heijden et al. 2008). The effects of microbial mutualists, in particular of rhizobia and arbuscular mycorrhizal fungi, and of pathogens on plant productivity are well-studied and there is evidence for strong effects of the microbial species identity (Vogelsang et al. 2006; Wagg et al. 2011b; Bever et al. 2013). Soil microbes can also affect plant community composition and functioning (Reynolds et al. 2003). One well-studied mechanism is plant-soil feedback (Van der Putten et al. 2013) which can occur through the influence of plants on the soil microbial community which, in turn, influences the future plant community (Bennett and Klironomos 2019). Indeed, the presence of a particular plant species can promote certain soil microbes (e.g. mutualistic partners or pathogens; Mills and Bever 1998; Bever 2002) and, due to the specificity of the interactions between plants and soil microbes (i.e. the existence or not of an interaction and the partner response to the interaction), this could favor or reduce the growth of the seedlings of this species. The existence of a negative plant-soil feedback could be a promoter of plant community diversity while a positive plant-soil feedback could give rise to the dominance of a species (Revillini et al. 2016).

The interdependence between plants and microorganisms for essential functions (e.g. nutrition) can be so strong that it could be useful to consider a plant and its associated microbes as a single functional and evolutionary unit, referred to as a holobiont (Zilber-Rosenberg and Rosenberg 2008; Vandenkoornhuyse et al. 2015). This is of particular interest in the study of the plant diversity-productivity relationship because it suggests that microbes could play a significant role in explaining this relationship. Many studies have highlighted the crucial role of plant pathogens and mutualists, especially fungi, in explaining either the existence (Maron et al. 2011; Wang et al. 2019) or the shape (Klironomos et al. 2000; Schnitzer et al. 2011) of the plant diversity-productivity relationship. These studies were focused on the link between plant species richness and productivity. But to the best of our knowledge, there is no study to date that investigated the effects of soil microbes on *i)* the link between plant genotypic diversity and productivity and *ii)* the interactions between plant species and genotypic diversity on productivity.

In this study, we wanted to determine if the effect of the interaction between species and genotypic diversity on productivity was influenced by the soil microbial community. Our experimental design involved pea-wheat mixtures varying for their levels of genotypic diversity in three soil microbial contexts (sterilised soil, native microbial community, exogenous microbial community). We first hypothesized that combining plant species and genotypic diversity could lead to non-additive

effects on ecosystem productivity and, more specifically, synergistic effects could arise through an increase in complementarity and resource use as compared with a case involving only one of the diversity levels. Then, we hypothesized that soil microorganisms could drive potential interactions between plant species and genotypic diversity based on the assumption that the effect of soil microbes on plant productivity could be genotype and/or species dependent and thus lead to changes in plant-plant interactions (e.g. competition enhancement or reduction). We expected the diversity effects on plants to be lower in a soil with low microbial diversity (i.e. sterilised soil) and different between a native and an exogenous microbial community due to differences in plant-soil feedbacks (Bennett and Klironomos 2019). Reciprocally, we also expected an effect of the plant community on soil microbial community structure and diversity as already shown in monoculture-mixture experiments using pea and wheat (Taschen et al. 2017).

MATERIALS AND METHODS

Experimental design

We grew two species which are commonly grown in mixtures: wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.), and for which positive plant-plant interactions have been described (Duchene et al. 2017). Three genotypes of each species were selected with contrasting phenotypic characteristics (described in Table S5.1). Genotypic monocultures and genotypic mixtures were set up in species monoculture or species mixture resulting in four species monoculture treatments: one wheat genotype (W1), one pea genotype (P1), three wheat genotypes (W3), three pea genotypes (P3); and four pea-wheat mixture treatments (W1P1, W1P3, W3P1, W3P3; Figure 5.1). W1 refers to the wheat genotype "Flamenko", W3 refers to the association of the wheat genotypes "Flamenko", "Renan" and "RE13088", P1 refers to the pea genotype "Cameor" and P3 refers to the association of the pea genotypes "Cameor", "Champagne" and "336.11". Note that the genotypes "Renan", "RE13088", "Champagne" and "336.11" were not grown in monoculture due to technical constraints (growth chamber capacity). The analyses were therefore adapted to take this limitation into account (see the "Biomass deviation from additivity" section). The

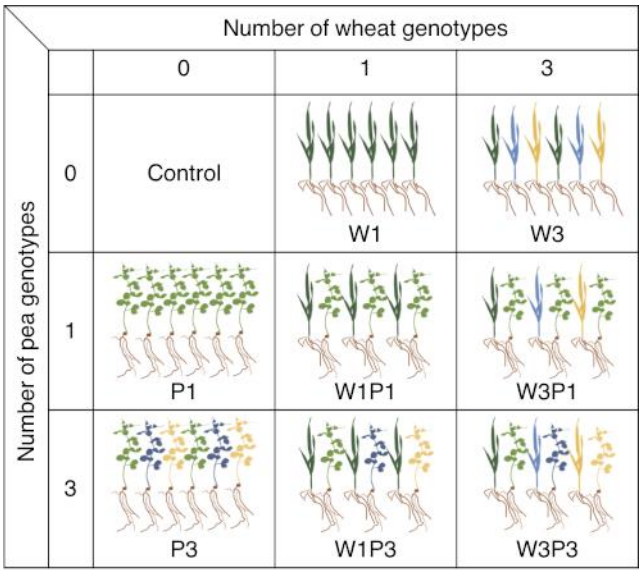


Figure 5.1 Experimental design. Two plant species, pea and wheat, were grown under two levels of species diversity, i.e. monoculture or pea-wheat mixture, and two levels of genotypic diversity, i.e. one or three genotypes per species. Abundance was fixed to six plants per pot. W: wheat; P: pea; 1: one genotype; 3: three genotypes. W1 and P1 refer to the genotypes Flamenko and Cameor respectively and W3 and P3 refer to the association of Flamenko, Renan, RE13088 and Cameor, Champagne, 336.11 respectively.

genotype that was chosen for being grown in monoculture for each species was the best performing in our experimental conditions (i.e. higher germination rate and emergence after transplantation) based on the results of a pre-experiment. The abundance was set to six plants per pot whatever the plant diversity and seedlings were planted in circle alternating genotypes and/or species, to ensure each individual had potentially the same neighborhood as the others. Each plant diversity treatment was repeated five times per microbial context (three different soil microbial communities, see "Microbial context" section) as well as a control treatment without plant (control, C), for a total of 135 pots.

Plant materials

Wheat seeds were obtained from Agri Obtentions (Guyancourt, France) and pea seeds from "Centre de Ressources Génétiques" (INRAE Dijon, France). Wheat seeds were placed on blotting paper soaked with osmotic water for six days (one day at 4 °C in the dark, three days at 18 °C in the dark, two days at 20 °C in constant light). Pea seeds were covered with osmotic water for two days (24 °C, in the dark). Wheat and pea were transplanted simultaneously; the first leaf emerged from the coleoptile in wheat seedlings and the radicle emerged from the pea seed. The transplantation time after sowing was determined according to a pre-experiment to avoid the dominance of one species from the very beginning of the experiment. Transplantation occurred 48 h after pot filling.

Microbial context

The soil used in this experiment was a sieved and gamma-sterilised (Ionisos, France) sandy soil from a natural meadow (CEREEP research station, Saint-Pierre-lès-Nemours, France), with the following characteristics: total organic carbon content: 14.7 $g.kg^{-1}$; total nitrogen content: 1.19 $g.kg^{-1}$; pH: 5.22; CEC: 4.08 $cmol.kg^{-1}$; texture: 6.9% clay, 19.0% silt, 74.1% sand. We wanted to test for an effect of the soil microbial context on the plant community. Thus, in order to avoid a possible confusing effect of soil characteristics, we used the same soil across the different microbial contexts and we inoculated, or not, a microbial community in this soil. The first microbial context, S for sterilised, corresponded to the sterilised soil without inoculation. In microbial context N (native), we inoculated a microbial community that came from the non-sterilised sandy soil cultivated in pea/wheat mixture for three weeks in the laboratory. In microbial context E (exogenous), the microbial community came from a field grown with pea/ wheat mixture (Époisses experimental station, INRAE, Bretenière, France). Six hundred ml of both inoculums were obtained by crushing four times 50 g of sieved soil in a blender for 3x30s with 150 ml of osmotic water (Philippot et al. 2013). The suspensions were then diluted ten times in osmotic water to reach a sufficient volume for the inoculation of the soil.

Growth conditions

Plants were grown in a climatic chamber for 22 days in one-liter pots filled with sandy soil. They were first watered with either 220 ml of osmotic water (S) or 120 ml of osmotic water and 100 ml of inoculum (N and E) which corresponded to 80% of the field capacity. Throughout the experiment, soil moisture was maintained between 60 and 90% of the field capacity, by watering daily with a fixed amount of water and re-adjusting water content after weighing twice a week. The conditions in the climatic chamber were: 70%-hygrometry, 12 h-photoperiod, 20 °C during the day and 18 °C at night.

Productivity measurement, soil nitrogen content assessment and microbial community analysis

Plants were harvested after 22 days of growth; shoots and roots were collected (roots were washed), dried at 50 °C for 48 h and weighed. Shoot biomass was measured for each individual separately whereas root biomass was characterised at the pot scale.

Soil mineral nitrogen content (nitrate and ammonium) at 22 days was measured by shaking 10 g of soil in 50 ml of KCl for 1 h, decantation for 45 min and analysis of the filtered supernatant with the analyser Global 240 (BPC Biosed, Rome, Italy).

Considering the colonization of the pots by plant root systems, we could hypothesize that the soil was under the influence of the plants in the entire pot. Thus, the soil of each pot was mixed and DNA was extracted from a sample of 280 mg (DNeasy PowerSoil Kit, Qiagen, Venlo, Netherlands). After ensuring that there was no PCR inhibition, 16S rRNA gene was amplified using the Pro341F/Pro805R couple of primers (targeting Bacteria, Takahashi et al. 2014). A second PCR allowed the barcoding of the samples by hybridization of specific couples of primers on the adapters carried by the Pro341F/Pro805R primers. PCR products were normalized (SequalPrep, ThermoFisher Scientific, Waltham, USA), pooled and sequenced on Illumina MiSeq (Microsynth, Balgach, Switzerland).

Bioinformatics analysis

Sequencing data was analysed following Goodrich et al. (2014) recommendations for 16S rRNA gene. Forward and reverse sequences were paired using PEAR (Zhang et al. 2014). Unassembled sequences and sequences smaller than 400 base pairs were discarded as well as chimeric sequences which were detected by reference OTU-picking against the Greengenes Gold database (VSEARCH; Rognes et al. 2016). OTU clustering was done at the 94% identity threshold (determined from the results obtained on a mock community of known species number). Taxonomy was assigned with Greengenes' representative set of sequences and based on the longest sequence of each OTU (highest base pair number). The number of sequences per sample ranged from 22,175 to 78,739. We worked on a rarefied OTU table at the level of 30,000 sequences per sample which excluded one sample from the dataset. α-diversity indices (number of observed species per sample, Simpson's reciprocal index, phylogenetic diversity) were the mean of ten independent calculations in QIIME (Caporaso et al. 2010). β-diversity

(difference in community composition between samples) was obtained by the construction of a Bray-Curtis dissimilarity matrix from the rarefied OTU table.

Biomass deviation from additivity

Diversity effects are usually defined as the difference between the observed yield in mixtures and the expected yield based on the average monoculture performances (Loreau and Hector 2001). As we did not grow all the monocultures, we defined the diversity effects as follows: the effect of genotypic diversity (GE for genotypic effect) in a given species was calculated as the difference in biomass in genotypic mixtures as compared to genotypic monocultures (i.e. W3 – W1 or P3 – P1). The effect of species diversity (SE) was the difference in biomass in the pea-wheat mixture as compared to the best of the two monocultures present in our design (i.e. W1P1 – W1), in line with what is done to study transgressive overyielding (Trenbath 1974). The effect of combining species and genotypic diversity (SGE) was calculated as the difference in biomass in mixtures involving both diversity levels (i.e. W1P3, W3P1 or W3P3) as compared with the best monoculture (W1). Mean effects and the associated 95% confidence intervals were obtained by bootstrapping (1000 calculations of each difference with replacement at each sampling event) and are showed in Table 5.1.

We determined if the effect of combining species and genotypic diversity (SGE) differed from the sum of the effect of species diversity (SE) on the one side and genotypic diversity (GE) on the other side, by calculating a "deviation from expected value" (Loreau and Hector 2001; Johnson et al. 2005). There is a deviation from additivity if "the total response of a variable is greater or less than the sum of the partitioned responses generated by the individual constituents" (Genung et al. 2010). We calculated this deviation as follows:

$$\Delta_{additivity_{ij}} = SGE_{ij} - \left(SE_j + GE_j\right)$$

With:

- $\Delta_{additivity_{ij}}$ the deviation from the additivity hypothesis in pot i in microbial context j,
- SGE_{ij} the effect of combining species and genotypic diversity in pot i in microbial context j i.e. the observed biomass in pot i in microbial context j minus the mean biomass in wheat genotypic monocultures in microbial context j.
- SE_j the mean effect of species diversity in microbial context j,
- GE_j the mean effect of genotypic diversity in microbial context j.

The expected GE depended on the mixture: the expected GE in W1P3 was the one attributed to pea (i.e. P3-P1), in W3P1 it was the one attributed to wheat (i.e. W3-W1) and in W3P3, the expected effect was the sum of the two effects previously mentioned. Note that this calculation method is based on the hypothesis that the genotypic diversity effect was constant regardless of the number of individual plants involved. We did not consider that the effect of genotypic diversity increased linearly with the number

of individuals in a mixture, as this supposed the absence of interaction effects among the individuals of a species.

Statistical analyses

Three pots over the 135 were removed from the dataset as a mistake occurred during the transplantation phase (one genotype instead of another). Fifteen pots on the remaining 132 presented an absence of growth of one of the six plants and three pots presented an absence of growth of two of the six plants. It represented 15.4% of the pots containing plants (5.1, 6.8 and 3.4% for S, N and E respectively). These 18 pots were included in the dataset to keep correct sample sizes (note that the analysis without these 18 pots gave consistent results).

Plant biomass, biomass deviation from additivity, soil mineral nitrogen content and α-diversity in the bacterial communities (observed species, phylogenetic diversity and Simpson's reciprocal index) were submitted to analyses of variance (ANOVA, type II sums of squares) including the plant treatment, the microbial context and their interaction as explanatory variables. Plant treatment refers either to plant diversity (i.e. a variable including the eight plant diversity treatments; note that for nitrogen content and α-diversity the control without plant was also included) or mixture diversity (i.e. a variable including the three plant diversity treatments involving both species and genotypic diversity) depending on the model. Wheat and pea per capita shoot biomass was submitted to analyses of variance with the species diversity (species monoculture or species mixture), the genotype, the microbial context and their interactions as explanatory variables. Each analysis of variance was followed by a post hoc Tukey's test. The β-diversity of the soil bacterial communities was analysed performing a PERMANOVA (including the plant diversity, the microbial context and their interaction as explanatory variables) with Bray-Curtis dissimilarity index, followed by pairwise comparisons. Data were visualised with a non-metric multidimensional scaling (NMDS).

We analysed the effect of soil bacterial richness on plant biomass with a linear model of plant total biomass explained by the microbial context, the plant species (pea, wheat or both), the bacterial community richness and their interactions. The slopes of the regression lines and the associated 95% confidence intervals were calculated holding the microbial context and the plant species effects constant on their average value.

One correlation network per microbial context was built based on the unrarefied OTU table of 16S rRNA gene sequencing. The aim of this analysis was to find statistical associations between pairs of OTU based on the abundance (i.e. sequence counts) obtained from sequencing data. These statistical associations (i.e. partial correlations which are represented by edges in the network) indicate ecological interactions (either positive or negative) between the OTUs (represented by nodes) that we supposed to depend on the soil microbial context. Only the partial correlations higher than 0.075 in absolute value were kept for the representation for readability (note that it gave a good representation of the effect of

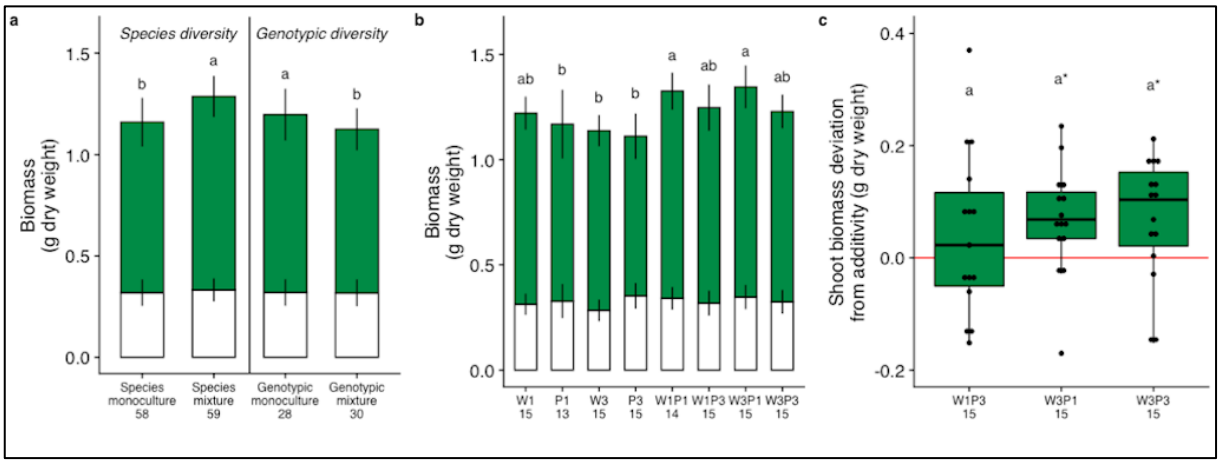
the microbial context as compared to the networks that included all of the partial correlations). To build the networks, the OTU table was filtered as follows: samples with less than 30,000 sequences were discarded as well as OTUs that represented less than 0.5% of the sequences. An abundance matrix was obtained from the filtered OTU table and described with a Poisson log-normal model (Chiquet et al. 2019) including an offset term to take the differences in sequencing depth between samples into account (the offset of a sample corresponded to the logarithm of the total number of counts in this sample). The best model was selected with a Stability Approach to Regularization Selection (StARS; Liu et al. 2010).

All the analyses were performed with R software version 3.6.1 and the following packages: car for ANOVAs (Fox and Weisberg 2019), agricolae for Tukey's tests (de Mendiburu 2020), vegan for PERMANOVA and NMDS (Oksanen et al. 2018) and mctoolsr for pairwise comparisons (Leff 2017), stats for linear models, t-tests and correlation tests, emmeans (Lenth 2021) and sjPlot (Lüdecke 2020) for regression slope description, PLNmodels for network analysis (Chiquet et al. 2019). Correlation networks were visualised with Cytoscape (Shannon et al. 2003).

RESULTS

Plant community diversity effect on plant biomass production

Overall, we registered overyielding but not transgressive overyielding, that is, total biomass production increased by 11% in pea-wheat mixtures as compared to species monocultures (Figure 5.2a; $t = -5.2$, $p_{df=115} = 1.1 \times 10^{-6}$) but, the best mixture (W3P1; 1.35 ± 0.13 g) was not significantly more productive than the best of the two grown monocultures (W1; 1.22 ± 0.10 g; Figure 5.2b). The increase



in biomass in mixtures was mainly due to an increase in shoot biomass in mixtures (+13%; $p_{df=115} = 2.2$

Figure 5.2 Plant diversity effect on plant biomass. Plant biomass at the pot scale according to **a)** species or genotypic diversity (species monoculture: W1, P1, W3, P3; species mixture: W1P1, W1P3, W3P1, W3P3; genotypic monoculture: W1, P1; genotypic mixture: W3, P3) and **b)** plant diversity treatment (W: wheat; P: pea; 1: one genotype; 3: three genotypes). Bottom: root biomass; top: shoot biomass. Letters represent significant differences in total biomass production ($\alpha = 0.05$). Bars represent the standard deviation. **c)** Shoot biomass deviation from the additivity hypothesis (i.e. the effect of combining species and genotypic diversity on plant biomass is equal to the sum of the effects of species diversity and genotypic diversity) depending on the mixture diversity. Letters represent significant differences between the treatments ($\alpha = 0.05$). Asterisks represent significant differences from zero. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. Sample sizes are given on the bottom of the graphs.

$\times 10^{-7}$) as there was no change in root biomass production ($p_{df=115} = 0.23$; Figure 5.2a). There was a negative effect of genotypic diversity on total biomass production (−6% in genotypic mixtures as compared to genotypic monocultures; Figure 5.2a; $t = 2.0$; $p_{df=56} = 0.048$) which, as for species diversity effect, occurred through a change in shoot biomass (−8%; $p_{df=56} = 0.023$; no change in root biomass: $p_{df=56} = 0.93$). There was no significant effect of increasing genotypic diversity on total biomass production once wheat and pea were considered separately (i.e. biomass in W3 and P3 did not differ from those of W1 and P1 respectively; Figure 5.2b).

The deviation of biomass from the additivity hypothesis, i.e. the existence of interaction effects between the two plant diversity levels, was not influenced by the interaction between the mixture diversity and the soil microbial context whatever the plant part (shoots, roots or total; Table S5.2), therefore we evaluated both effects separately. There was no effect of the mixture diversity on shoot biomass deviation from additivity as all the mixtures (W1P3, W3P1 and W3P3) showed a similar gain in shoot biomass as compared to what was expected under the additivity hypothesis (Figure 5.2c). However, this gain in shoot biomass was significant in W3P1 and W3P3 (+0.07 ± 0.10 g and + 0.07 ± 0.11 g respectively i.e. +7.2 and + 8.4%) but not in W1P3 (+0.04 ± 0.15 g i.e. +4.6%; Figure 5.2c). Unlike shoot biomass, the direction of root biomass deviation from additivity depended on the mixture diversity ($p_{df=2} = 6.2 \times 10^{-4}$; Table S5.2; negative deviation values for W1P3 and W3P3 and positive deviation value for W3P1; see Figure S5.1). Overall, shoot and root biomass deviation from additivity were positively correlated (two-sided Pearson's correlation test, $r = 0.42$, $p_{df=43} = 4.3 \times 10^{-3}$).

Differences in soil bacterial community between the microbial contexts

The three microbial contexts differed in terms of bacterial community structure ($p_{df=2} = 1.0 \times 10^{-3}$; $R^2 = 0.59$; Table S5.3; Figure S5.2a and b) and in terms of number of observed species, phylogenetic diversity and Simpson's reciprocal index (lower values in S than N and E and differences in N and E for the three indices; Table S5.4). An analysis based on correlation networks revealed a similar network structure in the native and exogenous communities with similar numbers of nodes and edges (47 nodes, 63 edges in the native community and 54 nodes and 78 edges in the exogenous community; Figure 5.3b and c) and the same proportion of negative partial correlations (respectively 19.0 and 19.4% in N and E). The network constructed from the data stemming from the sterilised soil included a higher number of nodes and edges than the other networks (100 nodes, 176 edges; Figure 5.3a) and a smaller proportion of negative partial correlations (2.3%). Note that the number of nodes and edges in the networks is not related to the community richness (Karimi et al. 2017) and that the networks draw a picture of an emerging pattern at the microbial context scale rather than the pot scale (at which α-diversity indices are calculated). The three networks showed high proportions of specific

nodes: 72, 51 and 44% in network S, N and E respectively (calculated as the proportion of specific nodes over the total number of nodes in a given network; Figure 5.3d).

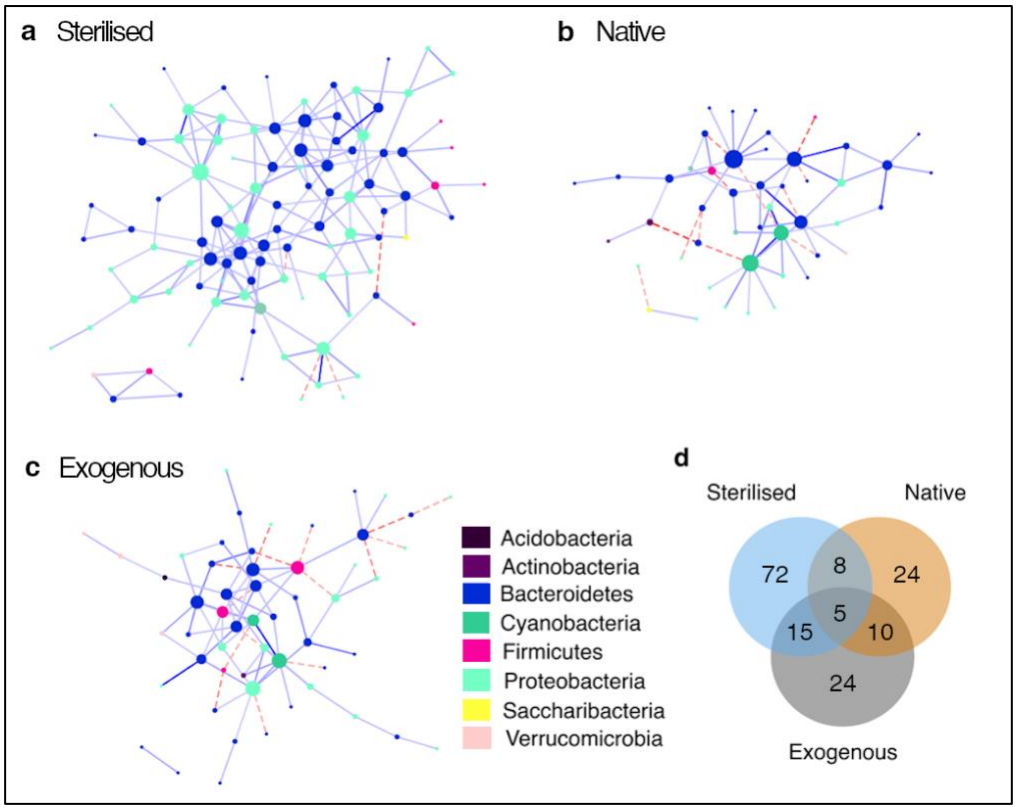


Figure 5.3 Correlation networks of the soil bacteria depending on the microbial context. **a)** Sterilised **b)** Native **c)** Exogenous. Nodes represent OTUs, edges represent partial correlations (threshold = 0.075; dashed line: <−0.075; solid line: >0.075). Node colour depends on the OTU phylum and node size depends on the number of nodes connected to the considered node. The networks were built based on the partial correlation values given by a Poisson log-normal model selected with StARS method. **d)** Venn diagram representing the number of specific and shared nodes in the three networks. Only partial correlations superior to 0.075 in absolute value were represented for readability but note that the obtained networks and the corresponding Venn diagram give a good representation of the differences between the three microbial contexts as compared to what is obtained when all partial correlations are considered (i.e. similar relative number of nodes and edges, similar relative proportion of negative partial correlations and similar levels of node specificity).

Soil microbial context effect on plant biomass and soil nitrogen content

Our results showed that the microbial context significantly influenced plant biomass (Table S5.5, Figure 5.4). Overall, shoot biomass was increased by 10.6% and 5.5% in the exogenous (E) and native (N) microbial contexts respectively as compared to the sterilised soil (S; Figure 5.4). There was an interaction between the soil microbial context and the plant diversity ($p_{df=14} = 4.9 \times 10^{-3}$; Table S5.5). Indeed, wheat species monocultures (W1 and W3) tended to respond differently than the other plant diversity treatments to the soil microbial context (Figure 5.4a). The lowest wheat shoot biomass occurred with the native microbial community while biomass tended to be higher and similar in S and E (Figure 5.4a). The overall effect on root biomass was the opposite of that on shoot biomass: we observed a higher root biomass in S (0.35 ± 0.07 g) than in E (0.30 ± 0.06 g) with an intermediate value in N (0.33 ± 0.05 g; Figure 5.4b). Thus, the microbial context influenced the shoot-root ratio ($p_{df=2} = 3.3$

$\times 10^{-9}$; Table S5.5; Figure 5.4c), i.e. plant resource allocation, but not the total biomass production (shoots + roots; $p_{df=2} = 0.36$; Table S5.5). To go beyond the three microbial contexts, we noticed that the soil bacterial community richness influenced positively plant total biomass ($p_{df=1} = 0.048$; slope = 1.89×10^{-4}; adjusted $R^2 = 0.17$; Figure S5.3). It suggested a possible influence of the bacterial community diversity per se on plant productivity, which requires further dedicated investigations.

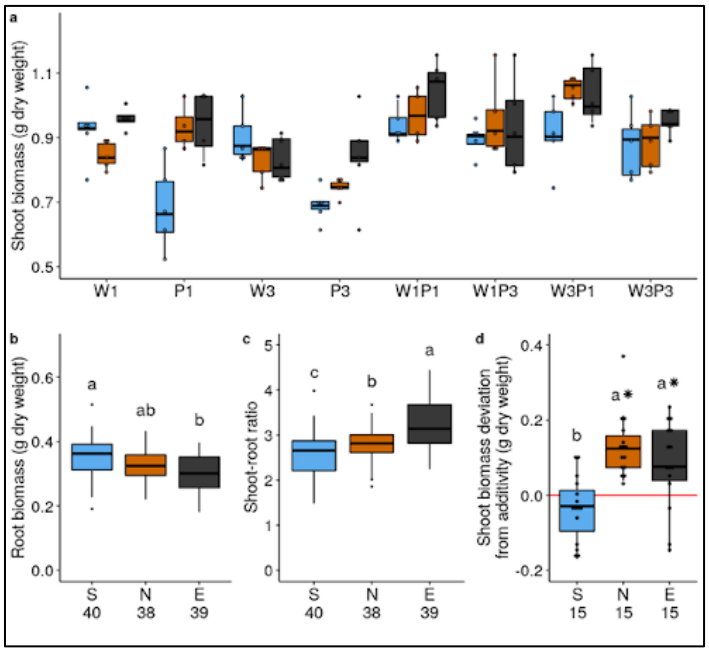


Figure 5.4 Plant biomass response at the pot scale to the soil microbial context. a) Shoot biomass depending on the plant diversity, **b)** Root biomass, **c)** Shoot-root ratio. S: sterilised; N: native; E: exogenous. Letters represent significant differences ($\alpha = 0.05$). Letters were not shown on fig. 6.4a for readability. There was no significant difference within plant diversity treatments except between S and N, E in P1. **d)** Shoot biomass deviation from the additivity hypothesis (i.e. the effect of combining species and genotypic diversity on plant biomass is equal to the sum of the effects of species diversity and genotypic diversity). Letters represent significant differences between the microbial contexts ($\alpha = 0.05$). Asterisks represent significant differences from zero. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. For panel a, sample sizes are n=5 except for P1 N and E and W1P1 N for which n = 4. The sample sizes for the other panels are given on the bottom of the graphs.

Total soil mineral nitrogen depended on the microbial context ($p_{df=2} = 2.4 \times 10^{-7}$; Table S5.6) and on the plant diversity (i.e. the eight plant diversity treatments + control; $p_{df=8} < 2.2 \times 10^{-16}$; Table S5.6). It was reduced in S and N as compared to E (respectively 5.2 ± 9.7, 6.3 ± 8.9 and 10.3 ± 10.3 $mg.kg^{-1}$ dry soil on average; Figure S5.4). Soil mineral nitrogen content was also reduced in pots containing plants as compared to control pots (respectively 4.3 ± 5.5 and 29.7 ± 6.3 $mg.kg^{-1}$ dry soil on average, of which respectively 91.3 and 85.6% were ammonium; Figure S5.4). Interestingly, pea species monocultures (i.e. P1 and P3) tended to show a higher soil mineral nitrogen content than the other treatments involving plants. This effect seemed to be driven by high nitrogen content in the presence of the exogenous microbial community (Figure S5.4; $p_{df=16} = 0.065$ for the interaction between plant diversity and microbial context; Table S5.6).

Interactions between plant diversity effects and soil microbes

The microbial context did not only affect plant productivity but also the existence of plant diversity effects on plant biomass. Indeed, there was no significant effect of increasing either species or genotypic diversity on shoot biomass in the sterilised soil, but positive effects in microbial contexts N and E (Table 5.1). In the native microbial community, we also registered a negative effect of increasing pea genotypic diversity (Table 5.1). The effect of mixing both diversity levels on shoot biomass (i.e.

change in biomass in W1P3, W3P1 and W3P3 as compared with W1) was never significant in S, significant in W3P1 in E and both in W3P1 and W1P3 in N (Table 5.1).

Table 5.1 Plant species and genotypic diversity effects on shoot biomass at the pot scale depending on the microbial context.

		Sterilised	Native	Exogenous
Wheat genotypic diversity		-0.017 g [-0.12;0.091]	-0.017 g [-0.072;0.035]	-0.13 g [-0.18;0.074]
Pea genotypic diversity		+0.0034 g [-0.11;0.12]	-0.19 g [-0.26;-0.13]*	-0.10 g [-0.26;0.044]
Species diversity		+0.015 g [-0.069;0.11]	+0.12 g [0.043;0.20]*	+0.089 g [0.0082;0.17]*
Species and genotypic diversity	W1P3	-0.029 g [-0.12;0.060]	+0.11 g [0.023;0.22]*	-0.021 g [-0.13;0.10]
	W3P1	-0.012 g [-0.13;0.10]	+0.20 g [0.16;0.25]*	+0.080 g [0.0074;0.16]*
	W3P3	-0.041 g [-0.15;0.074]	+0.041 g [-0.027;0.11]	-0.010 g [-0.052;0.031]

The shoot biomass deviation from the additivity hypothesis, i.e. the existence of an interaction effect between species and genotypic diversity, depended on the microbial context ($p_{df=2} = 9.4 \times 10^{-5}$; Table S5.2). There was a positive deviation from additivity in N and E (+0.13 ± 0.09 g and + 0.08 ± 0.12 g respectively i.e. +15.8 and + 8.9%) whereas there was no effect in the sterilised soil (−0.03 ± 0.09 g i.e. -3.7% which was not significantly different from zero; Figure 5.4d). Interestingly, whereas the highest shoot biomass occurred with the exogenous community (Figure 5.4a), the highest deviation from additivity tended to be achieved with the native community (Figure 5.4d).

An assessment of shoot biomass per capita allowed investigating potential mechanisms underlying these deviations from additivity. We showed that pea shoot biomass was not affected by the association with wheat ($p_{df=1} = 0.91$) whereas wheat shoot biomass increased when associated with pea ($p_{df=1} = 4.4 \times 10^{-9}$) which seemed to depend on the microbial context ($p_{df=2} = 0.06$ for the interaction term; Table S5.7). More particularly, the shoot biomass of the genotype RE13088 increased in mixture as compared to monoculture only in N and E (Figure 5.5). It led to a reduction in the productivity differential between the three wheat genotypes (standard deviations in Figure 5.5). However, this was not confirmed by the three-way interaction genotype*microbial context*species diversity ($p_{df=4} = 0.35$; Table S5.7).

Plant effect on soil bacterial communities

The soil bacterial OTU richness in pots with plants was similar to that of the control without plant (1208 ± 316 and 1195 ± 340 OTUs respectively). The OTU richness in the inoculated soils at the end of the experiment was lower than the OTU richness in the initial inoculums (i.e. the liquids used for soil inoculation; 2023 ± 333 and 2288 ± 104 OTUs in inoculums N and E respectively) but again, it did not differ between pots with plants and control pots (respectively −743 ± 134 and − 744 ± 141 OTUs

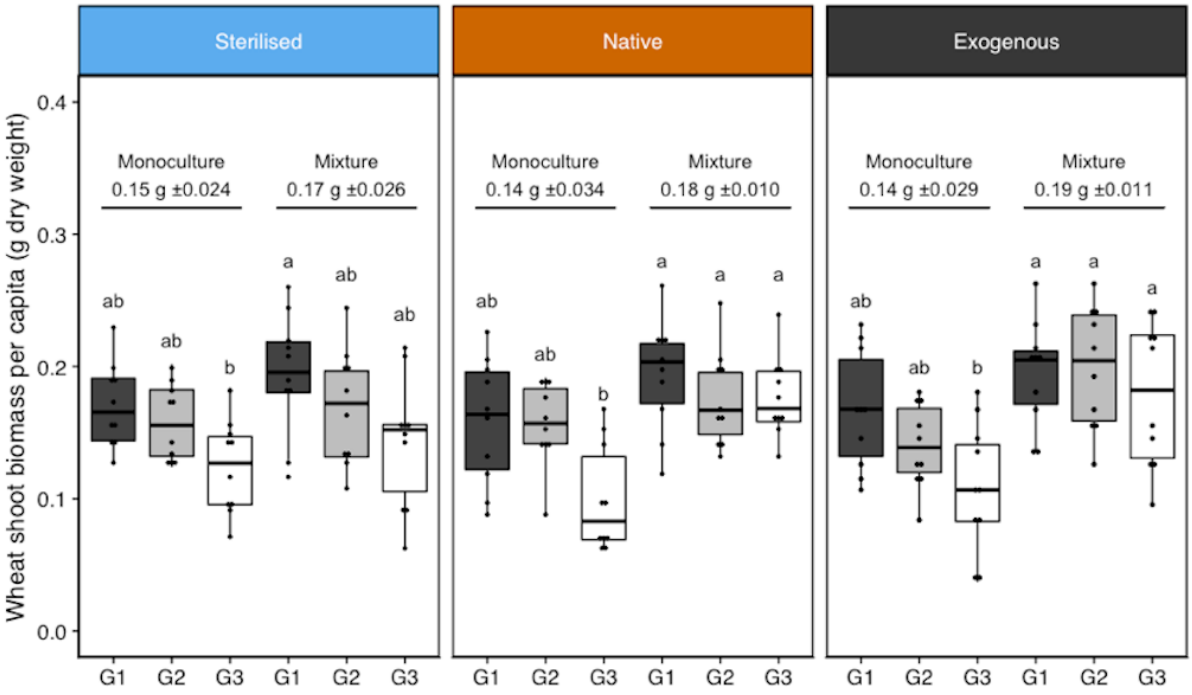


Figure 5.5 Wheat shoot biomass per capita depending on species diversity and soil microbial context. Monoculture: W3; Mixture: W3P1, W3P3. G1: Flamenko (black); G2: Renan (grey); G3: RE13088 (white). Letters represent significant differences (α = 0.05), they are presented for each microbial context independently for readability but were obtained from a post-hoc test following a global model including the three microbial contexts. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. n = 10. For each microbial context, the average shoot biomass per capita in monocultures and mixtures is given ± inter-genotype standard deviation.

as compared to the mean initial richness). Moreover, there was no difference in the bacterial community structure either considering the detail of the plant diversity treatments (the eight plant diversity treatments + control) or the plant species treatments (wheat, pea, mixture, control). We detected significant effects of the plants on soil bacterial communities performing PERMANOVA but these effects were weak (R^2 = 0.06 and 0.03; Table S5.3 and Table S5.8) and not detected by pairwise comparisons after correction for multiple testing. However, we noticed that there was a stronger divergence of the bacterial community structure across treatments in the sterilised soil than in the two other microbial contexts (Figure 5.6; see Figure S5.5 for a detailed view of each microbial context).

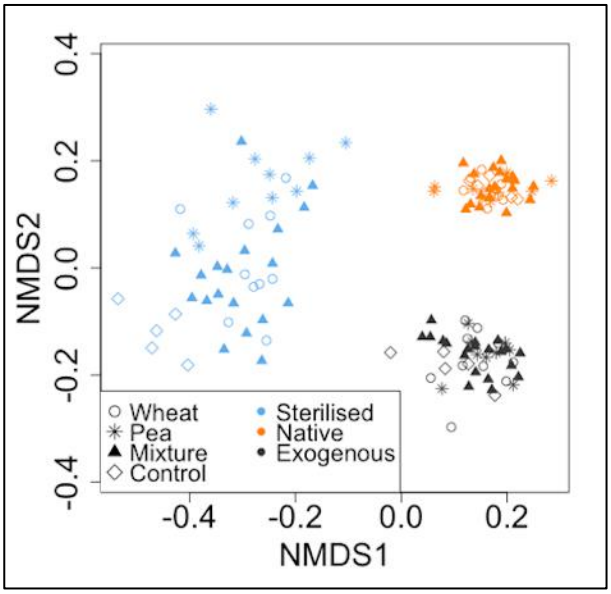


Figure 5.6 Non-metric multidimensional scaling of soil bacterial community structure. Dissimilarities were calculated with Bray-Curtis index. One point per pot. Stress = 0.11.

DISCUSSION

The purpose of this study was to assess the effect of soil microbes on the interaction between plant genotypic and species diversity. We found that the influence of the soil microbial context on plant productivity occurred in three ways: *i)* through effects on shoot and root biomass (and thus differences in shoot-root ratio; Figure 5.4c), *ii)* by regulating the significance of species and genotypic diversity effects on shoot biomass (Table 5.1) and *iii)* by modulating the effect of the interaction between species and genotypic diversity on shoot biomass (Figure 5.4d).

Soil sterilisation and inoculation or not with a soil suspension successfully created three different microbial contexts in terms of bacterial community diversity and structure, as attested by the bacterial community diversity metrics and suggested by the network analysis. We found fewer nodes and edges in the networks of inoculated soils than in the network of sterilised soil, i.e. less OTUs that were involved in direct interactions (Chiquet et al. 2021). As the environment was controlled and plant diversity had no effect on soil bacterial communities, it is likely that the differences in network structures reflect the effect of soil inoculation at the beginning of the experiment. We propose that the higher node and edge number in network S could suggest less constraints in soil colonisation dynamics (which could be due to the absence of species with high competitive abilities at the beginning of the experiment due to the absence of inoculation). These differences in soil microbial context determined plant shoot/root ratio as biomass production was more root-oriented in the sterilised soil than in inoculated soils. A substantial difference between the microbial contexts occurred through the establishment of a Rhizobium-legume symbiosis in inoculated soil only (N and E). We can hypothesize that it played a role in the observed differences in plant biomass between the microbial contexts, both in pea monocultures and pea-wheat mixtures. Beyond the possible role of mutualist interactions and despite the fact that our experiment was not specifically designed to assess the effect of microbial community richness on plant productivity, we noticed that the relationship between plant total biomass and bacterial community richness tended to be positive within each microbial context (Figure S5.3), giving rise to a positive overall relationship. Another study, that experimentally manipulated arbuscular mycorrhizal fungi diversity, showed that the fungal community richness affected plant biomass production (either positively or negatively depending on the plant species; Wagg et al. 2011a). More recently, a study highlighted that pea yield (grain biomass per plant) was influenced by the microbial community richness both in a wild-type genotype and a non-nodulating non-mycorrhizing mutant under drought stress (Prudent et al. 2020). Taken together, these results suggest that soil microbial richness per se could be of importance for plant productivity. This effect of richness could occur through sampling effects (e.g. increase in the probability of the presence of a beneficial microbial partner) or involve complex microbe-microbe interactions (e.g. higher availability of a nutrient allowed by the joint presence of several microbial species).

We highlighted that the existence of an effect of either plant species or genotypic diversity on shoot biomass was determined by the soil microbial context. In particular, shoot biomass did not respond to plant diversity in the sterilised soil contrary to what happened in inoculated soils where species diversity had a positive effect on shoot biomass (Table 5.1). This is consistent with the results of a previous study which showed no effect of the species richness (from one to three grassland species) or evenness on shoot biomass per plant in a sterilised soil, whereas there was a positive diversity-productivity relationship when the soil was inoculated with a soil microbial community (Wang et al. 2019). In this study, the authors also included a treatment in which the soil was inoculated with a combination of arbuscular mycorrhizal fungi isolates and they showed no plant diversity effect on productivity, as observed here for the sterilised soil. These results suggest that the plant diversity-productivity relationship is more likely influenced by a complex soil microbial community (e.g. stemming from field soil) rather than a simplified one (e.g. built in the laboratory). By contrast, other studies integrating higher levels of plant species richness (up to eight tree seedling species (Liang et al. 2019) and up to 15 herbaceous plant species (Schnitzer et al. 2011) registered an increase in plant total biomass with species richness increase even in sterilised soils. But, in Schnitzer et al. (2011), the shape of the diversity-productivity relationship was still affected by soil sterilisation. In the sterilised soil, productivity increased linearly with species diversity whereas, in inoculated soils, the diversity-productivity relationship was saturating. The authors suggested that, in the sterilised soil, the positive plant diversity-productivity relationship was mainly driven by plant complementarity whereas, in field soil, microbes had a major role and drove the relationship by strongly reducing plant productivity at low diversity levels (in which disease incidence was higher) giving rise to a non-linear relationship. Together, these results suggest that the diversity-productivity relationship could be more influenced by soil microbes in plant communities with low diversity levels or including species involved in strong mutualistic interactions (e.g. legumes).

To go further, our study provided evidence that the effect of the interaction between species and genotypic plant diversity on productivity can be modulated by the soil microbial community. Indeed, we did not detect plant species and genotypic diversity interaction in the sterilised soil whereas, in inoculated soils, plant productivity in species and genotypic mixtures was higher than expected in the absence of interaction. We expected the interaction effect on productivity to be different between the two inoculated soils but the gain of shoot biomass due to species and genotypic diversity interaction was similar. In both inoculated soils, the interaction effect seemed to be partly driven by changes in the effect of wheat genotypic diversity in pea/wheat mixtures as compared to wheat monoculture. While wheat genotype performances changed when associated with pea in inoculated soils, the relative performances remained similar to those observed in wheat monoculture in the sterilised soil. In the light of the knowledge on pea/wheat mixture (Bedoussac and Justes 2010; Bedoussac et al. 2015; Duchene et al. 2017), we suggest that the presence of nodules on pea roots in inoculated soils allowed niche complementarity to occur in pea/wheat mixtures thus reducing competition, and more specifically

intraspecific competition (Mahaut et al. 2020), for nitrogen. This interpretation is supported by very low levels of mineral nitrogen in the sterilised soil as compared to inoculated soils. Previous studies showed that complementarity allowed a better resource use which could explain a gain in productivity in mixtures as compared to monoculture (Mason et al. 2020), here our results suggest that complementarity in nitrogen use in species mixture could prevent nitrogen-limitation (which is likely to occur in our experiment in pots) and allow positive interactions between genotypic and species diversity.

Rather than a synergy between two positive effects of species and genotypic diversity, we observed that species mixture compensated the negative effect of genotypic diversity. Previous studies highlighting interactions showed that increasing genotypic diversity in a species mixture could change the effect of species diversity on above-ground productivity, either in a positive (switch from a negative to a positive relationship; Crawford and Rudgers 2012) or a negative way (decrease in net biodiversity effect; Schöb et al. 2015). The underlying mechanisms imply changes in interspecific competition and in species dominance patterns (Fridley and Grime 2010; Schöb et al. 2015). Here, we provided a first evidence that changes in dominance patterns could also be involved in the species diversity effect on the genotypic diversity-productivity relationship, and that, these mechanisms can be driven by the soil microbial communities.

A classical objective of diversity-productivity research is to shed light on genotypic and species interactions among plants. Some studies are interested in including plant interactions with other organisms, such as microbes, as modulators of this relationship. Based on our results and in the light of the concept of holobiont, it would be particularly interesting to assess the relative weight of inter-kingdom (plant-microbes) interactions as compared to intra-kingdom (plant-plant) interactions in explaining the biodiversity-ecosystem functioning relationship.

SUPPLEMENTARY MATERIAL

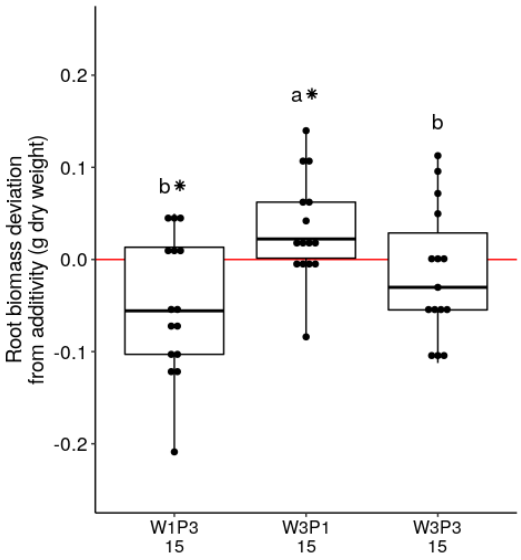


Figure S5.1 Root biomass deviation from additivity depending on the mixture diversity. There is a deviation from additivity if the effect of combining species and genotypic diversity on plant biomass is different from the sum of the effects of species diversity and genotypic diversity. Letters represent significant differences between the treatments (α=0.05). Asterisks represent significant differences from 0. First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars). Sample sizes are given on the bottom of the graph.

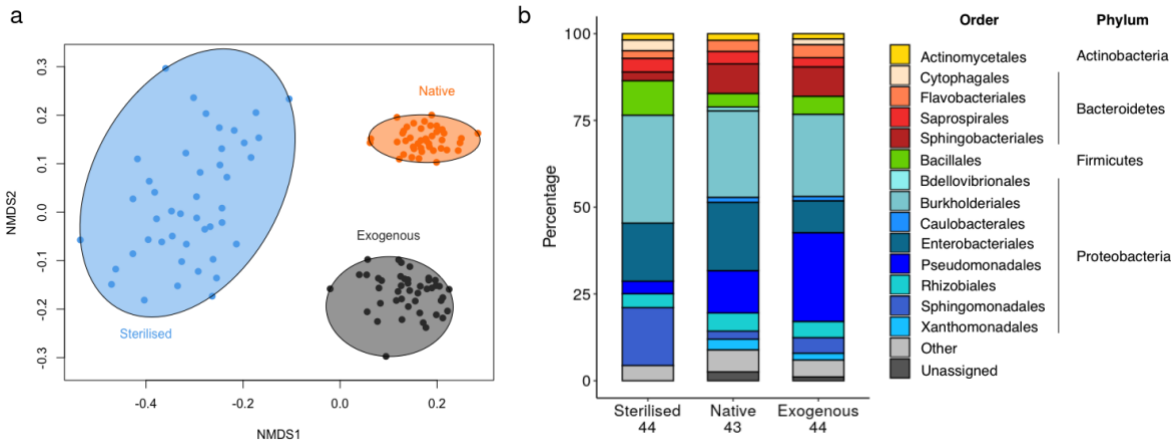


Figure S5.2 Bacterial community description depending on the soil microbial context based on 16S rRNA gene sequencing at the end of the experiment. a) Non-metric multidimensional scaling of soil bacterial community structure. Dissimilarities are calculated with Bray-Curtis index. One point per pot. Blue: sterilised; orange: native; gray: exogenous. Stress=0.11. **b)** Bacterial community composition. The taxonomy is presented at the order level. Percentages were calculated in a given microbial context considering the number of sequences of a given order over the total number of sequences (given that the number of sequences in a sample was normalised to 30,000). Orders representing less than 1 % of the sequences in a given microbial context are grouped under the category "Other".

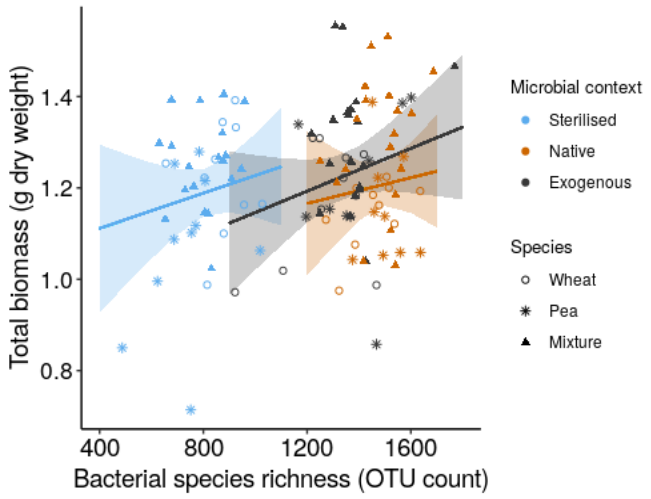


Figure S5.3 Plant biomass (shoots and roots) depending on the soil bacterial community richness. One regression line and one colour per microbial context (Sterilised: $y = 1.9 \times 10^{-4}x + 1.0$; Native: $y = 1.4 \times 10^{-4}x + 1.0$; Exogenous: $y = 2.3 \times 10^{-4}x + 0.91$). Point shapes depend on the plant species (Wheat, Pea or Pea-wheat mixture). 95 % confidence intervals are represented around the regression lines. Slopes were calculated holding the "plant species" factor at its average value for each microbial context. The overall equation is: $y = 1.9 \times 10^{-4}x + 0.99$ ($p_{df=1}$=0.048; adjusted R^2=0.17).

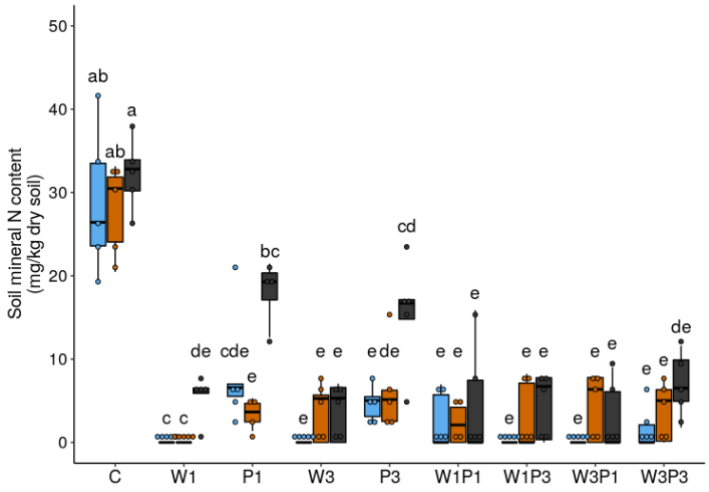


Figure S5.4: Soil mineral nitrogen content (ammonium and nitrate) depending on the plant diversity and the microbial context. W: wheat; P: pea; 1: one genotype; 3: three genotypes. Blue: sterilised; Orange: native; Grey: exogenous. Letters represent significant differences (α=0.05). First quartile, median and third quartile are represented as well as minimal and maximal values (ends of the bars) within 1.5 times the interquartile range. Points outside of the boxes represent outliers. n=5 except for P1 N and E and W1P1 N for which n=4. Ammonium contributed largely to the variable "soil mineral nitrogen" since 88 % of the samples showed nitrate content below the detection level.

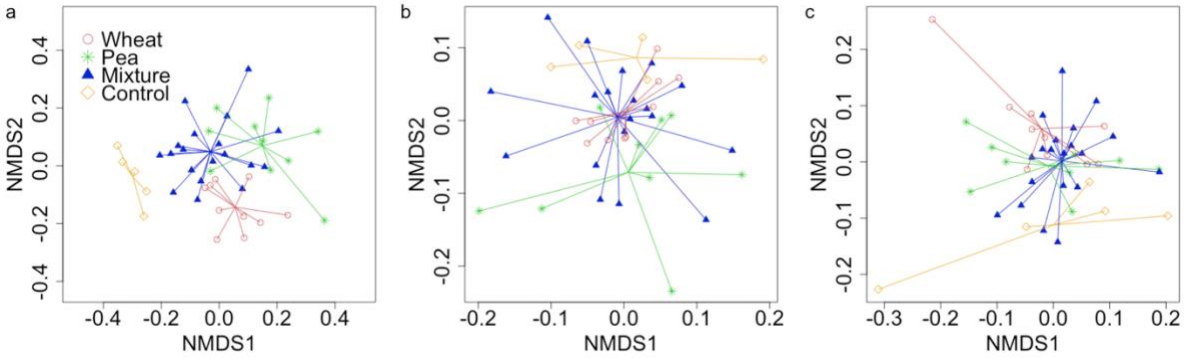


Figure S5.5 Non-metric multidimensional scaling of soil bacterial community structure. Dissimilarities were calculated with Bray-Curtis index. One point per pot. Stress values are 0.22, 0.18 and 0.18 for **a)** sterilised soil, **b)** native and **c)** exogenous community respectively (no convergent solutions were found in the three cases). Note that the axis scales are different between the three microbial contexts.

Table S5.1 Phenotypic characteristics of the pea and wheat genotypes selected for the experiment. Wheat genotype characteristics were defined relatively to a panel of wheat genotypes grown in the field during a previous experiment. We first selected four genotypes of each species displaying differences in the characteristics presented in the table and then kept the three best performing genotypes for each species in the experimental conditions (i.e. higher germination rate and emergence after transplantation).

Pea (*Pisum sativum* L.)				**Wheat (*Triticum aestivum* L.)**				
Genotype	**Season**	**Use**	**Photoperiod sensitivity**	**Genotype**	**Heading earliness**	**Height**	**Yield**	**Protein content**
Cameor	Spring	Garden pea	Not applicable	**Flamenko**	Early	Mid	High	Low
Champagne	Winter	Field pea	Yes	**Renan**	Intermediate	Mid	Low	High
336.11	Winter	Protein pea	Yes	**RE13088**	Early	High	High	Low

Table S5.2 Plant biomass deviation from additivity in species and genotypic mixtures. Analysis of variance (ANOVA, type II sums of squares) of the deviation from the additivity hypothesis (i.e. the effect of combining species and genotypic diversity on plant biomass is equal to the sum of the effects of species diversity and genotypic diversity) in response to the mixture diversity (W1P3, W3P1, W3P3 with W: wheat; P: pea; 1: one genotype; 3: three genotypes), the microbial context (sterilised, native, exogenous) and their interaction.

		Deviation of total biomass			Deviation of shoot biomass			Deviation of root biomass		
	df	Sum sq	F	p	Sum sq	F	p	Sum sq	F	p
Mixture diversity	2	0.099	3.3	**0.046**	0.0080	0.45	0.64	0.052	9.1	**6.2×10^{-4}**
Microbial context	2	0.43	14	**2.4×10^{-5}**	0.21	12	**9.4×10^{-5}**	0.066	11	**1.4×10^{-4}**
Mixture diversity * microbial context	4	0.13	2.2	0.091	0.072	2.0	0.11	0.026	2.3	0.081
Residuals	36	0.53			0.32			0.10		
		R^2=0.45			*R^2=0.37*			*R^2=0.49*		

Table S5.3 Soil bacterial community structure analysis. Analysis of variance (PERMANOVA) of the OTU occurrence depending on the plant diversity (W1, P1, W3, P3, W1P1, W1P3, W3P1, W3P3, C; with W: wheat; P: pea; C: control; 1: one genotype; 3: three genotypes), the microbial context (sterilised, native, exogenous) and their interaction. Pairwise comparisons showed that the three microbial contexts differed from each other ($p<0.05$ after correction for multiple testing with Bonferroni method).

	df	Sum sq	Mean sq	F	R^2	p
Plant diversity	8	1.1	0.14	2.8	0.056	**1.0×10^{-3}**
Microbial context	2	12	6.0	116	0.59	**1.0×10^{-3}**
Plant diversity * microbial context	16	2.0	0.12	2.3	0.095	**1.0×10^{-3}**
Residuals	104	5.4	0.052		0.26	

Table S5.4 α-diversity indices of the soil bacterial community depending on the microbial context at the end of the experiment. Means are given ±SE. Letters represent significant differences (α=0.05).

	Observed species	Phylogenetic diversity	Simpson's reciprocal index
Sterilised	805±19 c	48±0.92 c	11±0.62 c
Native	1,477±16 a	80±0.76 a	19±0.91 b
Exogenous	1,350±22 b	74±1.1 b	23±1.2 a

Table S5.5 Plant biomass response to the plant diversity and the soil microbial context. Analysis of variance (ANOVA, type II sums of squares) of plant biomass at the pot level in response to the plant diversity (W1, P1, W3, P3, W1P1, W1P3, W3P1, W3P3 with W: wheat; P: pea; 1: one genotype; 3: three genotypes), the microbial context (sterilised, native, exogenous) and their interaction.

		Total biomass			Shoot biomass			Root biomass			Shoot-root ratio		
	df	Sum sq	F	p	Sum sq	F	p	Sum sq	F	p	Sum sq	F	p
Plant diversity	7	0.72	6.6	**$2.6x10^{-6}$**	0.63	12	**$1.7x10^{-10}$**	0.051	2.4	**0.029**	8.2	6.1	**$7.0x10^{-6}$**
Microbial context	2	0.032	1.0	0.36	0.16	10	**$1.1x10^{-4}$**	0.047	7.6	**$8.8x10^{-4}$**	9.3	24	**$3.3x10^{-9}$**
Plant diversity * microbial context	14	0.31	1.4	0.15	0.27	2.5	**0.0049**	0.046	1.0	0.41	4.8	1.8	0.052
Residuals	93	1.4			0.73			0.29			18		
		R^2=0.28			*R^2=0.49*			*R^2=0.17*			*R^2=0.45*		

Table S5.6 Soil mineral nitrogen content response to the plant diversity and the soil microbial context. Analysis of variance (ANOVA, type II sums of squares) of soil mineral nitrogen (ammonium and nitrate) in response to the plant diversity (W1, P1, W3, P3, W1P1, W1P3, W3P1, W3P3, C with W: wheat; P: pea; C: control; 1: one genotype; 3: three genotypes), the microbial context (sterilised, native, exogenous) and their interaction.

		Soil mineral nitrogen		
	df	Sum sq	F	p
Plant-diversity	8	9 544	65	**$<2.2x10^{-16}$**
Microbial context	2	650	18	**$2.4x10^{-7}$**
Plant diversity * microbial context	16	491	1.7	0.065
Residuals	105	1 932		
		R^2=0.81		

Table S5.7 Analysis of the effect of the microbial context and the species diversity on pea and wheat shoot biomass. Analysis of variance (ANOVA, type II sums of squares) of the response of wheat and pea shoot biomass per capita to the microbial context (sterilised, native, exogenous), species diversity (i.e. monoculture: W3 for wheat and P3 for pea or mixture; W3P1 and W3P3 for wheat and W1P3 and W3P3 for pea), the genotype (Flamenko, Renan, RE13088 or Cameor, Champagne, 336.11) and their interactions.

	Wheat shoot biomass				Pea shoot biomass			
	df	Sum sq	F	p	df	Sum sq	F	p
Microbial context	2	$9.3x10^{-4}$	0.27	0.76	2	0.0096	3.5	**0.034**
Species diversity	1	0.065	39	**$4.4x10^{-9}$**	1	$2.0x10^{-5}$	0.012	0.91
Genotype	2	0.054	16	**$4.4x10^{-7}$**	2	0.41	148	**$<2x10^{-16}$**
Microbial context * species diversity	2	0.0097	2.9	0.060	2	0.0041	1.5	0.23
Microbial context * genotype	4	$9.1x10^{-4}$	0.13	0.97	4	0.0071	1.3	0.28
Species diversity * genotype	2	0.0066	1.9	0.15	2	0.0025	0.91	0.40
Microbial context * species diversity * genotype	4	0.0076	1.1	0.35	4	0.0032	0.57	0.69
Residuals	162	0.27			147	0.20		
	R^2=0.28				*R^2=0.65*			

Table S5.8 Soil bacterial community structure analysis. Analysis of variance (PERMANOVA) of the OTU occurrence depending on the plant species (wheat, pea, mixture, control), the microbial context (sterilised, native, exogenous) and their interaction.

	df	Sum sq	Mean sq	F	R^2	p
Plant species	3	0.62	0.21	3.6	0.031	**$1.0x10^{-3}$**
Microbial context	2	12	6.0	105	0.59	**$1.0x10^{-3}$**
Species*microbial context	6	1.0	0.17	3.0	0.051	**$1.0x10^{-3}$**
Residuals	119	6.8	0.057		0.33	

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SUMMARY IN FRENCH

Introduction

L'organisation en communauté

En écologie, une ***communauté*** peut être simplement définie comme la collection d'espèces présentes à un endroit donné. Cependant, certaines définitions intègrent également la nécessité de cooccurrence des espèces dans le temps et l'existence d'interactions entre les espèces pour qu'un groupe d'espèces puisse être défini comme une communauté. Ces divergences de définitions sont en fait liées à des divergences historiques de conception de ce qu'est une communauté. D'une part, une vision « individualiste » de la communauté proposée par Gleason suggère que les espèces présentes à un endroit donné sont celles qui sont capables de persister dans les conditions de l'environnement. D'autre part, une communauté peut être perçue comme une entité qui, grâce aux interactions entre espèces, possède un développement analogue à celui d'un organisme, c'est la vision proposée par Clements. Ces deux visions de la communauté reposent sur l'importance qui est accordée aux interactions entre les espèces et leur environnement abiotique d'une part ou entre les espèces elles-mêmes d'autre part. Dans les faits, les espèces sont en interaction avec leur environnement biotique et abiotique et la force de ces interactions peut varier au cours du temps. Les deux visions proposées sont donc pertinentes et peuvent être envisagées comme les extrêmes d'un continuum. Initialement, le concept de communauté a été développé pour décrire des assemblages de plantes mais il peut être utilisé pour l'ensemble des règnes et y compris pour décrire des assemblages multi-règnes (plantes, animaux, microbes). L'***écologie microbienne*** a longtemps été considérée à part de l'écologie, cependant à partir de la fin du 20ème siècle, le lien entre ces deux champs de recherche s'est établi. En effet, les microorganismes peuvent être de bons modèles pour tester des théories d'écologie et, à l'inverse, les théories construites à partir de l'étude des plantes ou des animaux peuvent permettre de comprendre le fonctionnement des communautés microbiennes. Dans cette thèse, nous nous sommes appuyés sur des études empiriques portant sur des communautés microbiennes, et plus particulièrement bactériennes, et nous avons mobilisé des théories issues de l'écologie au sens large.

On parle de ***propriété intrinsèque*** ou propriété émergente de la communauté lorsque cette propriété n'est prédictible ni à partir des caractéristiques des espèces qui composent la communauté ni à partir de la somme de ces caractéristiques. Il peut s'agir de la formation de biofilm par exemple. L'émergence de ces propriétés uniques au mode de fonctionnement en communauté est permise par l'existence d'interactions entre espèces ou ***interactions interspécifiques***. Les interactions entre deux espèces sont classiquement définies en fonction de la nature de leur effet sur les espèces impliquées (positif, neutre ou négatif). Chez les microorganismes, comme chez les plantes et les animaux, des interactions négatives peuvent se produire via la compétition (-/-) ou l'amensalisme (0/-), des interactions positives interviennent via le mutualisme (+/+) ou le commensalisme (0/+). Il peut y

également y avoir des interactions de type « gagnant/perdant » (+/-) via la prédation ou le parasitisme. Même si les interactions deux à deux ont été beaucoup étudiées, notamment la compétition et le mutualisme, dans les communautés complexes des interactions d'ordre supérieur mais également des interactions avec l'environnement abiotique entrent en jeu ce qui diminue la prédictibilité des propriétés des communautés.

Des traits spécifiques à la présence de plusieurs espèces peuvent également être considérés comme des propriétés émergentes des communautés. C'est le cas notamment de la ***diversité*** qui rend compte des espèces présentes et de leurs abondances relatives. En théorie, une augmentation de la diversité d'une communauté est associée à une augmentation du niveau d'une fonction de cette communauté. Cela peut intervenir via deux catégories de mécanismes : les ***effets de complémentarité ou de facilitation*** et les ***effets de sélection***. Dans le premier cas, le niveau d'une fonction peut augmenter sous l'effet d'interactions positives au sein de la communauté. Dans le second cas, il peut augmenter sous l'effet d'une augmentation de la probabilité d'intégrer une espèce dominante avec un fort impact sur la fonction étudiée lorsque la diversité augmente. Expérimentalement, il a été montré via l'étude de communautés végétales mais également microbiennes que la relation diversité/productivité était saturante. Cela s'explique par la ***redondance fonctionnelle*** qui peut exister entre espèces. En effet, à partir d'un certain nombre d'espèces, l'ajout d'une espèce dans la communauté peut ne pas induire d'augmentation du niveau de la fonction étudiée si cette espèce porte des fonctions déjà représentées dans la communauté (autrement dit s'il n'y a pas de complémentarité). Toutefois, cela peut avoir un effet positif sur la ***stabilité*** de la communauté. Plus la redondance fonctionnelle est élevée, plus le risque de perdre une fonction via la perte d'une espèce décroit, de même que la variabilité dans la fonction.

L'évolution des communautés

Selon le concept d'évolution par ***sélection naturelle*** proposé par Darwin, chaque variation, si elle est bénéfique, est préservée. Cela peut se produire si trois conditions sont respectées : l'existence de variation phénotypique, son association avec un différentiel de fitness, qui doit être héritable. Les niveaux d'organisation biologique auxquels ces conditions peuvent s'appliquer ont fait l'objet d'un débat pendant plusieurs décennies. Plus particulièrement, s'il est admis que la sélection naturelle intervient au niveau de l'individu, sa pertinence au niveau du groupe a été et est toujours controversée. Il existe de nombreuses théories de la ***sélection de groupe***, dont certaines ont été largement remises en cause, ce qui a semé la confusion sur la pertinence de l'ensemble de ces théories. L'une des théories remarquables est celle du superorganisme proposée par Wilson et Sober. Elle est fondée sur l'idée qu'un groupe est organisé fonctionnellement comme un individu : de même que les individus peuvent être envisagés comme des ensembles de gènes, les groupes peuvent être considérés comme des ensembles d'individus. Par conséquent, la sélection naturelle pourrait intervenir au niveau du groupe et la réponse à la sélection interviendrait au niveau du ***phénotype de groupe***. Ce qui est communément admis est que

la sélection de groupe pourrait expliquer les transitions majeures de l'évolution (par exemple le passage des Procaryotes aux Eucaryotes) et qu'elle pourrait intervenir dans le contexte d'une ***sélection multi-niveaux***, c'est-à-dire d'une sélection intervenant simultanément à différents niveaux d'organisation biologique. Dans cette thèse, nous nous appuyons sur la définition de la sélection de groupe proposée par Wade (1978) : la sélection de groupe est un processus de changement génétique causé par l'extinction ou la prolifération différentielle de groupes d'organismes. En conditions naturelles, certaines conditions environnementales pourraient permettre aux trois conditions nécessaires à la sélection (variation, différentiel de reproduction, héritabilité) d'être remplies au niveau du groupe mais cela reste difficile à démontrer. Au laboratoire, il est possible d'appliquer une sélection artificielle de groupe en compartimentant des groupes d'individus et en ne propageant que les meilleurs au regard d'une fonction prédéfinie. Les premières études de ce type ont été menées sur des populations ou communautés de plantes ou d'animaux et ont montré que le phénotype d'une population ou communauté pouvait changer sous l'effet d'une sélection artificielle au niveau du groupe.

Par la suite, au début des années 2000, plusieurs expérimentations de sélection artificielle de groupe ont été mises en place sur des communautés microbiennes. Ce type de procédure comporte trois étapes principales : *i)* la création d'une population d'unités de sélection, *ii)* le phénotypage des unités (= communautés) selon la propriété ciblée par la sélection, *iii)* la sélection et la propagation des unités les plus performantes au regard de cette propriété. Les premières expérimentations ont visé à augmenter la dégradation d'un composé toxique, modifier le pH d'un milieu aquatique, modifier la biomasse aérienne d'*Arabidopsis thaliana* (via une sélection portant uniquement sur les microbes du sol). Les résultats de ces expérimentations ont mis en évidence que des communautés microbiennes pouvaient répondre à la sélection artificielle de groupe avec toutefois une instabilité dans les réponses observées. Par la suite, des études de modélisation ont mis en évidence qu'un ***équilibre entre variation et héritabilité*** était nécessaire pour que la sélection soit efficace. En effet, l'existence de variation phénotypique est une condition indispensable à la sélection, toutefois si cette variation n'est pas due à des déterminants transmissibles à la génération suivante, le phénotype sera perdu. Dans une communauté, la variation phénotypique héritable peut être apportée par des changements génétiques au sein des espèces mais également par des changements dans les fréquences relatives des différentes espèces. Depuis 2015, de nouvelles études sont venues s'ajouter aux quelques études existant sur le sujet. Ainsi, la sélection artificielle a été appliquée sur des communautés microbiennes afin de diminuer leurs émissions de CO_2, augmenter leurs activités enzymatiques ou encore leur capacité à promouvoir la croissance bactérienne. Par ailleurs, le même type de procédure est appliqué sur des microbiotes, c'est-à-dire des communautés microbiennes associées à un hôte (une plante par exemple). Toutes ces études ont grandement contribué à l'accroissement des connaissances sur le sujet et ont mis en évidence plusieurs limitations et donc possibilités d'amélioration des procédures de sélection. L'intérêt pour les communautés microbiennes est grandissant et, considérant leur rôle dans de nombreux processus, l'ingénierie des communautés microbiennes se développe dans de nombreux domaines (santé humaine, agriculture, industrie

pharmaceutique, traitements des déchets etc.). Dans ce contexte, les procédures de sélection artificielle constituent un outil prometteur qui ne requiert aucune connaissance a priori sur les communautés pour être appliqué et qui peut donc permettre de trouver des combinaisons d'espèces (c'est-à-dire de gènes) qui n'auraient pas pu être trouvées autrement.

Interactions entre écologie et évolution

Il est de plus en plus admis que les dynamiques écologiques et évolutives des communautés sont intimement liées. En effet, l'effet de l'écologie sur l'évolution est reconnu depuis longtemps puisque les conditions écologiques sont à l'origine des pressions de sélection conduisant à l'évolution des organismes. De plus, l'effet de l'évolution sur l'écologie reçoit de plus en plus d'attention puisqu'il a été montré que les deux pouvaient intervenir à des pas de temps similaires contrairement à ce qui était envisagé auparavant. On peut donc parler de ***dynamiques éco-évolutives*** ou, lorsqu'il y a des interactions complexes entre écologie et évolution, de boucles de rétroaction éco-évolutives (= feedbacks éco-évolutifs). Ces boucles de rétroaction peuvent être définies comme les interactions cycliques entre écologie et évolution telles que des changements dans les interactions dirigent l'évolution des traits des organismes ce qui, en retour, altère les interactions écologiques. De nombreuses études illustrent les interactions complexes qui existent entre écologie et évolution, en particulier au regard de la diversité des communautés et des interactions entre espèces. En effet, l'évolution peut influencer la diversité des communautés via des effets sur leur assemblage, leur composition ou leur structure. En retour, la diversité peut influencer l'évolution via des effets sur le taux d'évolution des espèces ou en modifiant l'importance relative de différents processus évolutifs. De même, les interactions entre espèces peuvent influencer l'évolution de ces espèces en augmentant les pressions de sélection par exemple.

Dans les approches de sélection artificielle au niveau des communautés, il y a un intérêt à comprendre comment l'écologie des communautés peut influencer leur évolution et donc potentiellement leur réponse à la pression de sélection artificielle. Par exemple, il a été montré que la dynamique écologique d'une communauté au sein d'un cycle de sélection pouvait influencer l'efficacité de la sélection. De même, il semblerait qu'une certaine ***stabilité de la structure de la communauté*** soit nécessaire pour la sélection artificielle puisse être efficace. En effet, plusieurs études suggèrent que tant que la structure de la communauté fluctue d'un cycle de sélection à l'autre, la sélection artificielle ne fonctionne pas. Les interactions entre espèces semblent également jouer un rôle important dans la réponse des communautés à la sélection artificielle. En effet, il a été montré que, dans certains cas, la réponse évolutive d'une communauté n'était pas prédictible à partir des réponses évolutives des espèces qui la composent. De plus, une étude expérimentale a montré qu'une réponse de la communauté pouvait être enregistrée sans aucun changement dans les espèces composant cette communauté, cela suggère que les interactions en elles-mêmes peuvent évoluer. Certains auteurs suggèrent même que l'évolution

des interactions favoriserait la ressemblance entre les communautés parentales et les communautés descendantes dans les procédures de sélection artificielle et serait donc un déterminant de l'héritabilité.

Objectifs de la thèse

Le but de ce travail est de contribuer aux connaissances sur la sélection artificielle au niveau de la communauté, notamment via l'étude du fonctionnement et de l'évolution des communautés, et de mettre en évidence les connaissances que ce domaine de recherche peut apporter au domaine plus large de l'étude des dynamiques éco-évolutives. Pour cela, nos objectifs sont *i)* d'étudier comment améliorer l'efficacité des procédures de sélection artificielle et *ii)* d'étudier les déterminants des réponses évolutives des communautés. Ces objectifs seront traités dans trois chapitres dont les résumés sont présentés ci-dessous.

Chapitre 1 : Effet de la méthode de reproduction dans une procédure de sélection artificielle au niveau de la communauté

Des expérimentations de sélection artificielle de communautés microbiennes ont montré des résultats prometteurs. Cependant, une absence de maintien au cours temps de la réponse des communautés à la sélection laisse à penser que des améliorations pourraient être apportées à la procédure afin d'augmenter l'efficacité de la sélection. Les procédures de sélection s'articulent en cycles successifs au cours desquels *i)* une population de communautés est établie, *ii)* un phénotypage intervient après une période d'incubation prédéterminée, *iii)* les communautés ayant le phénotype le plus proche du phénotype cible sont identifiées et *iv)* utilisées pour établir une nouvelle population de communautés. Plusieurs choix expérimentaux relatifs à ces différentes étapes s'imposent et peuvent affecter l'efficacité de la sélection artificielle. Deux d'entre eux ont fait l'objet d'études expérimentales : la taille de l'échantillon prélevé dans la communauté d'intérêt afin de créer une nouvelle génération et la durée de la phase d'incubation. De plus, des approches de modélisation ont été utilisées pour déterminer l'effet de l'intensité de la sélection, c'est-à-dire du nombre de communautés choisies sur le nombre total de communautés dans la population pour créer une nouvelle génération, ou encore l'effet d'assembler ou non plusieurs communautés. Dans cette étude, nous avions pour objectif d'étudier expérimentalement ce dernier point, autrement dit, ***l'objectif était de caractériser l'effet de la méthode de reproduction sur l'efficacité de la sélection artificielle de communautés microbiennes.*** La méthode de reproduction, par analogie avec la sélection d'organismes à reproduction sexuée, fait ici référence à la méthode de création d'une nouvelle génération d'unités de sélection (= de communautés) à partir d'unités parentales. Deux méthodes peuvent être envisagées : soit les unités filles sont toutes issues de la même unité parentale (analogue à une reproduction asexuée), soit les unités filles sont issues d'une combinaison de plusieurs unités parentales (analogue à une reproduction sexuée). Ces deux méthodes sont respectivement

nommées méthode « ***propagule*** » et méthode « ***migrant pool*** ». Étant donné que la sélection artificielle de communauté peut agir sur les interactions entre espèces, nous avons fait l'hypothèse que la méthode permettant la plus grande efficacité serait celle qui préserverait au mieux les interactions potentiellement impliquées dans le phénotype d'intérêt, c'est-à-dire la méthode propagule. Toutefois, l'efficacité de la sélection n'est pas uniquement une question de fidélité de transmission des déterminants du phénotype d'intérêt (c'est-à-dire d'héritabilité) mais également une question de variation. Il était donc également possible de faire l'hypothèse que la méthode migrant pool permettrait de maintenir une plus grande diversité au cours de l'expérimentation et donc potentiellement une plus grande variabilité associée à une meilleure efficacité de sélection. Il était donc attendu que les différentes méthodes de reproduction soient associées à différents équilibres entre variation et héritabilité.

Une procédure de sélection artificielle a été appliquée sur des communautés microbiennes du sol cultivées en laboratoire et propagées au cours de 14 cycles de sélection selon les deux méthodes de reproduction. La propriété cible de la sélection était la ***production de biomasse*** (estimée par mesure de densité optique) et différents traitements de sélection ont été appliqués : deux traitements de sélection directionnelle visant à augmenter (H pour High) ou diminuer (L pour Low) la production de biomasse, un traitement de sélection stabilisante (S pour Stabilizing), un contrôle impliquant une sélection aléatoire des communautés parentales (R pour Random). Pour chacun de ces traitements, les deux méthodes de reproduction ont été appliquées : P pour méthode propagule avec laquelle une communauté parentale était sélectionnée parmi dix pour créer dix unités filles (procédure répétée trois fois) et M pour méthode migrant pool avec laquelle trois unités parentales étaient sélectionnées parmi 30, assemblées et utilisées pour créer 30 unités filles. Cette expérimentation incluait donc huit traitements au total : HP, HM, LP, LM, SP, SM, RP, RM. La production de biomasse des communautés a été caractérisée à la fin de chaque cycle de sélection et la diversité des communautés aux cycles 3, 7, 10 et 14 a été analysée par une approche de « fingerprinting ».

Les profils de changement de production de biomasse au cours de la procédure ont mis en évidence des difficultés à mettre en place une sélection artificielle efficace, comme dans les études expérimentales précédentes. En effet, des variations de productivité erratiques au cours des cycles ont été observées et les divergences entre les différents traitements étaient seulement transitoires. Des calculs d'héritabilité ont permis de mettre en évidence que celle-ci était très faible dans notre expérimentation ce qui était probablement à l'origine de la faible efficacité de sélection. L'une des pistes d'amélioration de ces procédures serait de diminuer la part des variations de phénotype qui ne sont pas dues à des déterminants héritables. Par exemple, pour éviter les erreurs d'échantillonnage, les unités filles pourraient être inoculées à partir d'une quantité fixe de cellules via la cytométrie de flux ce qui éviterait de créer des variations quantitatives entre les unités filles qui interviennent certainement lorsque l'inoculation se fait par pipetage. Ainsi, dans notre étude, la ressemblance enfants/parents était certainement trop faible pour que la sélection soit efficace. Nous avons également observé un déclin global dans la productivité des communautés au début de l'expérimentation quel que soit le traitement

appliqué (bien que ce déclin ait été moins important dans les communautés sélectionnées pour une haute production de biomasse). Cela indique qu'un manque d'acclimatation et/ou d'adaptation des communautés aux conditions de culture a également pu interférer avec la sélection artificielle. Si nous avons observé des effets transitoires des traitements de sélection sur la production de biomasse, nous avons également observé des effets sur la diversité des communautés. En effet, la diversité des communautés a décliné au cours des cycles de sélection pour l'ensemble des traitements avec toutefois un maintien de la diversité dans le temps supérieur dans le traitement L ce qui indique que certains phénotypes de communautés seraient plus susceptibles d'être établis par un ensemble d'espèces que d'autres. Concernant la méthode de reproduction, nos résultats indiquent qu'elle interagit avec le traitement de sélection, de plus, nous n'avons observé que des différences transitoires entre les deux méthodes ce qui complique l'émergence d'une conclusion globale. Toutefois, nos résultats indiquent que la méthode migrant pool serait plus propice à une sélection ciblant une augmentation d'un niveau de fonction (+5% de productivité par rapport à une sélection avec la méthode propagule). De plus, une augmentation de la richesse de la communauté en fin d'expérimentation a été observée dans le traitement HP. Cela indique que l'interaction positive entre la méthode migrant pool et la sélection pour une production de biomasse élevée était accompagnée d'un effet favorable sur la diversité de la communauté.

En conclusion, la préservation du phénotype des communautés sélectionnées est un enjeu clé de la réussite d'une procédure de sélection artificielle. Le fait d'assembler ou non plusieurs communautés parentales lors de l'étape de reproduction ou encore le choix du nombre de communautés à assembler sont des éléments qui peuvent être déterminants et qui doivent donc faire l'objet d'une attention particulière lors de la conception des expérimentations. En particulier, la méthode de reproduction peut avoir un impact sur la structure et la diversité des communautés et ainsi influencer l'efficacité de la sélection.

Chapitre 2 : La diversité de la communauté détermine l'évolution de communautés bactériennes synthétiques sous sélection artificielle

La sélection artificielle de communautés microbiennes peut être appliquée sans aucun a priori sur la composition ou le fonctionnement des communautés sous sélection. Cependant, étudier la diversité des communautés au cours d'une procédure de sélection peut permettre de mieux comprendre la dynamique des communautés dans ce contexte. Il est connu que les composantes de la diversité des communautés, telles que la richesse, la composition ou l'équitabilité, influencent plusieurs fonctions telles que la productivité ou la stabilité. Augmenter la diversité d'une communauté augmente la probabilité d'observer des effets de complémentarité ou de facilitation entre espèces de même que la probabilité d'intégrer une espèce très performante au regard d'une fonction. Ainsi, une diversité élevée pourrait être associée à des niveaux élevés d'une fonction. Dans les procédures de sélection artificielle,

au-delà d'un effet potentiel de la diversité sur le niveau initial d'une fonction, il pourrait également y avoir un effet sur la réponse de la communauté à la sélection. Le terme « évolution » est parfois restreint aux changements génétiques au sein des espèces au cours des générations. Cependant, dans le contexte de la sélection de communautés, d'autres sources de variation peuvent intervenir dans la réponse évolutive de la communauté à partir du moment où elles sont transmissibles d'une génération à l'autre. En effet, le phénotype d'une communauté dépend de sa composition allélique et des interactions intragénomiques, de la composition des populations et des interactions intraspécifiques, et de la composition en espèces et des interactions interspécifiques. Toutes ces sources de variation possible dans la réponse des communautés à la sélection sont en lien direct avec la diversité des communautés. Augmenter la diversité des communautés sous sélection pourrait donc permettre d'augmenter les sources possibles de variation phénotypique et donc la probabilité d'observer des valeurs extrêmes de la fonction cible de la sélection. En conséquence, si l'héritabilité est non nulle, la réponse à la sélection pourrait être augmentée. Dans cette étude, ***nous avions pour objectif d'explorer le lien entre la diversité d'une communauté et l'efficacité de la sélection artificielle***. Dans la littérature, la sélection artificielle est la plupart du temps appliquée sur des communautés naturelles complexes (issues d'un prélèvement de sol par exemple) qui sont cultivées en laboratoire. Plusieurs études ont proposé un suivi de la diversité des communautés au cours du temps mais aucune d'entre elles n'a intentionnellement manipulé la diversité des communautés. Nous avons conçu une expérimentation alliant les approches de sélection artificielle de communautés microbiennes et celles utilisées pour étudier les liens biodiversité/fonctionnement des écosystèmes afin de déterminer l'effet d'une augmentation de la richesse des communautés sur l'efficacité de la sélection.

Des communautés bactériennes de ***différents niveaux de richesse*** (2, 4, 8 ou 16 espèces) ont été construites à partir de 18 souches de laboratoire. L'ensemble des communautés (6 communautés différentes par niveau de richesse) et des 18 monocultures ont été soumises à une procédure de sélection artificielle visant à augmenter la ***production de biomasse*** (estimée par mesure de densité optique). 40 cycles de sélection d'une durée de 3,5 jours ont été appliqués. L'efficacité de la sélection a été définie comme le changement de production de biomasse au cours des cycles de sélection mise au regard du changement obtenu dans un traitement contrôle dans lequel aucune sélection artificielle n'était appliquée. À la fin de la procédure, les monocultures et communautés évoluées ont été caractérisées par rapport aux monocultures et communautés ancestrales (c'est-à-dire avant évolution) via une étude de leurs dynamiques de croissance, de leurs capacités métaboliques et de leur composition.

Nos résultats ont montré que la sélection artificielle avait un effet sur la production de biomasse moyenne des communautés (+16% par rapport au contrôle), en revanche aucun effet sur le changement de production de biomasse au cours du temps n'a été détecté. L'absence d'effet au cours de la procédure rend compte de l'absence d'efficacité de la sélection quel que soit le niveau de richesse initiale. En effet, nos résultats ont indiqué qu'une grande part de la variation phénotypique observée n'était pas transmissible ce qui a limité considérablement la portée de la sélection. La richesse initiale des

communautés avait un effet à la fois sur la production de biomasse moyenne et sur le changement de production au cours du temps. Tout d'abord, les résultats de l'étude de la composition des communautés indiquent que la dominance était élevée dans les communautés dès le début de l'expérimentation. En effet, dès lors que la composition initiale d'une communauté incluait à la fois une souche d'*Escherichia coli* et une souche de *Pseudomonas* sp. ADP, quasiment aucune autre souche n'était détectable. D'autres études ont montré des résultats similaires et suggèrent que des interactions positives existent entre les souches d'Enterobacteriaceae et de Pseudomonadaceae. Ainsi, il est probable que, dans notre expérimentation, l'effet positif de la richesse initiale sur la productivité et les capacités métaboliques des communautés ait été dû à une augmentation de la probabilité d'intégrer ce binôme de souches avec l'augmentation du nombre d'espèces. Nos résultats ont indiqué que la diversité avait également une influence sur la dynamique évolutive des communautés. En effet, le sens du changement de la production de biomasse (augmentation ou diminution au cours des 40 cycles) dépendait de la richesse initiale des communautés mais également de leur composition. Malgré un manque global d'efficacité de la sélection, nos résultats indiquaient également que la richesse des communautés pourrait avoir un effet sur celle-ci. En effet, la divergence entre le traitement de sélection et le traitement contrôle était affectée (de manière non-linéaire) par l'augmentation de la richesse initiale des communautés. Par ailleurs, le niveau de corrélation entre le phénotype parental et le phénotype des communautés filles dépendait également du niveau de richesse initiale. Cela suggère que la recherche d'un équilibre variation/héritabilité pourrait être conduite en modulant la diversité des communautés. D'après nos résultats, l'augmentation de la richesse initiale des communautés conduisait à une augmentation de leur ressemblance en termes de composition et donc à une diminution de la variation phénotypique entre communautés d'un même niveau de richesse. Cela signifie donc potentiellement une baisse du niveau d'exploration des trajectoires possibles. Il faudrait donc s'assurer en début de procédure qu'il existe une divergence de composition suffisante entre les différentes populations de communautés pour explorer au mieux le paysage structure/fonction.

Dans cette étude, nous avons montré que la diversité des communautés pouvait jouer un rôle dans les procédures de sélection artificielle. En effet, la richesse des communautés avait à la fois un effet direct sur la propriété sous sélection et un effet sur la dynamique évolutive des communautés. Nos résultats suggèrent également que la richesse pourrait influencer l'efficacité de la sélection mais un compromis entre augmentation de la richesse et maintien de la variabilité de composition rend l'effet non linéaire. Une fois cette limitation dépassée, appliquer la sélection sur des communautés de différents niveaux de diversité pourrait permettre d'explorer différents équilibres entre variation et héritabilité. Des améliorations sont encore nécessaires pour rendre la sélection artificielle de communautés efficace, des études portant sur le lien entre la dynamique écologique des communautés et leur trajectoire évolutive permettraient d'ouvrir la voie à une ingénierie efficace des communautés microbiennes et des microbiotes.

Chapitre 3 : Le rôle central de l'évolution des interactions interspécifiques dans l'évolution des communautés

Pour que le phénotype d'une communauté évolue, il faut que les communautés soient discrètes (c'est-à-dire distinctes spatialement les unes des autres), différentes les unes des autres, capables de se reproduire et il doit y avoir une ressemblance entre les communautés parentales et les communautés filles. Ces conditions peuvent être remplies en laboratoire et certaines conditions écologiques pourraient également favoriser leur existence *in natura*. L'évolution du phénotype d'une communauté peut impliquer ou non une évolution des interactions entre espèces. D'un point de vue théorique, il est admis que l'évolution des communautés peut intervenir via des changements génétiques chez les espèces qui composent la communauté (par exemple via des mutations, des transferts horizontaux de gènes, des pertes de gènes). En fonction des auteurs, des changements dans les abondances relatives des différentes espèces sont soit considérés comme partie prenante de l'évolution des communautés soit comme un « tri écologique ». En plus des changements dans les gènes et les fréquences des espèces, des études de sélection artificielle de communautés ont suggéré que les interactions interspécifiques pouvaient également contribuer à la réponse d'une communauté à l'évolution. En parallèle, d'autres études dont l'objectif était d'étudier les dynamiques évolutives des communautés, en particulier en réponse à des changements environnementaux, ont présenté des résultats détaillés concernant l'évolution des interactions dans des communautés microbiennes synthétiques. Ainsi, les interactions peuvent évoluer : sous l'effet de l'évolution de l'une des espèces de la communauté, sous l'effet de l'évolution de plusieurs espèces dans la communauté, ou encore par coévolution, c'est-à-dire adaptation et contre-adaptation chez des espèces en interaction. De plus, l'environnement abiotique peut influencer l'évolution des interactions. Par exemple, une mutation chez une espèce affectant son interaction avec une autre espèce peut être sélectionnée ou non en fonction du niveau de structuration de l'environnement. Pour aller plus loin, l'environnement abiotique peut être modifié par une espèce ce qui peut ensuite influencer l'évolution des autres espèces de la communauté : c'est la construction de niche. Ainsi, des boucles de rétroactions éco-évolutives peuvent également être à l'origine de l'évolution des interactions. Il y a de nombreuses études qui illustrent l'évolution des interactions interspécifiques, la question n'est donc plus de savoir si les interactions peuvent évoluer mais plutôt à quel point l'évolution des interactions est importante dans l'évolution des communautés. Dans cette étude, ***nous avions pour objectif d'étudier la prévalence de l'évolution des interactions interspécifiques dans l'évolution du phénotype d'une communauté***. Nous avons ré-isolé des souches bactériennes qui avaient évolué en communauté lors de l'expérimentation présentée dans le Chapitre 2 afin de discuter de l'évolution des interactions deux à deux. Nos hypothèses étaient les suivantes : *i)* les interactions interspécifiques ont influencé l'évolution du phénotype des communautés, *ii)* cette influence s'est produite au travers d'une évolution des interactions en elles-mêmes et *iii)* l'évolution du phénotype de la communauté était dépendante de l'environnement abiotique.

Huit binômes de souches bactériennes issues de communautés différentes ont été étudiés. Pour chaque binôme de souches, onze phénotypes ont été caractérisés dans deux environnements différents. La ***production de biomasse*** (estimée par mesure de densité optique) a été mesurée pour chacune des deux espèces d'un binôme dans sa version ancestrale (= avant évolution), évoluée en isolée (= en monoculture) et évoluée en communauté (soit six traitements au niveau de l'individu). La production de biomasse des communautés (= des deux souches d'un binôme en co-culture) a été mesurée lorsque les deux souches étaient en version ancestrale, évoluée en isolée et évoluée en communauté. De plus, la production de biomasse de communautés mixtes ancêtres/évoluées a également été mesurée. Ces mesures ont été réalisées dans l'environnement abiotique dans lequel les communautés ont évolué et dans un second environnement différant pour le volume du milieu de culture et l'agitation de ce milieu.

Tout d'abord, nos résultats ont indiqué que l'évolution des souches bactériennes au sein d'une communauté dépendait des interactions interspécifiques. En effet, une évolution en monoculture ne produisait jamais le même phénotype qu'une évolution en communauté, cela illustre l'importance de l'environnement biotique dans l'évolution des individus. En catégorisant les deux espèces membres d'une communauté en fonction de leur productivité initiale en monoculture, une bonne prédiction de leur réponse évolutive a été observée. Nos résultats indiquaient que l'espèce la plus productive des deux avait un rôle dominant dans le phénotype de la communauté mais également dans la réponse de la communauté à l'évolution. Au regard des résultats rapportés dans d'autres études, le taux d'évolution d'une espèce et donc son rôle dans l'évolution du phénotype d'une communauté pourrait être prédit à partir de sa fréquence dans la communauté, au moins dans les premiers temps de l'établissement d'une communauté. Nos résultats ont également indiqué que l'évolution du phénotype d'une communauté dépendait des interactions interspécifiques. Cependant cet effet des interactions est intervenu différemment en fonction des communautés. Le phénotype de la communauté a évolué dans sept communautés sur huit soit *i)* via l'évolution d'une espèce conditionnellement à la présence de la seconde sans évolution de l'interaction (deux communautés sur huit), *ii)* via l'évolution des deux espèces conditionnellement à leur présence respective sans évolution de l'interaction (1/8), *iii)* via l'évolution de l'interaction sous l'effet d'une espèce ou des deux (4/8). Ainsi, l'évolution du phénotype de communauté a impliqué une évolution des interactions interspécifiques dans la moitié des communautés étudiées ce qui suggère qu'elles ont un rôle important dans les dynamiques évolutives des communautés. La réponse évolutive des communautés lorsque les interactions ont évolué a donné lieu à une variation de production de biomasse d'environ 35% par rapport aux communautés ancestrales soit environ 15% de plus que ce qui aurait pu être prédit à partir des réponses individuelles uniquement. Toutefois, la direction de ce changement était communauté-dépendante. De même, l'effet de l'environnement abiotique sur les réponses évolutives des communautés et des souches composant ces communautés était également communauté-dépendant. En effet, certains changements de phénotype de souches ou de communautés étaient observés quel que soit l'environnement abiotique tandis que d'autres étaient non-détectables ou intervenaient dans la direction opposée sous l'effet d'un changement d'environnement

abiotique. Fait intéressant, dans trois des quatre communautés dans lesquelles une évolution des interactions a été détectée, la réponse évolutive de la communauté était détectable quel que soit l'environnement contrairement à celle des souches composant la communauté.

Ainsi, nous avons enregistré une évolution des interactions interspécifiques dans 50% des communautés étudiées. De plus, nous avons mis en évidence que, même lorsque les interactions n'évoluaient pas en elles-mêmes, elles influençaient l'évolution de la communauté et des souches qui la composaient. Cela met en évidence les liens complexes qui existent entre écologie et évolution. Nos résultats suggèrent également que les communautés au sein desquelles les interactions évoluent sont plus susceptibles d'être indépendantes de l'environnement abiotique dans l'expression du phénotype évolué. Cela pourrait présenter un fort intérêt dans le cadre des procédures de sélection artificielle et de leurs applications et cela met en évidence l'intérêt possible de considérer les communautés comme des unités de sélection.

Discussion

Améliorer la sélection artificielle de communautés

Dans les deux procédures de sélection artificielle de communautés microbiennes que nous avons mises en place (Chapitres 1 et 2), nous avons eu des difficultés à enregistrer une amélioration de la fonction cible de la sélection au cours du temps. Nous pouvons identifier plusieurs facteurs qui ont pu limiter notre capacité à mettre en œuvre une sélection efficace. Tout d'abord, et comme souvent dans ce type de procédure, nous avons observé des variations erratiques de la valeur de la propriété sous sélection au cours du temps. Cela signifie que le phénotype des communautés parentales n'est pas fidèlement reproduit par les communautés filles. Cette absence de ressemblance peut être la conséquence de caractéristiques intrinsèques des communautés et/ou de facteurs extrinsèques liés aux conditions expérimentales. En effet, un phénotype résulte de facteurs génétiques et de l'environnement. Ce qui est héritable (au sens large), c'est la part de variation phénotypique qui est due à une variation dans les facteurs génétiques. Autrement dit, pour que les communautés filles ressemblent à leurs parents, la variation phénotypique entre les parents (= les unités de sélection) doit être due aux espèces composant la communauté (leurs gènes, leurs abondances relatives, leurs interactions). Si ce n'est pas le cas, par exemple si la variation phénotypique est due à des ***variations spatiales dans les conditions environnementales***, l'unité parentale la plus performante au regard de la fonction cible de la sélection sera l'unité qui a les conditions les plus favorables et donc la variation ne sera pas héritable. Cela peut également se produire si la variation est introduite par des ***erreurs de mesure***. Des variations dans les conditions environnementales d'un cycle à l'autre peuvent également affecter la ressemblance parents/enfants en créant du « ***bruit expérimental*** ». L'une des manières d'enregistrer le « signal » de la sélection malgré ce « bruit » peut être d'augmenter le nombre de cycles de sélection. Un manque de ressemblance parents/enfants peut également être dû à une ***erreur d'échantillonnage*** intervenant au

moment de créer des communautés filles à partir d'une communauté parentale d'intérêt. Cela pourrait être évité en utilisant la cytométrie de flux plutôt que le pipetage par exemple. Au-delà des limitations causées par des facteurs expérimentaux, la biologie des communautés sous sélection peut également jouer sur la ressemblance parents/enfants. En effet, plusieurs auteurs suggèrent que la composition et la structure des communautés doivent être stables pour pouvoir appliquer une sélection efficace. Il a été montré par des approches de modélisation et expérimentales que cette stabilité pouvait être favorisée par la mise en place de plusieurs cycles successifs de croissance avant de commencer la procédure de sélection. Malgré cela, il peut y avoir des perturbations de la ressemblance parents/enfants liées au fonctionnement intrinsèque des communautés. En effet, certains auteurs suggèrent que de très petites variations dans les conditions initiales chez les communautés filles, induites par un effet d'échantillonnage par exemple, peuvent être amplifiées par la dynamique complexe des communautés : c'est l'***effet papillon***. La taille de l'échantillon utilisé pour créer les unités filles peut également avoir une influence. En effet, plus une communauté est petite, plus elle va être soumise à la ***dérive écologique et génétique***, c'est-à-dire à des fluctuations stochastiques dans la fréquence des espèces et des gènes. Ainsi, une petite taille d'échantillon est associée à un fort risque de perdre les déterminants du phénotype parental. Enfin, la ***sélection naturelle*** au niveau de l'individu peut également intervenir au sein des communautés au cours des cycles de sélection artificielle de communautés et provoquer des dissimilarités entre communautés filles et communautés parentales.

Le paradoxe dans les procédures de sélection artificielle de communautés est que des variations entre enfants et parents peuvent empêcher la transmission du phénotype d'intérêt mais, dans le même temps, il doit y avoir de la variation entre unités de sélection pour que la sélection fonctionne. L'une des possibilités pour ***introduire de la variation phénotypique*** entre unités de sélection (= entre communautés parentales) est de créer la population de communautés initiale (= la métacommunauté) à partir d'échantillons d'origines diverses. L'avantage de cette méthode est qu'elle crée une importante variation phénotypique qui peut être à l'origine d'une forte réponse à la sélection, toutefois cette variation est très vite érodée et la procédure revient à éliminer les communautés les moins performantes plutôt qu'à améliorer les meilleures d'entre elles. Dans certaines études, la sélection artificielle est appliquée sur plusieurs lignées en parallèle, c'est-à-dire plusieurs métacommunautés, toutes issues du même échantillon initial, comme dans le Chapitre 1. Dans le Chapitre 2, nous avons également appliqué la procédure sur plusieurs lignées, à la différence que ces lignées étaient issues de différents échantillons de départ. Théoriquement, c'est la variation au sein d'une lignée qui a une importance pour l'efficacité de la sélection, toutefois nous nous sommes demandé si le fait de maintenir plusieurs lignées pouvait avoir un intérêt. Des résultats complémentaires à ceux présentés dans les Chapitres et obtenus à partir des données du Chapitre 2 indiquent que l'établissement de plusieurs lignées peut permettre d'intégrer des lignées dans lesquelles il existe naturellement une forte variation propice à la sélection. Une autre possibilité est d'introduire volontairement de la variation via l'application d'une perturbation par exemple. Une fois que la communauté la plus performante au regard de la propriété sélectionnée a été

identifiée dans une métacommunauté, des variants de cette communauté pourraient être créés en modifiant le matériel génétique des espèces ou la composition de la communauté par exemple. Cela peut être fait en favorisant le transfert horizontal de gènes, en ajoutant ou en retirant une espèce en particulier ou encore en appliquant une perturbation environnementale. Le choix de la perturbation (choc thermique, application d'un antibiotique etc.) peut permettre de contrôler le niveau de variation induite afin de ne pas perdre le phénotype cible.

Le ***choix des traitements contrôles*** est important dans les procédures de sélection de communautés. On trouve deux types de contrôles dans la littérature. La plupart du temps, le contrôle est un traitement où les communautés parentales sont sélectionnées au hasard, comme dans le Chapitre 1. L'autre possibilité est d'inclure un traitement dans lequel aucune sélection de communauté n'est appliquée : toutes les communautés sont transférées au cycle suivant, comme dans le Chapitre 2. Ces deux contrôles permettent de répondre à des questions différentes. Le contrôle sans sélection artificielle permet de répondre à la question : est-ce que les résultats obtenus par sélection artificielle au niveau de la communauté sont différents de ce qui est obtenu par sélection naturelle au sein des communautés ? Le contrôle avec sélection aléatoire permet quant à lui de répondre à la question suivante : est-ce que l'effet d'une sélection directionnelle entre les communautés est différent de l'effet d'une sélection aléatoire ? Dans l'idéal, il faudrait inclure les deux traitements mais cela augmente considérablement l'effort d'échantillonnage. Lorsqu'il n'est pas possible d'inclure les deux contrôles, le choix du contrôle doit être déterminé par la question de recherche et les interprétations doivent être faites avec attention au regard du contrôle choisi.

Comprendre les dynamiques éco-évolutives des communautés

L'écologie microbienne synthétique permet de travailler sur des communautés simplifiées dans le but de comprendre leur fonctionnement et le rôle des interactions dans l'établissement des propriétés de communauté. Le nombre d'espèces composant une communauté synthétique est considérablement réduit par rapport à celui des communautés naturelles et les communautés synthétiques sont étudiées dans des conditions extrêmement contrôlées. Malgré cela, il est clair que l'étude des communautés synthétiques peut contribuer grandement à la compréhension de l'écologie et de l'évolution des communautés. Dans le Chapitre 3, nous avons montré que l'évolution des communautés et des membres d'une communauté était dépendante d'une histoire évolutive commune des espèces via deux mécanismes : un ***effet des interactions sur l'évolution*** des phénotypes (effet de l'écologie sur l'évolution) et une ***évolution des interactions interspécifiques*** elles-mêmes (effet de l'évolution sur l'écologie). Dans le premier cas, la présence d'une espèce influence l'évolution d'une seconde espèce de telle manière que la réponse évolutive à l'échelle de la communauté est prédictible à partir des réponses individuelles. Nos résultats ainsi que ceux de la littérature suggèrent que certaines caractéristiques des espèces pourraient permettre de prédire leur réponse évolutive en tant que membre

d'une communauté. En effet, dans une communauté à deux espèces, l'espèce dominante (qui peut être celle qui a le taux de croissance et/ou la productivité le plus élevé en monoculture) est plus susceptible d'avoir une réponse évolutive plus importante que la seconde espèce et, nos résultats suggèrent que cette réponse implique souvent une adaptation à l'environnement abiotique. Nos résultats suggèrent également que, en réponse, la seconde espèce est susceptible de s'adapter à la présence de l'espèce dominante. Lorsque les interactions interspécifiques évoluent, la réponse évolutive de la communauté est plus difficile à prédire, même dans des communautés synthétiques. En effet, il a été montré qu'une évolution des interactions pouvait mener à une augmentation d'une fonction de la communauté. Cependant, dans l'étude présentée en Chapitre 3, une diminution de la productivité concomitante avec l'évolution des interactions a été observée dans deux communautés sur huit. Ainsi, l'évolution des interactions ne conduit pas nécessairement à l'augmentation du niveau de fonctionnement d'une communauté. Toutefois, nos résultats suggèrent qu'elle peut conduire à une meilleure stabilité de la fonction portée par une communauté, mais cela pourrait dépendre du type d'interaction qui est en jeu. Il est complexe de prédire l'effet de l'évolution des interactions sur le phénotype d'une communauté et, il est également difficile de prédire dans quels cas nous pouvons attendre une évolution des interactions étant donné que cela semble dépendre de multiples facteurs liés à l'environnement biotique et abiotique. Étudier les interactions deux à deux est un bon point de départ pour comprendre les mécanismes qui pourraient influencer l'évolution des communautés, cependant l'existence d'interactions d'ordre supérieur dans des communautés complexes rend encore plus difficile d'en prédire le fonctionnement.

La ***diversité*** est l'un des objets d'étude principaux en écologie des communautés. En accord avec les données de la littérature, nous avons enregistré une augmentation du fonctionnement de nos communautés bactériennes synthétiques avec l'augmentation de leur richesse. Pour aller plus loin, nous avons également observé un lien entre la richesse des communautés et leur évolution. En effet, la direction des changements de productivité au cours de l'évolution expérimentale de communautés était affectée par la richesse initiale de celles-ci. Cependant, cet effet n'était pas linéaire et nous avons également observé des différences entre communautés d'un même niveau de richesse variant pour leur composition. À partir des données de la littérature, il apparaît que l'effet de la diversité sur l'évolution des communautés peut dépendre de l'environnement abiotique, de l'identité des espèces composant la communauté, des interactions interspécifiques et de leur évolution. Si l'effet de la diversité sur l'évolution des communautés est peu connu, il y a plus de travaux sur l'effet de la diversité sur l'évolution des membres d'une communauté. D'un point de vue théorique, une augmentation de la richesse d'une communauté pourrait affecter positivement le taux d'évolution d'une espèce via l'augmentation des pressions de sélection dues à la présence d'autres espèces ou l'augmentation de la probabilité d'observer un transfert horizontal de gène. Au contraire, on peut s'attendre à un effet négatif de l'augmentation de la richesse d'une communauté sur le taux d'évolution d'une espèce sous l'hypothèse qu'une augmentation de la richesse conduit à une diminution de la taille de population par espèce. Un autre effet négatif peut intervenir via la limitation de la coévolution – qui est connue pour

promouvoir une évolution rapide des espèces impliquées - due à l'existence d'un réseau d'interactions complexe avec des espèces impliquées dans des interactions avec de nombreuses autres espèces. Des études expérimentales suggèrent que l'évolution d'une espèce pourrait être favorisée par la présence de quelques autres espèces mais que, au-delà d'un certain nombre d'espèces, une augmentation de la richesse pourrait diminuer la force des interactions biotiques et le taux d'évolution. Ainsi, il apparaît qu'il y a un lien étroit et complexe entre la diversité et l'évolution des communautés et des membres des communautés, un changement de diversité (via l'introduction ou la perte d'une espèce) pourrait donc avoir un impact à long terme.

Dans une étude récente portant sur la diversité dans une communauté végétale (voir résumé ci-dessous), nous avons montré que la diversité interspécifique interagissait avec la ***diversité intraspécifique*** dans la relation diversité/productivité. Dans nos expérimentations sur communautés bactériennes, il est très probable que la diversité intraspécifique (c'est-à-dire la diversité dans les populations bactériennes) ait joué un rôle dans le fonctionnement et l'évolution des communautés. En effet, nous avons remarqué la présence de deux sous-populations de *Pseudomonas* à la fin de son évolution en monoculture. Chez les plantes, il a été montré qu'une augmentation de la diversité intraspécifique pouvait avoir un effet sur la compétition interspécifique et sur la dominance dans les communautés. À l'inverse, nous avons également montré qu'augmenter la diversité interspécifique pouvait changer les interactions entre génotypes. Chez les bactéries, certaines études suggèrent que les interactions interspécifiques pourraient affecter l'évolution au sein des espèces en favorisant ou réduisant la diversité intraspécifique, des études complémentaires permettraient d'aller plus loin dans la compréhension des interactions entre diversité inter- et intraspécifique et de leur effet sur l'évolution des communautés.

Nous avons discuté des liens étroits qui existent entre la diversité, les interactions interspécifiques et le fonctionnement et l'évolution des communautés et avons mis en évidence la complexité du mode d'organisation en communauté et les défis associés à la prédiction de la dynamique des communautés. Dans ce contexte, il est pertinent de se demander quel pourrait être l'impact d'introduire une communauté obtenue par sélection artificielle dans un environnement dans lequel une communauté est déjà établie. En effet, si l'environnement en question est un milieu naturel et ouvert tel qu'une plante ou un sol, nous devons prendre en considération la communauté native et les effets possibles d'une inoculation sur celle-ci. La mise en contact de deux communautés qui ne l'étaient pas auparavant est appelée ***coalescence*** des communautés. L'effet de la coalescence peut dépendre des conditions environnementales, du ratio avec lequel les communautés sont mixées, de l'interface d'interaction mais aussi de la dynamique temporelle de la coalescence. Dans le contexte de l'introduction d'une communauté microbienne dans un environnement, nous pouvons nous attendre à ce que la communauté microbienne native bénéficie d'un effet de priorité qui limite les risques d'invasion par l'inoculant. Cela est confirmé par des approches expérimentales portant sur l'inoculation de sols agricoles. Toutefois, il a été montré que, bien que la communauté introduite ne soit rapidement

plus détectée, la structure de la communauté native est impactée et, bien souvent, à long terme. Cela a une importance majeure car nous pouvons nous attendre à ce qu'un changement de structure de communauté influence le fonctionnement et l'évolution de la communauté de même que les processus écosystémiques associés. De plus, il semblerait que l'effet d'une inoculation puisse être contexte-dépendent et ainsi avoir des résultats inattendus. Par exemple, certains microorganismes connus pour établir des associations mutualistes avec leurs hôtes peuvent devenir pathogènes en fonction des conditions environnementales. Par ailleurs, des transferts de microbes intestinaux chez l'humain ont produits des résultats contrastés avec, chez certains patients, une détérioration de l'état clinique et parfois de sévères effets secondaires indésirables liés au transfert involontaire de bactéries portant des multi-résistances. Il y a donc des points de préoccupation majeurs concernant l'effet de l'introduction d'une communauté sur les communautés natives, les risques d'invasion et leurs conséquences, et les possibles effets imprévisibles. Ainsi, l'ingénierie des communautés microbiennes, y compris via la sélection artificielle, nécessitera de considérer les effets possibles de la communauté d'intérêt sur l'environnement biotique et abiotique dans lequel elle sera introduite.

Conclusion

Les microorganismes jouent des rôles clés dans le domaine de la santé, en agriculture, dans l'environnement, dans l'industrie… Le fonctionnement en communauté permet l'émergence de propriétés qui font l'objet d'un intérêt particulier au regard de l'importance des microorganismes dans de nombreux processus. Dans ce contexte, la sélection artificielle de communautés est une approche prometteuse qui pourrait permettre une ingénierie des communautés pour répondre à des problématiques appliquées. Dans cette thèse, nous avons mis en évidence des limitations qui persistent avant d'être capable d'appliquer une sélection artificielle efficace. Toutefois, plusieurs pistes d'amélioration ont déjà été mises en évidence et il y a une littérature grandissante à ce sujet de telle manière qu'il sera probablement possible d'utiliser cette approche pour l'ingénierie des communautés microbiennes et des microbiotes. Cela va soulever des questions éthiques liées à l'utilisation de ces communautés portant des fonctions d'intérêt (par exemple : devons-nous les introduire en milieu naturel ? seulement dans des systèmes fermés ? quel degré de manipulation est acceptable ?). Au-delà des applications possibles, les expérimentations de sélection artificielle à l'échelle des communautés peuvent contribuer à la compréhension des dynamiques de communautés. En effet, l'organisation en communauté confère des propriétés uniques mais également des défis uniques lorsqu'il s'agit d'en comprendre l'écologie et l'évolution. Dans cette thèse, nous avons illustré les nombreuses interactions qui existent entre l'écologie et l'évolution des communautés et apporté des éléments qui portent à considérer les communautés comme des unités de sélection. Au vu de la complexité de l'écologie et de l'évolution des communautés, l'étude au laboratoire de communautés simplifiées peut contribuer grandement à la compréhension du fonctionnement des communautés au sens large.

Annexe : Les microbes du sol dirigent l'effet de l'interaction entre diversité végétale spécifique et génotypique sur la productivité

Chez les plantes, la diversité en espèces, ou diversité spécifique, est connue pour avoir des effets positifs sur le fonctionnement des écosystèmes. Plus particulièrement, de nombreuses études ont montré un ***effet positif de la diversité spécifique sur la productivité*** avec notamment de forts effets de la richesse et de la dissimilarité fonctionnelle. Les mécanismes impliqués sont les effets de complémentarité/facilitation et les effets de sélection. Les effets de complémentarité, et plus particulièrement la complémentarité dans l'utilisation des ressources, sont très étudiés car ils constituent des leviers potentiels de l'amélioration du prélèvement des ressources disponibles dans les sols et de la diminution des pertes de nutriments. De même que pour la diversité spécifique, des effets de complémentarité ou de sélection peuvent intervenir lorsque la diversité génotypique au sein d'une espèce est augmentée. En effet, bien que la variabilité entre génotypes soit supposée plus faible que la variabilité entre espèces, plusieurs études ont montré des ***effets positifs d'une augmentation de la diversité génotypique sur la productivité***. Pour aller plus loin, il semblerait que la diversité spécifique et la diversité génotypique puissent interagir et conduire à des modifications des patrons de dominance et de compétition entre espèces. Il a également été montré que ces modifications pouvaient conduire à des modifications de la productivité des communautés, positives ou négatives. Ainsi, comprendre les ***interactions entre diversité spécifique et génotypique*** est nécessaire pour comprendre la relation diversité/productivité. Les microorganismes du sol peuvent également avoir une influence sur la productivité des plantes via des effets directs (microorganismes mutualistes ou pathogènes) ou indirects (microorganismes libres qui agissent sur la disponibilité en ressources). Les effets des microorganismes mutualistes, en particulier des rhizobia et des champignons mycorhizogènes à arbuscules, et des pathogènes sur la productivité sont très étudiés et il semble y avoir un fort effet de l'identité de l'espèce considérée. Les microorganismes du sol peuvent également avoir un effet sur la composition et le fonctionnement des communautés végétales. L'un des mécanismes les plus étudiés est la rétroaction plante-sol ou « plant-soil feedback » qui se produit lorsque les plantes modifient les communautés microbiennes du sol ce qui, en retour, influence la future communauté de plantes. En effet, la présence d'une espèce végétale peut favoriser certains microbes (mutualistes ou pathogènes par exemple) et, de par la spécificité d'interaction entre plantes et microbes, promouvoir ou réduire la croissance des plantules de cette espèce. L'existence d'une boucle de rétroaction négative peut promouvoir la diversité dans une communauté végétale, à l'inverse une boucle de rétroaction positive peut favoriser la dominance d'une espèce. L'interdépendance entre plantes et microorganismes pour des fonctions essentielles telles que la nutrition peut être tellement forte qu'il peut être utile de considérer une plante et ses microbes associés comme une seule unité fonctionnelle et évolutive que l'on nomme holobionte. Cela présente un intérêt particulier dans le cadre de l'étude du lien diversité/productivité chez les plantes car cela suggère un potentiel rôle des microorganismes dans cette relation. Plusieurs études ont montré

l'importance des microorganismes mutualistes et pathogènes, en particulier des champignons, dans l'existence mais aussi la forme de la relation diversité des plantes/productivité. Ces études étaient centrées sur le lien entre richesse spécifique des plantes et productivité mais, à notre connaissance, aucune étude ne présente le lien entre les microorganismes du sol et *i)* le lien entre diversité génotypique et la productivité des plantes et *ii)* l'effet des interactions entre diversité spécifique et génotypique sur la productivité des plantes. Dans cette étude, ***l'objectif était de déterminer si l'effet de l'interaction entre diversité spécifique et génotypique des plantes sur la productivité était influencé par les microorganismes du sol***. Nous avons d'abord fait l'hypothèse que combiner diversité spécifique et génotypique dans une communauté végétale pouvait mener à des effets non-additifs sur la productivité. Plus spécifiquement, des effets de synergie pourraient se produire via une augmentation dans la complémentarité d'utilisation des ressources en comparaison avec une communauté n'incluant qu'une des deux sources de diversité. Ensuite, nous avons fait l'hypothèse que les microorganismes du sol pouvaient diriger les interactions entre diversité spécifique et diversité génotypique des plantes en se basant sur l'idée que l'effet des microorganismes du sol pouvait être spécifique à une espèce ou un génotype de plante et donc que les interactions plante-plante pouvaient être modifiées. D'après les données issues de la littérature, nous nous attendions également à observer un effet de la communauté végétale sur la diversité des communautés microbiennes du sol.

Nous avons mis en culture ***deux espèces végétales*** qui sont couramment cultivées en association : le ***blé*** (*Triticum aestivum* L.) et le ***pois*** (*Pisum sativum* L.) et pour lesquelles des interactions positives ont été décrites dans la littérature. ***Trois génotypes de chaque espèce*** ont été sélectionnés car possédant des caractéristiques phénotypiques contrastées. Des monocultures ou des mélanges de génotypes ont été cultivés en monoculture ou en mélange d'espèces ce qui résultait en huit traitements de diversité végétale : un génotype de blé (W1), un génotype de pois (P1), trois génotypes de blé (W3), trois génotypes de pois (P3), et quatre mélanges de blé et de pois (W1P1, W1P3, W3P1, W3P3). Un traitement contrôle sans plante a également été inclus. Chacun de ces traitements a été mis en place dans ***trois contextes microbiens*** différents : en sol stérilisé, en sol stérilisé inoculé avec la communauté microbienne native de ce sol et en sol stérilisé inoculé avec une communauté exogène issue d'un champ cultivé en association blé/pois. Les plantes ont été cultivées en pots pendant 22 jours en chambre climatique. À la fin de la période de croissance, les ***biomasses aériennes et racinaires*** après séchage ont été mesurées, l'***azote minéral du sol*** quantifié et l'ADN extrait pour séquençage du gène de l'ARNr 16S chez les bactéries.

Nous avons détecté une influence des communautés microbiennes du sol sur la productivité des plantes via *i)* des effets sur la biomasse aérienne et racinaire (et donc un effet sur le ratio parties aériennes/parties racinaires), *ii)* une régulation des effets de la diversité spécifique et génotypique des plantes sur la biomasse aérienne et *iii)* une modulation des effets de l'interaction entre diversité spécifique et génotypique sur la biomasse aérienne. Tout d'abord, la stérilisation du sol et sa ré-inoculation ou non avec une suspension de sol a effectivement permis de créer trois contextes microbiens

différents en termes de structure et de diversité des communautés bactériennes. Ces différences étaient associées à des différences de ratio biomasse aérienne/biomasse racinaire avec une production de biomasse plus orientée vers les racines en sol stérilisé qu'en sol inoculé. Il faut noter qu'en sol stérilisé, aucune association pois/Rhizobium n'a été observée contrairement aux sols inoculés. Cela a pu jouer un grand rôle dans les différences de productivité observées mais nous avons également des résultats qui suggèrent que la richesse bactérienne en elle-même peut également avoir un rôle. Nous avons montré que l'existence d'un effet de la diversité spécifique des plantes ou de leur diversité génotypique sur la production de biomasse aérienne dépendait des microorganismes du sol. Plus particulièrement, la biomasse aérienne n'était pas influencée par la diversité de la communauté en sol stérilisé contrairement aux sols inoculés. Pour aller plus loin, nos résultats suggèrent que l'effet de l'interaction entre diversité spécifique et diversité génotypique sur la productivité peut être modulé par les microorganismes du sol. En effet, nous n'avons pas détecté d'effet de l'interaction entre les deux sources de diversité dans le sol stérilisé tandis que, dans les sols inoculés, la productivité des plantes en mélanges d'espèces et de génotypes était supérieure à ce qui était attendu en absence d'interaction. Nous nous attendions à des effets différents dans les deux sols inoculés mais le gain de productivité dû à l'interaction entre diversité spécifique et génotypique était similaire. Dans ces deux sols, l'effet d'interaction semblait au moins en partie dirigé par un changement de l'effet de la diversité génotypique du blé en mélange blé/pois par rapport à la monoculture de blé. En sol inoculé, les performances relatives des trois génotypes de blé étaient modifiées par l'association avec le pois alors que cet effet n'a pas été observé en sol stérilisé. Nous suggérons que l'association pois/Rhizobium en sol inoculé a permis la mise en place d'une complémentarité de niche entre le blé et le pois ce qui a pu réduire la compétition, en particulier la compétition intraspécifique, pour l'azote du sol. Nous avons en effet enregistré des taux d'azote minéral du sol très bas en sol stérilisé. Plutôt qu'un effet synergique entre deux effets positifs, nous avons enregistré une compensation d'un effet négatif de la diversité génotypique par l'augmentation de la diversité spécifique. Ainsi, des changements dans les patrons de dominance peuvent être impliqués dans l'effet de la diversité spécifique sur la relation diversité génotypique/productivité et ces effets peuvent être dirigés par les communautés microbiennes du sol.

L'un des objectifs classiques de l'étude de la relation diversité/productivité chez les plantes est de comprendre les interactions plante/plante. Certaines études intègrent également les interactions entre plantes et d'autres organismes tels que les microbes comme modulateurs de la relation diversité/productivité. Au vu de nos résultats et dans le cadre conceptuel de l'holobionte, il pourrait être particulièrement intéressant d'étudier le poids relatif des interactions inter-règnes (plantes-microbes) et intra-règne (plantes-plantes) dans l'explication de la relation diversité/fonctionnement de l'écosystème.

Abstract

In agriculture, the plants and animals that are bred come from artificial selection procedures that aim at producing individuals with desirable traits. Such procedures can also be applied at the community level, especially on microbial communities. However, there is a need for improvements of the process.

The aim of this work was to identify the ways of improving the artificial selection of microbial communities through a better understanding of community ecology and evolution.

In a first experiment, we showed that the reproduction step, i.e. the way offspring communities are created from parental ones in an artificial selection experiment, can be a key determinant of the outcome of the artificial selection. In a second experiment, we showed that community diversity positively influenced the level of the function under selection and also affected community evolutionary dynamics. We also had evidence that it could affect the efficiency of the artificial selection. In a third experiment, we highlighted that the evolution of pairwise interspecific interactions was widespread in community evolution as it was detected in 50% of the studied communities. All together these results give several avenues for improving artificial selection: choosing the reproduction method according to the desired direction of change in a function, promoting community diversity, promoting the evolution of the interspecific interactions.

Improving our understanding of community eco-evolutionary dynamics will open the way to an efficient artificial selection of communities and promote its use as a tool for microbial community engineering.

Keywords: artificial selection, community evolution, diversity, interspecific interactions, microbial community

Résumé

En agriculture, plantes cultivées et animaux d'élevage sont issus de procédures de sélection artificielle qui ont pour but de produire des individus portant des traits d'intérêt. Ces procédures peuvent être appliquées au niveau de la communauté, en particulier chez les communautés microbiennes. Toutefois, des améliorations sont nécessaires afin de rendre ces procédures efficaces.

L'objectif de cette thèse était d'identifier des pistes d'amélioration des procédures de sélection artificielle via une meilleure compréhension de l'écologie et l'évolution des communautés.

Nous avons d'abord montré que la méthode de création de communautés filles à partir de communautés mères peut être un déterminant du résultat de la sélection. Ensuite, nous avons montré que la diversité des communautés influençait positivement le niveau de la fonction sous sélection et affectait également la dynamique évolutive des communautés et possiblement l'efficacité de la sélection. Enfin, nos résultats ont indiqué que l'évolution des interactions interspécifiques était commune dans l'évolution des communautés puisque détectée dans 50% des communautés étudiées. Ensemble, ces résultats ont apporté plusieurs pistes d'amélioration des procédures de sélection artificielle : faire le choix de la méthode de reproduction en fonction de la direction du changement souhaité dans le phénotype cible de la sélection, favoriser la diversité des communautés, favoriser l'évolution des interactions.

Améliorer notre compréhension des dynamiques éco-évolutives des communautés ouvrira la voie à une sélection artificielle de communautés efficace et permettra ainsi son utilisation pour l'ingénierie des communautés microbiennes.

Mots-clés : sélection artificielle, évolution des communautés, diversité, interactions interspécifiques, communautés microbiennes