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Deciphering Biotic Interactions and Their Role in Soil Microbial Community Assembly and Functions

Sarah Huet

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Doctorat de Biologie des Populations et Écologie

Par

Sarah HUET

Deciphering Biotic Interactions and Their Role in Soil Microbial Community Assembly and Functions:
Using Community Manipulation to Steer Soil Microbial Communities

Thèse présentée et soutenue à Dijon, le 6 Décembre 2022

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Dedication

To whomever has felt that you are not enough: you are not - you are part of a community.

So interact!

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Bonne lecture :)

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List of Abbreviations

Abbreviations	Definition
AHL	N-AcylHomoserine Lactone
AMO	Ammonia Mono-Oxygenase
AOA	Ammonia Oxidizing Archaea
AOB	Ammonia Oxidizing Bacteria
BLO	Bdellovibrio and Like Organisms
CI	Confidence interval
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization
HSD	Honestly Significant Difference
NGS	Next-Generation Sequencing
NOB	Nitrite-Oxidizing Bacteria
NOR	Nitrite OxidoReductase
OTU	Operational Taxonomic Unit
PTLB	Phage Tail-Like Bacteriocins
PCoA	Principal Component Analysis
PCR	Polymerase Chain Reaction
pH	Potential of Hydrogen
rDNA	Ribosomal DNA
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
s.e.	Standard Error

1

1.1 Soil Microbial Communities

1.1.1 Role of Soil Microbial Communities

The Earth's environment as we know it did not appear in a snap. It took billions of years to alter the raw elements of the early Earth into the environment we live in today. Microorganisms played a huge part in those alterations, from the ancestors of cyanobacteria producing dioxygen in the Earth's early atmosphere [1] through Earth's biogeochemical cycles [2], to soil pedogenesis [3]. Nowadays, microorganisms are the second major component of biomass on Earth after plants [4] and they not only drive major biogeochemical cycles but also influence plant, animal, and human welfare either directly or indirectly [2], [5], [6].

Microorganisms that live in the soil and deep subsurface account for approximately 20% of the global biomass alone [4]. Soil microorganisms belong to all three domains of life and represent the highest terrestrial diversity with billions of individuals of thousands of different species in one gram of soil [7], [8]. They are essential for plant growth and health as they can impede or sustain plant nutrient acquisition and growth and can act as pathogens or protect the plant from pathogens. Furthermore, they perform the ultimate steps of chemical transformations in soils as they decompose organic matter, which releases nutrients that they transform. Soil microorganisms play a key role in Earth cycles, including the two main ones: carbon and nitrogen cycles. These two cycles are interlinked and the steps performed by microorganisms likely influence the whole Earth ecosystem [2], [9]. In the carbon cycle, soil microorganisms contribute to both (i) efflux of carbon dioxide from the soil to the atmosphere through respiration and (ii) influx into soil stable carbon pool as microbial necromass or through microalgae photosynthesis and plant growth promotion [10]. They also produce humic substances that stabilize carbon in soil, making soil the highest terrestrial carbon pool [11]. Soil microorganisms are also the main drivers of nitrogen cycle from which N-cycling microbial guilds perform decisive steps [12]. In this thesis, we mostly focused on the nitrogen cycling, as a proxy of soil microbial activities, from which we choose three crucial steps: the nitrogen fixation, the nitrification, and the denitrification. While dinitrogen (N_2) constitutes about 78% of Earth's atmosphere, this gaseous form is not readily available for plants and animals and it first need to be converted into ammonium (NH_3/NH_4^+). This transformation is mainly accomplished by the nitrogen-fixing bacteria and catalysed by nitrogenase that are encoded by a variety of *nif* genes such as the *nifH* gene. The ammonium can then be directly assimilated by plants and other microorganisms or can enter the nitrification pathway. Nitrification is the aerobic oxidation of ammonia to nitrate (NO_3^-) via nitrite (NO_2^-) and is performed by two functionally defined group of microbes. The first step is the oxidation of ammonia to nitrite, performed by Ammonia Oxidizing Archaea (AOA) and Ammonia Oxidizing Bacteria (AOB) and catalysed by the ammonia monooxygenase (AMO) encoded by the *amoA* archaeal

and bacterial genes, respectively. This first step is considered as the rate-limiting step in nitrification [13]. The second step of nitrification is the oxidation of nitrite to nitrate, which is performed by nitrite-oxidizing bacteria (NOB) using nitrite oxidoreductase (NOR). Note that the long-sought-after and recently discovered comammox process refers to the complete oxidation of ammonia to nitrate in one organism [14], [15]. At the opposite, denitrification is the reduction of nitrate and nitrite into gaseous nitrogen oxides (NO , N_2O , and N_2). A key step in the denitrification pathway is the transformation of soluble NO_2^- to gaseous NO , which is catalysed by the nitrite reductase, encoded by either the *nirK* or *nirS* genes.

Despite the vital role that carbon and nitrogen cycles play in ecosystem functioning, anthropogenic activities are strongly impacting these cycles at a level that crosses certain biophysical thresholds, leading to potentially disastrous consequences for humanity [16], [17]. Soil ecosystem functioning is threatened by anthropogenic activities that either alter directly (e.g., agriculture or pollution) or indirectly (e.g., climate change) the microbial processes driving carbon and nitrogen cycling [17]. For example, loss of biodiversity alters both carbon and nitrogen cycles. The decrease in microbial diversity can slow the decomposition of the litter, changing the efficiency of carbon use [18], [19] and can deplete microbial guilds that perform crucial steps of the nitrogen cycle [20]–[22]. In 2015, the Food and Agriculture Organization of the United Nations (FAO) declared that soil is a living resource that “constitutes the foundation for agricultural development, essential ecosystem services, and food security”, advocating that “effective policies and actions for the sustainable management and protection of soil resources” are urgently needed [23].

1.1.2 Community Definition: Emergent Properties and Biotic Interactions

A community is a multi-species assemblage, in which organisms live together in a contiguous environment (co-occurrence in space and time) and potentially interact with each other [24]–[26]. The importance of biotic interactions in the definition of a community is not clear, and opposite views arise from the literature. In the early 20th century, Clement suggested that members of a community were so tightly interconnected that communities can be viewed as supra-organisms, considering whole communities as a single organism [27]. At the opposite, Gleason preferred an individualistic perspective, arguing that species co-occur in one habitat because they tolerate the same environmental conditions but without necessarily interacting with each other [28]. Later, the supra organism view was supported by the idea that a community is not defined spatially by one habitat but rather by the range of interactions occurring between individuals, thereby the community boundaries corresponding to the interaction fading [29]. Recently, Liautaud *et al.* [30] proposed a unifying community theory suggesting that the supra-organismal and the individualistic view are ends of a continuum of interaction strength. A high variance in biotic interaction strength will lead to a supra-organismal form of the community while weak and uniform biotic interactions will lead to an individualistic form of the community. Either way, both ends of this continuum are defined by biotic interaction strength.

Furthermore, the importance of biotic interactions in the definition of a community is further supported by the concept of community emergent properties, which is defined as the characteristics that are intrinsic to a community but that cannot be identified or predicted by analysing the individual members of the community. These intrinsic community properties are inherently due to the biotic interactions that occur between the community members [24], [31]–[33]. As an example, due to interspecific interactions, biofilm formation and antimicrobial resistance were unpredictably enhanced in multi-species biofilm compared to single-species biofilm [34], [35]. Therefore, biotic interactions potentially occurring between the community members could be of importance both for the community itself and for its properties.

1.1.3 Community Assembly: Species Coexistence and Biotic Interactions

Microbial communities are not static but dynamic systems that assemble through the influence of several processes. Three main theories could explain the processes that influence the assembly of microbial communities [26], [36], [37]. First, neutral theory asserts that species are ecologically equivalent (i.e., no difference in their population dynamics), implying that community assembly is mainly driven by stochastic processes of birth, death, colonization, extinction and speciation [38], [39]. At the opposite, niche-based theory assumes that species occupy different ecological niches. A species niche is the combination of both (i) the "fundamental niche" (or "requirement niche"), which represents the environmental conditions under which the species exhibits positive response or growth, and (ii) the "realized niche" (or "impact niche"), which represents the additional ecological constraints on the species (e.g., biotic interactions), as well as the impact of the species on its environment [40], [41]. This theory implies that communities are assembled through deterministic processes, defined by abiotic and biotic factors. Neutral and niche-based theories are not mutually exclusive and are more likely part of continuum [37], [42]. A third theory, the modern co-existence theory, reunites the two previous ones [42]–[44]. This theory explains the coexistence of species within a community by equalizing mechanisms which lower relative fitness differences (neutral theory) and stabilizing mechanisms which reduce niche overlap (niche-based theory) [36], [45]–[47]. Equalizing mechanisms often relate to competition strategies that prevent one competitor to drive the other extinct. For instance, when a fast- and a slow-growing species compete for iron, the slow-growing species can produce siderophore to corner iron, which confer a fitness benefit, thereby reducing the relative fitness differences between these two species [48]. On the other hand, stabilizing mechanisms occurs mainly through resource partitioning. For instance, labour sharing in a community can lead to the coexistence of multiple species on a single resource, each species performing a specific step of the resource degradation [49]. According to this modern co-existence theory, community assembly encompasses different processes ruling community membership (i.e., allowing or not species co-existence in a community), and the deterministic processes involved are commonly referred to as abiotic and biotic filters. These filters likely interact with each other simultaneously, and distinguishing their effects is puzzling [36], [50].

The abiotic filter depends upon the ability of a species to colonize and thrive in the community abiotic environment, referring to the requirement niche and fitness differences. In terrestrial ecosystems, an emphasis has been placed on the understanding of the role of abiotic filters for microbial community assembly. Indeed, the abiotic edaphic conditions were shown to impact soil microbial communities, with the soil pH being a major factor [51], [52]. Kraft *et al.* [53] reviewed 258 articles studying the abiotic filter on different prokaryotes and eukaryotes communities. To establish abiotic filter *sensu stricto*, they assess whether studies included evidence that species could tolerate the considered abiotic conditions in the absence of potentially interacting species. They conclude that the impact of biotic interactions between the community members could not be withdrawn in 85% of the studied cases. Their conclusion highlights the potential pervasive and important role of interactions between microorganisms in community assembly and the need to investigate mechanisms aside from the abiotic filter. A growing body of evidence further supports their conclusion [54]–[58].

The biotic filter depends on the interactions between organisms, such as competition or cooperation, and therefore refers to the impact niche and species niche overlap. In natural communities, species with overlapping niches are competing against each other. Competition was described as the main type of interaction occurring within a community [59]–[61] and leads to two different outcomes during community assembly: niche differentiation or competitive exclusion. Niche differentiation, also known as niche partitioning, has been described as the main process supporting the coexistence of competing species [36], [45], [62]–[64]. Niche differentiation is the reduction of the niche overlap between two competing species (i.e., sta-

bilizing mechanisms), allowing them to co-exist despite their competition. When species initially share the same niche, they can differentiate their niche using different resources or using them differently (i.e., spatially, or temporally). Thereby, niche differentiation is based on resource partitioning (i.e., nutrient, prey, or habitable space) and can be metabolically, spatially, and temporally defined [45], [62], [63], [65]–[67]. Species can specialize in consuming different substrates to reduce competition between them [49], [68], [69]. Also, in a heterogeneous environment, such as soil, competing species can spatially compartmentalize habitable space, enabling them to coexist in the same community [63], [65]. Temporal partitioning can arise through seasons. For instance, in a tropical lowland evergreen rain forest, the community is dominated by decomposers, anaerobic saprophytes and N_2 -fixers during the rainy season, while in the dry season, members of the Beijerinckiaceae family were dominating [70]. Therefore, changes in soil nutrient and moisture regimes related to seasonal precipitations led to a cyclic dominance in which a different group of species dominate the community at one time, while all species co-exist in the community throughout the year. Alternatively to species co-existence, competitive exclusion predicts that the taxa with the optimal trait will eliminate the taxa without this optimal trait [36]. Competitive exclusion is the first formulated assembly principle [71]–[74] and recent studies further support the role of competitive exclusion in microbial community assembly [54], [58], [75]. By studying competition between eight bacterial species, Friedman *et al.* [75] showed that competitive exclusion occurred in 1/3 of the cocultures while the other 2/3 of the pairwise cocultures resulted in the coexistence of both species.

Cooperation has been described as a common interaction type within microbial communities, therefore also playing a role in community assembly [60], [76] and maintaining biodiversity [77], [78]. Cooperation between organisms could increase species tolerance to niche overlap and equilibrate the species fitness ratio, thereby acting as both a stabilizing and an equalizing mechanism [60], [76]. For example, bacteria might commonly secrete costless by-products, offsetting competition for nutrients [57] and the exchange of metabolites between species could be a major driver of species coexistence [79]. However, there is still no consensus on which type of interaction dominates in microbial communities and more importantly, the balance between the interaction types can vary [76]. For example, the trade-off between competition and cooperation among bacteria can depend on the nutrient availability [80], [81]. Also, the proportion of individuals cooperating within a given community is influenced by the dispersal rate and the relatedness between the individuals [82]. Either way, the study of biotic interactions occurring between microorganisms could help to further understand microbial community assembly.

1.2 Interactions Between Microorganisms

1.2.1 The Interaction Compass and Interaction Definition

An interaction can be defined by its outcome (i.e., the impact each species has on each other's fitness). The outcome can be negative or positive on a species fitness, resulting thereby in a deleterious or a beneficial interaction, respectively. The interaction compass, designed by Lidicker in 1979 [31] has widely been used to define interactions [60], [83], [84]. This compass provides an array of all possible interactions, represented by “whether the interactants experience a positive, negative, or neutral effect as result of the interaction” [31]. The compass ranges from mutualism which is beneficial for both interactants (+/+) to competition which is deleterious for both interactants (-/-), through commensalism (0/+), exploitation (i.e., parasitism or predation) (+/-) and amensalism (0/-) (Figure 1.1) [31], [60], [83], [85].

The compass metaphor allows us to graphically represent a pairwise interaction and to define quantitatively its type and its strength (Figure 1.1): one interaction is represented by a

point on the compass, its type (e.g., competition) is described by the angle of the point from the zero degree line (e.g., 90°) and the interaction strength is described by the distance of this point from the compass centre [31], [60]. Firstly, defining an interaction type by an angle allows to consider interactions in a continuum rather than clearly delineated [31], [60]. For instance, mutualism, commensalism, and parasitism have been described to be parts of the same continuum, and the transition along this continuum can occur across ecological and evolutionary times [86]–[89]. Secondly, the interaction compass allows to define interaction strength which is an important characteristic of interactions as it reflects the interaction reciprocity [90], [91]. Along the same line, competition, mutualism, and exploitation are considered as reciprocal interactions, where the two interacting species affect each other fitness. On the contrary, commensalism and amensalism are considered as unilateral interactions, where only one species affects the other.

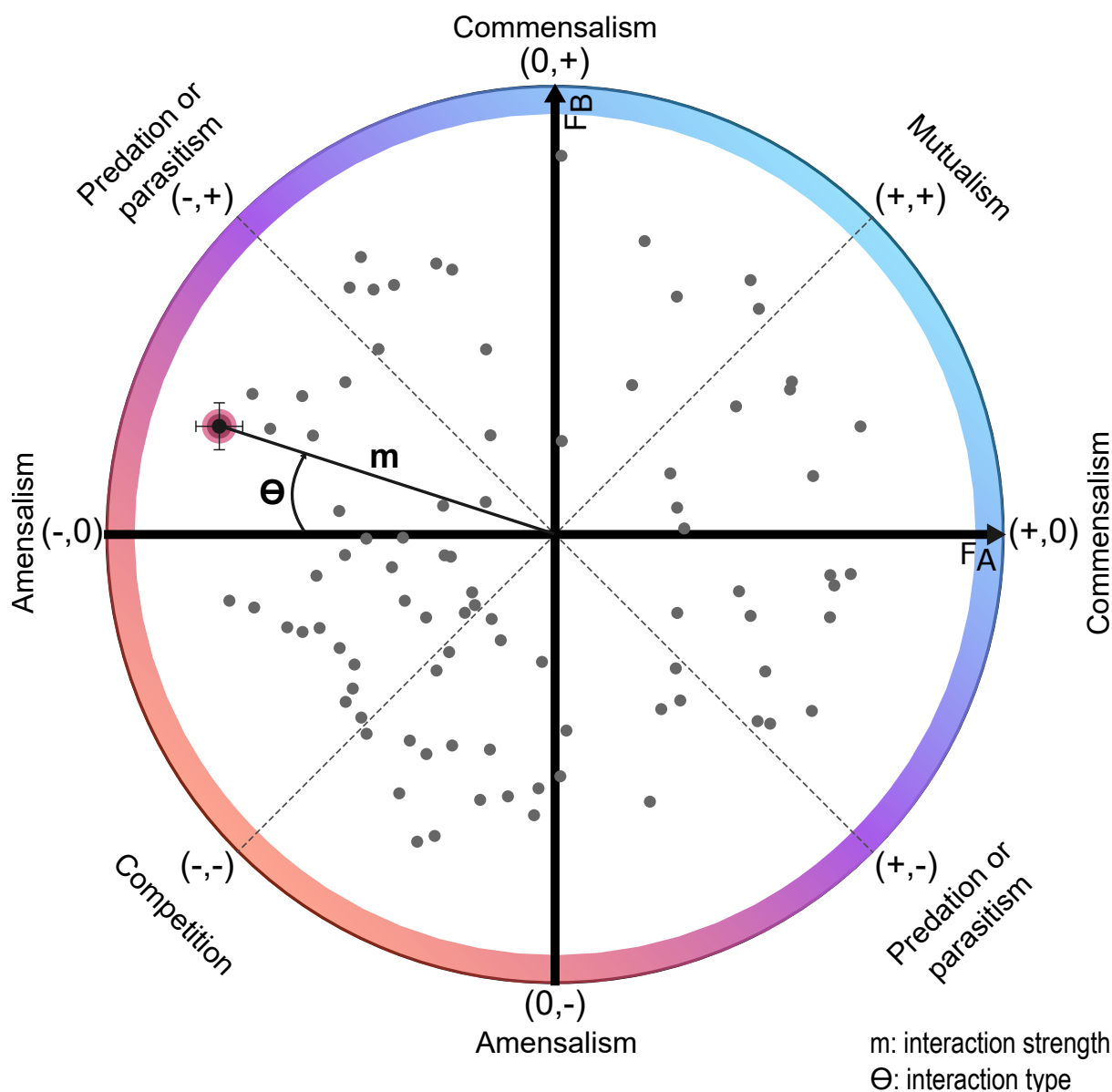


Figure 1.1: Interaction compass. The impact on the fitness of a species A (x-axis) and a species B (y-axis) of a pairwise interaction can be described qualitatively (interaction type, θ) and quantitatively (interaction strength, m). Adapted from Kehe *et al.* (2021) [60]

The interaction compass has two main limitations [57]. First, the interaction compass aim

to represent interaction outcome without considering the underlying mechanisms [31]. Thus, interactions relying on very different mechanisms are groups on the same position in the compass (e.g., predation and parasitism). Second, the interaction compass is presented in two dimensions. Thereby, it considers only one pairwise interaction at a time, withdrawing *de facto* interactions involving more than two species and multiple pairwise interactions occurring at the same time.

In this thesis, we focused on biotic interactions in highly complex communities, with thousands of potentially interacting species. Thus, while the interaction compass allow to define interactions objectively and operationally [31], we cannot use such two-dimensional framework here. Instead, we will define an interaction by its effect on a focal taxon fitness. For each taxon, we will assess whether it is positively or negatively affected as result of a positive or negative interaction, respectively.

1.2.2 Competition

Competition is a negative interaction as the outcome of competition is deleterious for both interactants (i.e., -/- in the interaction compass). There are two major mechanisms of competition: exploitative competition and interference competition. While exploitative competition can occur between different species (i.e., interspecific) as well as between member of the same species (i.e., intraspecific), interference competition has mostly been described as interspecific [92].

Exploitative competition is an indirect interaction in which two organisms compete for a limiting resource (e.g., nutrient or habitable space) [63], [92]. One of the main examples of such exploitative competition is the secretion of siderophore. Indeed, to corner iron, some species costly secrete in their environment iron-scavenging molecules (i.e., siderophores), impeding iron acquisition for strains that either (i) are unable to translocate siderophores into their cytoplasm or (ii) secrete siderophores with lower affinity for iron [63], [93]. Another example of exploitative competition is the production of molecules to colonize habitable space more efficiently (e.g., adhesins). For instance, a *Rhizobium leguminosarum* mutant with an overproduction of adhesion protein RapA1 had an increased competitiveness compared to control-production strain, leading to a greater nodule occupation of the adhesin-overproducer mutant [94].

On the other hand, interference competition is a direct interaction, where a competitor actively harms another [63], [92], using chemical, biological, and mechanical weapons [92]. The chemical weapons are toxins developed by microorganisms and are the first and most studied competition form between microorganisms since the discovery of penicillin in 1928 by Alexander Fleming [63], [92]. Toxins which are produced by bacteria target either the cell envelope (membrane or wall), or the cell core metabolism, leading to the target lysis or to the disruption of its cell growth and division. Bacteria can deliver the produced toxin to the target by either (i) releasing toxins in the environment, targeting all the surrounding competitors or (ii) directly injecting toxins into a specific target cell through different secretion system types, such as the Type IV secretion system [92]. Bacteria also use biological weapons such as prophages. The prophages are dormant form of the viral genome integrated into the host bacterial genome and replicate within the host cell without producing virulent progeny [95]. Only when the prophage is activated, as under stress conditions, it produces numerous virion progeny that are released in the cell surroundings, typically by host cell lysis. Host cell clonemates are often immune to infection by copies of the virus they carry [96]. Therefore, the released virion can infect only the host competitors, acting as a biological weapon to lyse competitor cells [92], [97], [98]. Bacteria can also use mechanical weapons such as phage tail-like bacteriocins (PTLBs) also known as tialocins, which are R-type pyocins that resemble Myoviridae bacteriophage tails. They are produced by bacteria and secreted in the environment where they bind to a target cell surface, contract their sheath, and fire their spike to physically perfo-

rate the target membrane and create a pore (Figure 1.2) [99], [100]. The pore thus created will deprive the target cell of its membrane potential, sentencing it to death [92], [100]–[102].

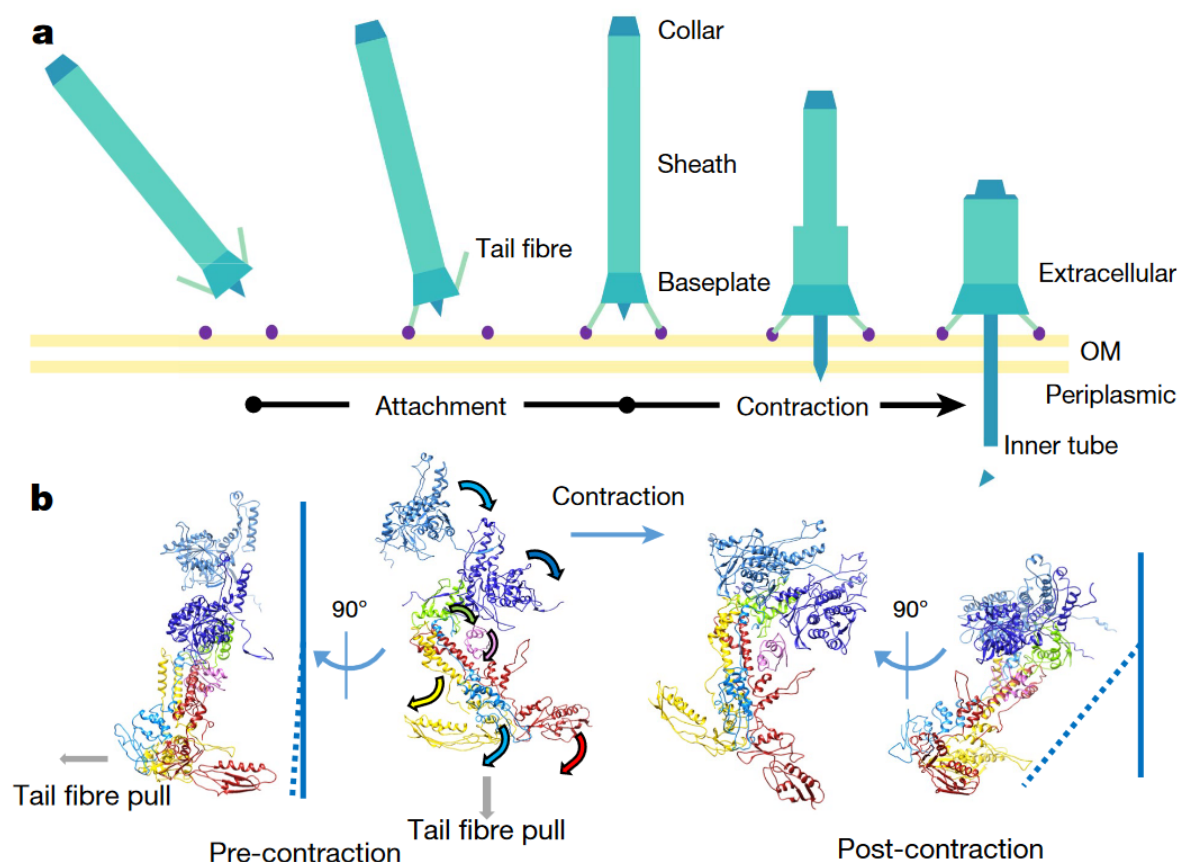


Figure 1.2: Baseplate transition from the pre-contracted to the post-contracted state. (a) Illustration of a pyocin landing on a bacterial cell and firing. Release of the spike and hub following injection is postulated on the basis of the lack of these structures on contracted particles that we observed *in vitro* (OM, outer membrane). (b) Ribbon diagram of the conserved baseplate components and sheath proteins in their pre-contracted and post-contracted states. Ripcord is believed to travel with the inner tube during the power stroke and therefore is not a conserved component of the baseplate after contraction. Arrows denote potential movements for sub-units in the same colours, respectively. From Ge *et al.* (2020) [100]

1.2.3 Cooperation

Cooperation between microorganisms is a positive interaction, as the outcome of cooperation is beneficial for both interactants (i.e., ++ in the interaction compass). Microorganisms can cooperate among individuals of the same species (i.e., intraspecific) or among individual of different species (i.e., interspecific).

Among intraspecific cooperation mechanisms, quorum sensing is probably the most studied one. Quorum sensing is a system by which bacteria communicate and obtain information on the bacterial density, through the production of signal molecules [103]–[105]. Signal molecules accumulate in the extracellular environment, are recognized by specific receptors and trigger a coordinated population response when accumulated above a critical threshold [104]. The signal molecules used can vary between Gram-negative and Gram-positive bacteria (N-acyl homoserine lactone family or oligopeptides interacting with two-component histidine

protein kinase signal transduction systems, respectively) [104]. Quorum sensing regulates numerous social phenotypes in both Gram-negative and Gram-positive bacteria, such as biofilm maturation, fruiting body development, pathogen virulence, bioluminescence, motility, etc. [104]. In general, even if the production of the quorum-sensing signal is costly for producers, it leads to a coordinated response beneficial for the whole population. Quorum sensing mechanisms have also been described between different bacterial species (i.e., bacterial cross-talk) or even between organisms belonging to different kingdoms (i.e., symbiosis) [104]. Another intraspecific cooperation mechanism is the production of public goods. Public goods are “products manufactured by an individual that can then be utilized by the individual or its neighbours” [106]. Thus, siderophore production can be considered not only as an interspecific competition mechanism but also as an intraspecific cooperation mechanism [106], [107]. Thus, the secretion of pyoverdine (i.e., a siderophore) by *Pseudomonas* is making iron available to individuals able to take up the pyoverdine-iron complex, mostly the producer’s own kin [82], [107] but also other species [108]. Pyoverdine production is costly for the producing individual but benefit the entire *Pseudomonas aeruginosa* colony.

Among interspecific cooperation mechanisms, symbiotic mutualism is the most extreme example that will be discussed in the “Interaction continuum” section below. Here, I will briefly develop another well-studied interspecific cooperation: division of labour (a.k.a. labour sharing). The division of labour is a collective phenotype divided between individuals that perform separate tasks [92]. An example of labour sharing is the two-step nitrification process [106]: (i) oxidation of ammonia into nitrite carried out by ammonia-oxidizing bacteria (AOB) or archaea (AOA) (e.g., *Nitrosomonas sp.*, *Nitrospira sp.*, etc.), then (ii) oxidation of nitrite into nitrate carried out by nitrite-oxidizing bacteria (NOB) (e.g., *Nitrospira sp.*, *Nitrobacter sp.*, etc.). By dividing in two the metabolic pathway of converting ammonia into nitrate, the two-step nitrification process is presumed to maximize the ATP production rate and therefore increase the growth rate [109]. Note that the long-sought-after and recently discovered comammox process cannot be considered as labour sharing because it refers to the complete oxidation of ammonia to nitrate in one organism [14], [15].

1.2.4 Predation

Predation among microorganisms can be considered as an antagonist or exploitative interaction, since the outcome of predation is beneficial for the predator and deleterious for the prey (i.e., +/- and -/+ in the interaction compass, respectively). Protists are well-known to predate bacteria, fungi or even other protists [17], [110]. But bacteria can also predate other bacteria. Among bacteria preying other bacteria, several species, and even entire genera, belonging to different classes have been described as bacterial predators [111]. Martin [112] classified the different mechanisms by which bacteria predate other bacteria into four predatory strategies: (i) wolfpack or group, (ii) epibiotic, (iii) direct invasion or diacytotic, and (iv) periplasmic predation strategies (Figure 1.3). Wolfpack or group predation strategy occurs when a pack of bacterial predators producing enzymes that will cause the lysis of the targeted cell(s), and then feed on the released prey nutrient. Several genera are known to use this strategy such as *Myxococcus* (Delta-Proteobacteria), *Lysobacter* (Gamma-Proteobacteria), and *Ensifer* (Alpha-Proteobacteria). The epibiotic predation strategy is a contact-dependent predation in which the predator attaches to the prey surface and assimilates the prey molecules through specialized structures (e.g., *Ensifer adhaerens* and *Vampirococcus sp.*) [111], [112]. In the direct invasion strategy or diacytosis, the predator (e.g., *Daptobacter sp.*) directly enters the prey cell, degrades the cytoplasm, and divides within the prey cell. The periplasmic predation strategy is used by *Bdellovibrio* and Like Organisms (BLOs), which are well studied predators of Gram-negative bacteria [113]–[115]. BLOs mostly refer to *Bdellovibrio spp.*, *Bacteriovorax spp.* and *Peridibacter spp.* that all belong to the Delta-Proteobacteria [111]. These obligate predators have a two-phase life cycle: (i) a free-swimming phase where the predator search

for preys, and (ii) an intraperiplasmic phase where the BLOs grow and replicate inside the periplasm of their Gram-negative prey.

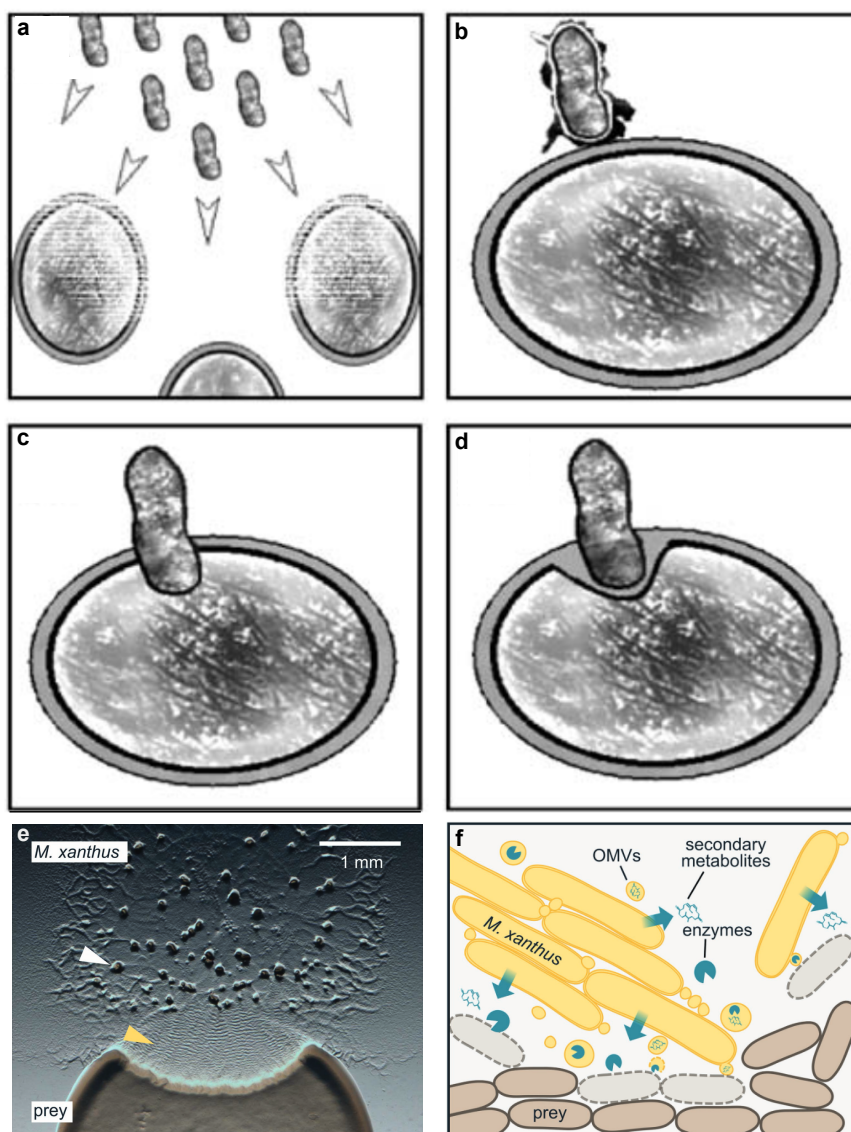


Figure 1.3: Different general strategies of predation by microorganisms. (a) Group or “wolfpack” predation (as observed with *Myxococcus*), (b) Epibiontic attachment (*Vampirococcus*), (c) Direct invasion of the cytoplasm (*Daptobacter*), and (d) Periplasmic invasion (*Bdellovibrio*). From Martin (2020) [112]. (e) *Myxococcus xanthus* cells that are placed next to *E. coli* on a CF agar plate, which only provides a minimal amount of nutrients, expand radially using gliding motility, enter the prey colony, and lyse prey cells. Multicellular fruiting bodies (white arrowhead), in which *M. xanthus* cells differentiate into spores, start to emerge near the inoculation spot. Preying *M. xanthus* induces regular cell reversals, which appear as macroscopic ripples within the prey area (yellow arrowhead). The image was taken 2 days after the initial inoculation of predator and prey. (f) *M. xanthus* secretes hydrolytic enzymes and secondary metabolites, which presumably kill and degrade prey cells for biomass acquisition. Outer membrane vesicles (OMVs) may contribute to the delivery of these lytic factors. *M. xanthus* cells typically move and prey in large clusters, but also individual cells are able to induce prey cell lysis. From Thiery & Kaimer (2020) [116]

1.2.5 Commensalism and Amensalism: Tipping Points of an Interaction Continuum

Commensalism has been described as the tipping point of a continuum between parasitism and mutualism, in symbiotic association (Figure 1.4) [117]. The molecular mechanisms used by symbionts are remarkably similar, regardless of the interaction type (i.e., parasitism, commensalism, or mutualism) [118], and the transition along this continuum could occur within an organism lifespan, depending on the ecological conditions [87], [89]. Such a continuum has been well described for symbiosis between microorganisms and plants or animals [88], [89] but could also occur between endosymbionts and hosts that are both microorganisms. For instance, the relationship between bacteria and prophage highlights the thin boundary between parasitism and mutualism. Prophages are well-known viral parasites of bacteria that reproduce in their host and trigger the cell lysis to spread in the environment. However, the interaction between prophage and its bacterial host is currently increasingly being considered as potential mutualism [119]. Indeed, while prophages can severely reduce a bacterial population, they can also promote horizontal gene transfer between bacterial species, including the transfer of genes that improve host fitness. Furthermore, in *Pseudomonas aeruginosa* biofilms, filamentous prophages interact with biofilm polymers outside of host cells, enhancing biofilm adhesion, desiccation survival, and antibiotic tolerance [120]. Another example is the relationships between fungi and bacteria. In soil, bacteria colonize the cell surface as it offers them available niches with nutrients and space, consuming, for example, the by-products of lignocellulose degradation, resulting therefore in a commensal interaction. Bacteria can also live inside the fungal cell, becoming symbionts and having mutualistic or parasitic effects on their host [119], [121]. For example, *Mycoavidus cysteinexigens* (Burkholderiaceae) can establish an endosymbiosis with members of the *Mortierella elongata* fungus species (Mortierellomycotina), consume their host metabolites therefore impeding the fungal growth [122]. At the opposite, *Candidatus Glomeribacter gigasporarum* (Burkholderiaceae), an endosymbiont of arbuscular mycorrhizal fungi, increases its host ATP production, elicits the detoxification of reactive oxygen species and potentially produces B-vitamins for its host [121]. Other members of Burkholderiaceae can have fluctuating effects. For instance, the endosymbiosis of *Paraburkholderia rhizoxinica* with *Rhizopus microsporus* can shift from mutualistic to antagonistic depending on the host's lipid metabolic changes occurring during the invasion stage [123].

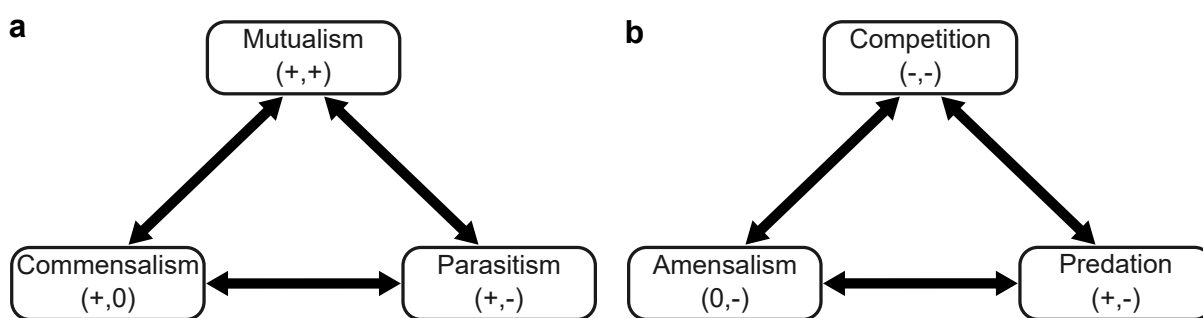


Figure 1.4: Interaction Continua between mutualisms-commensalism-parasitism (a) and between competition-amensalism-predation (b). Adapted from Hirsch (2004) [88]

Amensalism is “an interaction in which one organism inflicts harm to another organism without receiving any costs or benefits” [91]. Antibiosis is a major mechanism of amensalism which is identified as the production of secondary metabolites by microorganisms that inhibit the growth of or kill other microorganisms with specific to broad spectrum. A well-known example for industrial microbiologists is the production of lactic metabolites by lactic

acid bacteria that inhibits the growth of many other bacteria, preventing coculture of lactic acid bacteria with other bacteria [124]. Antibiosis can also be mediated by the secretion of antibiotic compounds affecting neighbouring species [125], [126]. Therefore, three different interactions are based on the release of antibiotic compounds in the environment: amensalism through antibiosis, interference competition through the secretion of antibiotics, and predation through the wolf-pack strategy. In the interaction compass, these three interactions result in a decreased fitness for the “target” while the impact on the producer fitness depends on the type of interaction: a decreased fitness in competition, a constant fitness in amensalism, and an increased fitness in predation. One way to differentiate between these three types of interaction could be to estimate the impact of the interaction on the fitness of the antibiotic producer. Such as commensalism, mutualism and parasitism can be considered as a continuum in a symbiotic relationship [88], [89], [117], competition, amensalism, and predation could be a continuum in antibiotic production (Figure 1.4).

1.3 Factors Influencing Biotic Interactions

The outcome and strength of biotic interactions can be influenced by several factors: abiotic environment (temperature, pH, type and concentration of nutrients), order of colonization, stochasticity in community assembly, habitat spatial structure, spatial distance between microorganisms, and relative population size (density dependence). For instance, as temperature and pH influence individual physiology, these environmental variables systematically affect species interactions [127]–[130]. Depending on the type and concentration of nutrients available in the environment, species can have high or low overlap between their niches, resulting in more or less competition or cooperation between them [57], [60]. Besides, the order of colonization of an environment can be important for biotic interactions: early colonizers may determine the fate of the latter ones, either by impeding or facilitating their establishment [131]. In this thesis, we manipulated physical distance between cells and density of microbial communities, and I will further detail how these factors might influence biotic interactions.

1.3.1 Distance Between Cells, Interaction Strength and Spatial Structure

The distance at which microorganisms can interact (i.e., interaction distance) is the distance over which a cell can affect the concentration of some solute [132]. This distance ranges from less than 100 μm in the rhizosphere for quorum sensing (Figure 1.5) [133], to a few centimetres in soil through volatile organic compounds [134], [135] and up to a few meters in water columns regarding substrate consumption and metabolite production [136]. In soil, it has been estimated that a single bacterium can have on average 120 other bacterial cells within a distance of ca. 20 μm , with an average distance of 12.46 μm between cells [137]. Bacteria interact at a distance ranging from 5 to 20 μm for contact-independent interactions [137], [138] and at a distance lower than 1 μm for contact-dependent interactions [139]. In general, microorganisms preferentially engage in short-range interactions with their immediate neighbours [140]. Therefore, increasing the distance between two interacting bacterial cells could reduce the interaction strength. For instance, the virulence of the oral pathogen species *Aggregatibacter actinomycetemcomitans* (Aa) is promoted as it feeds on lactate produced by the commensal species *Streptococcus gordonii* (Sg). However, Sg also produces hydrogen peroxide, which has antimicrobial properties at high concentration. In a murine infection abscess, Stacy *et al.* [141] shown that Aa spatially distance itself from Sg (>4 μm) to potentially both reduce exposure to inhibitory levels of H₂O₂ and maintain lactate supply. Increasing the distance between Aa and Sg thus reduced the strength of the amensalism of Sg towards Aa.

Soil microorganisms primarily live in spatially structured communities such as biofilm [142], [143]. Cells that cooperate tend to aggregate while those negatively affected by each

other tend to spatially segregate [141], [144]. Moreover, a poor competitor can spatially organize itself around a stronger competitor to commensally consume by-products of the main carbon source degradation. For instance, Christensen *et al.* [145] shown that a strain of *Pseudomonas putida* was outcompeted by a strain of *Acinetobacter* which was more efficient to consume benzyl alcohol but only when cultivated as suspended cultures. When cultured as mixed biofilms, the *P. putida* strain spatially organized around the colonies of *Acinetobacter* to consume the benzoate they produced from the degradation of benzyl alcohol. Furthermore, disrupting a community spatial structure may change an interaction outcome. For instance, Frost *et al.* [56] studied the competition between a carbenicillin-resistant and a sensitive strain of *Pseudomonas aeruginosa*, at intermediate antibiotic concentration. In spatially structured communities (i.e., agar plates), the local extracellular degradation of carbenicillin by the resistant cells allow the sensitive one to outcompete them while disrupting the community spatial structure (i.e., liquid broth) decreases the susceptible cell fitness. This effect of spatial structure on biotic interactions could be of tremendous importance in soil. Indeed, soil is a heterogeneous spatially structured environment with patchy and piled-up arrangements of mineral-associated organic matter that compartmentalize cell aggregates, directly influencing the potential interaction between them [146].

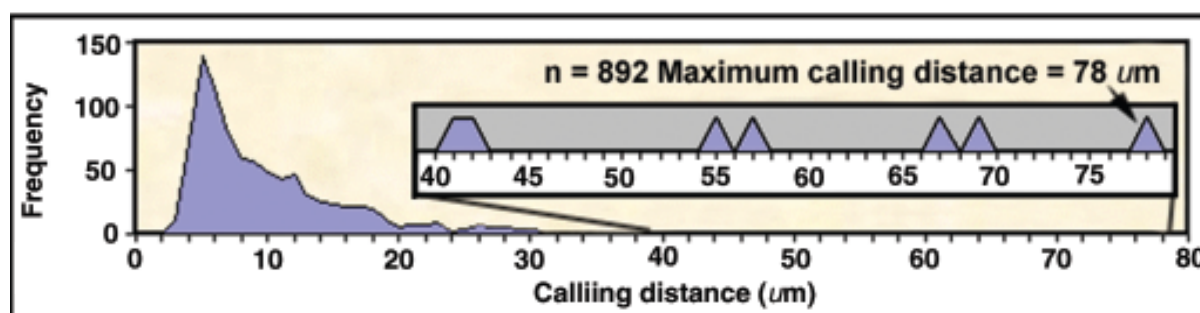


Figure 1.5: Frequency histogram of the range of calling distances for AHL-mediated communication between individual cells of the sensor and nearest neighbor source reporter strains on non-root-hair surfaces. The longer calling distances are indicated in the enlarged inset. From Gantner *et al.* (2006) [133]

1.3.2 Density-Dependent Interactions

Density dependence is the relationship between the population size and the *per capita* growth rate [147], [148]. This relationship can be negative or positive. The density-dependence can also arise only above a critical density threshold. Such density threshold dependence effect is known as Allee effect [149]. As discussed above in the “Cooperation” section, quorum sensing is a system by which bacteria detect and respond to changes in the density of surrounding cells [103], [150]. Quorum sensing is therefore an important mechanism the underlying density-dependent interactions.

Regarding cooperation, Darch *et al.* [151] experimentally manipulated the density of *Pseudomonas aeruginosa* and found that, through quorum-sensing regulation, the production of public goods was more efficient at higher cell density, thereby resulting in a higher global fitness benefit. However, higher density of public good producers can also give rise to social cheaters. For instance, among a population of *Pseudomonas aeruginosa* producing siderophores as public goods, the relative fitness of low-producing individuals (i.e., cheaters) was the greatest at higher population densities, as they were better able to exploit the cooperative siderophore production [152]. Positive density-dependent cooperation can also be subjected to Allee effects. For instance, two species cross-feeding mutualistically in a chemostat are subjected to

an Allee effect with a minimum density required to maintain both species population [153]. Under a critical density threshold, the consumption of the cross-fed nutrients exceeded their production, leading to the extinction of both species. Higher cell density can also increase the overall population resistance to toxic molecules. For example, *Roseobacter sp.*, a model organism for manganese oxidation, exhibits positive density-dependent resistance to nitrite toxicity [154]. The inoculum size of the strain is positively correlated to its growth rate in the presence of manganese oxide, as the strain can use manganese oxide to catalyse nitrite oxidation. At higher cell density, more nitrite is oxidized into nitrate, reducing the inhibition of the strain growth by nitrite.

Regarding competition, negative density-dependence has been theorized by animal population ecologists using consumer-resource models [155]. Briefly, when resources are unlimited, such as when a species colonizes a new environment, the observed pattern is an exponential population growth with the species growth rate at its maximum. However, in nature, resources are limited and therefore, populations experience a logistic growth: when the population size reaches half of the carrying capacity, the growth rate starts decreasing, due to the depletion of resources. Therefore, the increase in cell density triggers exploitative competition, reducing the population growth rate. This negative density-dependence of exploitative competition can outweigh other positive interactions. For instance, Trosvik *et al.* [156] studied co-culture of *Escherichia coli* and *Bacteroides uniformis* strains in liquid medium. At low abundances, *E. coli* removes the residual oxygen from the medium, favouring the growth of *B. uniformis* which is strictly anaerobic. At a higher level of *E. coli* abundance, this beneficial effect is outweighed by the competition for nutrients between the two strains, leading to a decrease in *B. uniformis* growth. Conversely, interference competition can trigger positive density-dependence, at the benefit of either toxin producers or sensitive strains. For instance, toxin producers at low density are not able to invade a sensitive population [157] while sensitive populations, if they exceed a certain density threshold, can successfully colonize a lethally concentrated antibiotic environment [158]. The relative abundance of competitors can also determine the outcome of a competitive interaction. Li *et al.* [98] described *in vitro* the competition occurring between *Duganella sp.* and *Curvibacter sp.* and showed that *Duganella sp.* is outcompeted in coculture at both low and high *Curvibacter sp.* initial density, but not at the intermediate initial density. This density-dependence pattern is due to the presence of a prophage sequence in the genome of *Curvibacter sp.*. The prophage can be reactivated and release in the environment by *Curvibacter sp.*, which cannot be re-infected, thereby infecting and lytically replicating into *Duganella sp.*. At low density of *Curvibacter sp.*, the released phages encountered proportionally more *Duganella sp.*, while at high density of *Curvibacter sp.*, a lot of phages are released, both situations leading to a decrease in the *Duganella sp.* population. Interference competition could also rely on mechanisms similar to quorum sensing: bacteria could sense competitor density [103], [159]. Through this competition sensing, bacteria could trigger an appropriate and density-dependent response, such as the secretion of toxins that need a high density of producers to be effective against sensitive competitors.

Regarding predation, the well-known Lotka-Volterra model describes the relationship between the population size of the prey and the population size of the predator in the same way as the consumer-resource pattern [160]–[162]. Briefly, when a prey population size is high, the growth rate of its predator will be at its maximum until it exceeds the prey growth rate. Once there is not enough prey left to sustain the predator population, the predator population declines. This leads to cyclic variations of both the predator and the prey population sizes. Such density-dependent dynamics have also been described for the prokaryote predator *Bdellovibrio* and Like Organisms (BLOs) and its preys [114]. For example, a prey density of $3 \cdot 10^6$ cells per mL is required for the BLOs to have a 50% chance of survival and maintain their population [163]. Preys can also be subjected to an Allee effect with a critical density threshold under which the population declines, either subjected or not to predation [164]. When subjected to

predation, a minimum population density is needed to lower the risk of predation and maintain the prey population. Without predation, a minimum population density is needed to trigger collective behaviour (e.g., quorum sensing) that will maintain the population growth rate.

Table 1.1: Different methods used in studies bringing insights about biotic interactions among the ones cited in this thesis

Method	Studies
Observation	Allee & Bowen, 1932 [149]; Elton, 1949 [155]; MacArthur, 1958 [73]; Schoener, 1974 [62]; Diamond, 1975 [74]; Bascompte <i>et al.</i> , 2006 [90]; Gantner <i>et al.</i> , 2006 [133]; Wakeham <i>et al.</i> , 2007 [136]; Burke <i>et al.</i> , 2011 [165]; Raynaud & Nunan, 2014 [137]; Zelezniak <i>et al.</i> , 2015 [79]; Graham <i>et al.</i> , 2016 [166]; Bahram <i>et al.</i> , 2018 [167]; Nuccio <i>et al.</i> , 2020 [67]; Szoboszlay & Tebbe, 2021 [168]; Ren <i>et al.</i> , 2022 [169]
Theory	Clements, 1916 [27]; Grinnell, 1917 [170]; Lotka, 1925 [160]; Gleason, 1926 [28]; Volterra, 1928 [161]; Gause, 1934 [71]; Vandermeer, 1972 [171]; Leibold, 1995 [40]; Chesson, 2000 [45]; Hubbell, 2001 [38]
Modelling	Abrams, 2009 [148]; Goberna <i>et al.</i> , 2014 [54]; Carrara <i>et al.</i> , 2015 [172]; Coyte <i>et al.</i> , 2015 [55]; Mougi, 2016 [173]; Momeni <i>et al.</i> , 2017 [174]; Letten & Stouffer, 2019 [175]; Pacheco <i>et al.</i> , 2019 [57]; Qian & Akçay, 2020 [76]; Vet <i>et al.</i> , 2020 [153]
Bottom-up approaches <i>Pairwise coculture</i>	Varon & Zeigler, 1978 [163]; Rainey & Rainey, 2003 [176]; Diggel <i>et al.</i> , 2007 [104]; Greig & Travisano, 2008 [157]; Ross-Gillespie <i>et al.</i> , 2009 [152]; Mongiardini <i>et al.</i> , 2009 [94]; Morgan <i>et al.</i> , 2010 [177]; Darch <i>et al.</i> , 2012 [151]; Cordero <i>et al.</i> , 2012 [178]; Foster & Bell, 2012 [59]; Hol <i>et al.</i> , 2016 [158]; Wright & Vetsigian, 2016 [179]; Kaul <i>et al.</i> , 2016 [164]; Kragh <i>et al.</i> , 2016 [180]; Russel <i>et al.</i> , 2017 [181]; García <i>et al.</i> , 2017 [124]; Frost <i>et al.</i> , 2018 [56]; Pacheco <i>et al.</i> , 2019 [57]; Dal Co <i>et al.</i> , 2020 [140]; Ranava <i>et al.</i> , 2021 [81]; Kehe <i>et al.</i> , 2021 [60]
<i>More than two species</i>	Kerr <i>et al.</i> , 2002 [77]; Griffin <i>et al.</i> , 2004 [82]; Bell <i>et al.</i> , 2005 [182]; Trosvik <i>et al.</i> , 2008 [156]; Foster & Bell, 2012 [59]; Lee <i>et al.</i> , 2014 [34]; Røder <i>et al.</i> , 2015 [35]; Mayfield & Stouffer, 2017 [183]; Friedman <i>et al.</i> , 2017 [75]; Mickalide & Kuehn, 2019 [184]; Ratzke <i>et al.</i> , 2020 [58]
Top-Down approaches <i>Community Manipulation</i>	Wubs <i>et al.</i> , 2016 [185]; Calderón <i>et al.</i> , 2017 [186]; Rocca <i>et al.</i> , 2020 [187]; Romdhane <i>et al.</i> , 2021 [188]

1.4 Approaches for Deciphering Biotic Interactions Between Microorganisms and Their Contribution to Community Assembly

The aforementioned insights about biotic interactions, the factors influencing them and their contribution to the assembly of microbial communities were obtained using different methods: theory inference from observations, *in silico* community modelling and hypothesis driven laboratory experiment (Table 1.1).

Traditional culture-based approaches have resulted in a flourishing literature of bottom-up experiments using pairwise cocultures of strains or mixing more than two species together (Table 1.1). The design of such synthetic microbial communities is expected to become easier and high-throughput, allowing bottom-up approaches with more complex communities [189]. These bottom-up approaches allow to simplify the highly complex system that are natural microbial communities to their fundamental building blocks and to measure interaction outcomes between small sets of well-identified species as well as the interaction underlying mechanisms. However, such reductionist approaches use coculture experiments with a handful of strains, which is far below the thousands of species observed in natural communities and require cultivable species that represent a phylogenetically fragmented and sparse part of natural biodiversity [190]. Therefore, it is uncertain whether they can fulfil their promise of capturing biotic interactions occurring in complex natural microbial communities [191], [192]. For instance, pairwise species interactions often fail to predict interactions in more complex systems [174], [175], [193]. Thereby, using bottom-up approaches with synthetic communities could not allow linking biotic interaction to community-level processes nor understanding the contribution of biotic interactions to the assembly of natural communities.

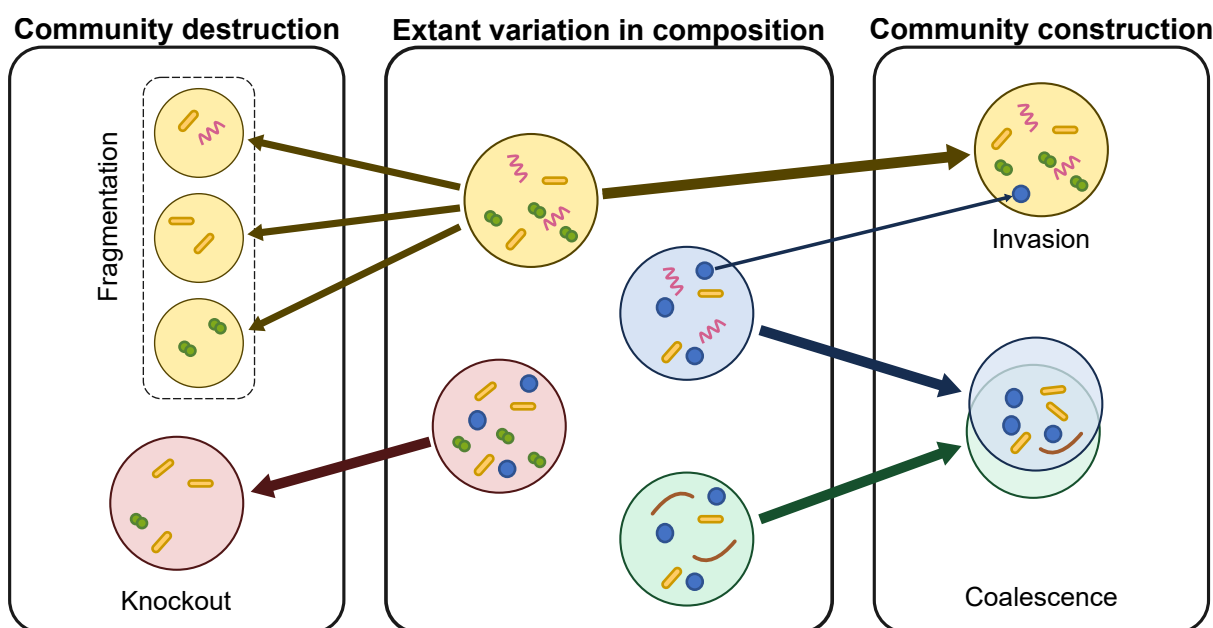


Figure 1.6: Top-down community manipulations from native communities. Each circle is a community, and the shapes represent different microbial taxa that inhabit those communities. Extant variation in community structure is illustrated in the central panel, while left and right panels show the different kinds of addition and removal manipulations. From Bell (2019) [192]

Therefore, other approaches are needed to decipher interactions between microorganisms occurring in complex communities and their role in the community assembly and functioning. Such approaches would help better predict microbial community assembly and decipher the interplay between community structure and functioning. To that end, top-down approaches

using manipulation of native microbial communities open new research avenues [192]. Native community manipulations can address three issues previously raised: (i) observational studies lacking hypothesis-driven experimental design [194], (ii) co-occurrence networks inferring correlations that need experimental validation for an ecological interpretation [83], and (iii) the need to use complex communities in laboratory experiments to decipher biotic interactions and their role on the community assembly and functioning [191], [192]. Community manipulation can take different forms either by community destruction or by community construction (Figure 1.6). From a native microbial community, community destruction may be achieved either by separating taxa (i.e., fragmentation) using serial dilution or micropore filters [186], [188], or by removing targeted taxa (i.e., knock-out) using biocidal treatments [188]. Community construction can be achieved either by inoculating invader taxa to a native microbial community (i.e., invasion) [195], or by mixing different communities (i.e., coalescence) [196].

1.5 Thesis Aim and Approach

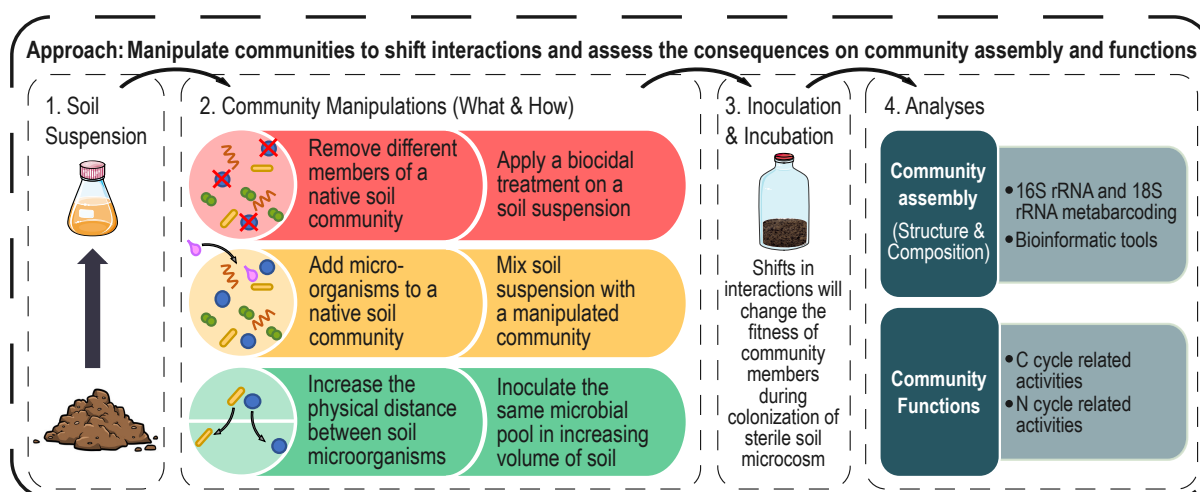


Figure 1.7: Schematic representation of the experimental approach used in the thesis.

The overall aim of this thesis is to assess the role of biotic interactions between microorganisms in soil microbial community assembly, through soil microbial community manipulations. Specifically, our objectives are: (i) to identify biotic interactions between microbial groups using community manipulation, (ii) to assess the potential contribution of biotic interactions to microbial community assembly and (iii) to assess the consequences of microbial community manipulation on soil functions. To address these objectives, we used an experimental top-down approach based on microbial community manipulations in order to modify interactions between community members (Figure 1.7). We used three different community manipulations and articulated them into three different chapters:

- Chapter 2: Experimental Community Coalescence Sheds Light on Microbial Interactions in Soil and Restores Impaired Functions
- Chapter 3: Insights into the Biotic Factors Driving the Outcome of Coalescence Events Between Bacterial Communities
- Chapter 4: Manipulating the Physical Distance Between Cells During Soil Colonization Reveals the Importance of Biotic Interactions in Microbial Community Assembly

In the second chapter, we investigated to which extent microbial interactions affect community assembly and functions. We used a two-step community manipulation based on successive removal and coalescence approaches to quantify interactions between microorganisms within a community and to validate the identified interactions, respectively. We formulated two hypotheses: (i) removing some taxa from a native microbial community will favour their competitors therefore increasing their relative abundance within the reassembled community and (ii) mixing the control and the manipulated communities will re-establish the original interactions, therefore decreasing the relative abundances of the poor competitors that were favoured by the depletion of some bacterial taxa.

In the third chapter, we assessed to which extent community diversity, composition and density influence the coalescence outcome between two communities. For this purpose, we mixed a native microbial community with various manipulated communities obtained from the same native microbial community but differing in their diversity, composition, and densities. We hypothesized that these properties of the coalescing communities (i.e., diversity, composition and density) are driving factors of the outcome of the coalescence via the modifications of the interactions between community members.

In the fourth chapter, we examined to which extent the physical distance between neighbouring species in soil determine the fitness of specific member species over others for a more general understanding of the role of biotic interactions in microbial community assembly. For this purpose, we inoculated the same species pool from a soil suspension into increasing volumes of sterilized soil. We hypothesized that experimentally manipulating the physical distance between microbial cells will modify the interaction strengths leading to differences in microbial community composition, with increasing distance between neighbours favouring poor competitors. Finally, to assess the relative competitiveness of the manipulated communities, we performed a coalescence experiment with a reciprocal transplant design by mixing microbial communities that assembled with different initial distances between cells.

2

Experimental Community Coalescence Sheds Light on Microbial Interactions in Soil and Restores Impaired Functions

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Abstract

Microbes typically live in communities where individuals can interact with each other in numerous ways. However, knowledge on the importance of these interactions is limited and derives mainly from studies using a limited number of species grown in coculture. Here, we manipulated soil microbial communities to assess the contribution of interactions between microorganisms for assembly of the soil microbiome. By combining experimental removal (taxa depletion in the community) and coalescence (mixing of manipulated and control communities) approaches, we demonstrated that interactions between microorganisms can play a key role in determining their fitness during soil recolonization. The coalescence approach between depleted and control microbial communities not only revealed the importance of density-dependent interactions in microbial community assembly but also allowed to restore partly or fully community diversity and soil functions. Microbial community manipulation resulted in shifts in both inorganic nitrogen pools and soil pH, which were related to the proportion of ammonia-oxidizing bacteria. Our work provides new insights into the understanding of the importance of microbial interactions in soil. Our top-down approach combining removal and coalescence manipulation also allowed linking community structure and ecosystem functions. Furthermore, these results highlight the potential of manipulating microbial communities for restoration of soil ecosystems.

Keywords: Microbial interactions; community manipulation; coalescence; restoration; soil functions; density-dependent interactions

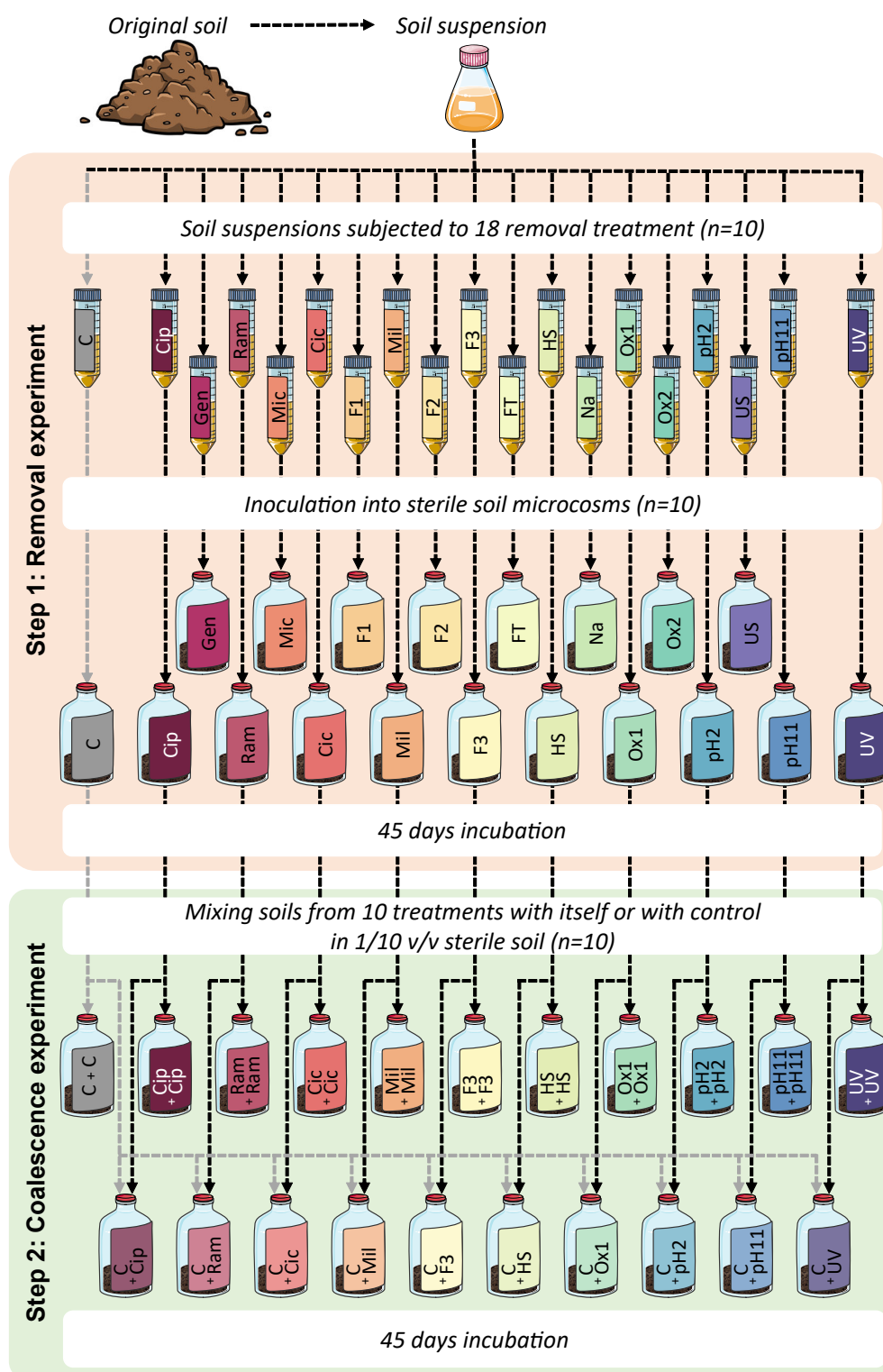


Figure 2.1: Schematic illustration of the experimental design. The soil microbial community was manipulated using a top-down approach in two steps. Step 1 consisted in a removal approach during which soil suspensions were either subjected to one out of 18 removal treatments (R, n=10) to deplete various microbial groups or were not treated (control, C, n=10). Step 2 consisted in a coalescence approach during which soils from 10 removal treatments were either mixed with themselves (R+R) or with the soil from the non-treated microcosms (R+C) and non-treated soil was mixed with itself (C+C) in 1/10 v/v sterile soil (n=10). Values in parentheses indicate the number of biological replicates.

2.1 Introduction

Microbes form complex and highly diverse communities that have an essential role in ecosystem functioning [2], [8]. In the last few decades, evidence has arisen that these functions performed by microbial communities are intrinsically related to their diversity and composition [20], [166], [197]. While microorganisms can interact with each other in numerous ways [55], [58], [198], only limited insights exist about the contribution of such biotic interactions to the assembly and composition of microbial communities. They are often obtained from simplified microbial systems in which pairwise interactions between strains are monitored [59], [179], [181]. Competition in particular has been suggested as the dominant type of interaction among microbial species [59], [66]. However, because of the simplicity of these systems, it is uncertain if these reductionist approaches can live up to their promise of providing a better understanding of interactions between microbes in their real habitat. For example, pairwise species interactions often fail to predict interactions in more complex systems likely due to higher-order interaction, which arises in the presence of additional species [184]. Therefore, a better understanding of interactions between microorganisms in complex communities is needed to better predict microbial community assembly, which is key to rationally engineer or manipulate these complex communities for our own ends.

Community coalescence is a recently introduced concept describing the encounter of previously separate microbial communities [196]. During these encounter events, novel interactions are generated and Rillig *et al.* [196] therefore proposed that an explicit consideration of coalescence could help better understand the complexity of microbial assemblages as well as the importance of microbial interactions. Since even the most degraded ecosystems are unlikely to be sterile, coalescence of microbial communities has also recently been investigated in relation to ecological restoration of degraded ecosystems [186]. Thus, due to their critical roles in biogeochemical cycling, microbial communities are now seen as a system component to be manipulated for promoting the recovery of ecosystems [199]. For example, Wubs *et al.* [185] tested the application of soil inocula in the field and showed coalescence could steer the soil community and promote nature restoration.

Here we used a two-step, top-down manipulation of soil microbial communities based on removal and coalescence approaches to assess the importance of interactions between microorganisms for soil microbial community assembly and functions (Figure 2.1). As proposed by Rillig *et al.* [200], community coalescence in our work stands for the mixing of soils containing manipulated and non-manipulated microbial communities. For this purpose, we first subjected a soil microbial community to 18 different removal treatments (Table 2.1) before reinoculation in its native, but sterilized, soil to allow the different populations to assemble during recolonization. We then applied a generalized linear mixed model to identify the OTUs with significant changes in relative abundance (used as proxy of the relative fitness) in the manipulated communities compared to the non-treated control community after 45 days of incubation. To test the hypothesis that depletion of competitors by the removal treatments was behind the observed increase in relative fitness of a large fraction of the dominant prokaryotic and eukaryotic OTUs across treatments, we then used a coalescence approach by mixing the depleted and control communities and postulated that it would re-establish the initial interactions. Finally, to assess to which extent treatment-induced changes in soil properties and functions were related to shifts in microbial communities, we applied a multi-omics integrative analysis, which supported the usage of removal and coalescence experiments for validating structure–function associations [192].

2.2 Material & Methods

Soil Sampling and Experimental Design. A sandy loam soil (6.9% clay, 19% loam and 74.1% sand, pH 5.5, and C and N content 14.7 and 1.19 g.kg⁻¹ dry soil, respectively) recognized as a reference [201] was collected at the CNRS Ecology and Environment Institute research station CEREEP, France (N48°16'59.5", E2°40'18.5") and sieved through 4 mm. In a first step, the soil microbial community was manipulated by applying 18 different removal treatments (Figure 2.1 and Table 2.1). Briefly, each removal treatment was applied independently to 1:10 suspensions of the sieved soil (n=10) and 14.2 ml of the treated suspension was inoculated into 147 ml plasma flasks containing 50 g of γ -sterilized soil (>60 kGy; Conservatome, Dagneux, France). Ten replicate microcosms inoculated with non-treated soil suspensions were used as controls. All of the 190 soil microcosms were closed with sterile lids allowing gas exchange and incubated at 23 °C at a soil moisture ranging between 60% and 80% of the soil water-holding capacity for 45 days. After incubation, soil microcosms and original soil were used for subsequent analyzes. In a second step, ten removal treatments were selected for the coalescence experiment (Figure 2.1). These ten treatments were selected to represent the variety of the Step 1 removal treatment types: antibiotics (Cip and Ram), fungicide (Cic), protisticide (Mil), filtration (F3), heat shock (HS), oxidative stress (Ox1), pH (pH2 and pH11) and shortwave (UV). For this purpose, 2.5 g of soil from a removal treatment microcosm (R) was thoroughly mixed with 2.5 g of the non-treated control soil (C) into 45 g of sterile soil microcosms, which corresponds to the coalescence treatment (R+C). Soils from removal treatments and the control were also mixed separately with 45 g of sterile soil to obtain the self-mixed removal treatments (R+R) and the self-mixed control (C+C), respectively. Soil microcosms from Step 2 were also replicated 10 times and incubated under the same conditions as Step 1 for 45 days.

Table 2.1: Description of the removal treatments applied to soil suspension in Step 1 and the corresponding abbreviations (Abbr.) used in figures and text.

Treatment	Abbr.	Description
Control	C	Not treated soil suspension
Ciprofloxacin	Cip	Antibiotic, 66,67 μ g/ml for 5 h
Gentamicin	Gen	Antibiotic, 69,44 μ g/ml for 5 h
Ramoplanin	Ram	Antibiotic, 69,44 μ g/ml for 5 h
Ciclopirox	Cic	Fungicide, 200 μ g/ml for 5 h
Micafungin	Mic	Fungicide, 66,67 μ g/ml for 5 h
Miltefosin	Mil	Protisticide, 69,44 μ g/ml for 5 h
Filtration 1	F1	Fraction > 5 μ m (5 μ m filter)
Filtration 2	F2	5 μ m > Fraction > 3 μ m (3 μ m filter)
Filtration 3	F3	3 μ m > Fraction > 1 μ m (1 μ m filter)
Freeze-Thaw	FT	6 x (15 min at -80 °C then 15 min at +30 °C)
Heat shock	HS	0 °C for 5 min / 70 °C for 15 min / 0 °C for 5 min
Osmotic	Na	NaCl, 0.1 g/ml for 2 h then wash (x3)
Oxidative 1	Ox1	H2O2 50 mM for 2 h
Oxidative 2	Ox2	H2O2 25 mM for 2 h
Alkaline	pH11	0,5 ml ammoniac 20% at 1 M for 2 h then wash (x3)
Acid	pH2	1 ml malic acid at 1 M for 2 h then wash (x3)
Sonication	US	9x(30 sec ultrasounds (Vibracell VC-500, 20 kHz) + 30 sec rest)
UV	UV	2 h exposure

Soil pH, Inorganic Nitrogen Pools and Carbon Cycle Related Activities. Impacts of microbial community manipulations on soil functions and properties were assessed using 5 replicate soil samples from each treatment. Soil pH was measured in water (ISO 10390:2005). Soil mineral nitrogen (NO₃⁻ and NH₄⁺) was extracted from 10 g of fresh soil using 50 ml of KCl (1 M), then shaken at 80 rpm for 1 h at room temperature, filtered and quantified by colorimetry (ISO standard 14256-2). Microbial respiration rates were measured according to the MicroResp method [202] using a plate reader (TECAN Infinite® M200 Pro) for three different C substrates: D-(-)Fructose, L-Arginine and Gallic acid.

Assessment of Microbial Community Composition and Diversity. DNA was extracted from 400 samples (ten original soil samples, 190 Step 1 microcosms and 210 Step 2 microcosms, n=10) using the DNeasy PowerSoil-htp 96 well DNA isolation kit (Qiagen, France) according to the manufacturer's instructions. To generate amplicons, a two-step PCR approach was used according to Berry *et al.* [203]. The hypervariable region V3-V4 of the 16S rRNA gene and the hypervariable region V4 of the 18S rRNA gene were amplified using primers 341F (5'-CCTACGGGRSGCAGCAG-3') and 805R (5'-GACTACCAGGGTATCTAAT-3') and EK-565F (5'-GCAGTTAAAAGCTCGTAGT-3') and 18S-EUK-1134-R - UnonMet (5'-TTTAAGTTTCAGCC TTGCG-5'), respectively. The amplicon size was checked with 2% agarose gel and the DNA concentration was estimated using Quant-IT™ dsDNA HS Assay kit (Invitrogen™, Carlsbad, CA, USA). Final PCR products were purified and their concentration normalized using the SequalPrep Normalization plate kit (Invitrogen™, Carlsbad, CA, USA). Sequencing was performed on MiSeq (Illumina, 2 x 250 bp and 2 x 300 pb for 16S and 18S rRNA amplicons, respectively) using the MiSeq reagent kit v2. Demultiplexing and trimming of Illumina adaptors and barcodes was done with Illumina MiSeq Reporter software (version 2.5.1.3). Sequence data from the 400 soil samples were analyzed using an in-house developed Python pipeline (available upon request). Briefly, 16S rRNA and 18S rRNA gene sequences were assembled using PEAR (version 0.9.8) [204] with default settings. Further quality checks were performed using the QIIME pipeline (version 1.9.1) [205] and short sequences were removed (< 400 bp for 16S and < 475 bp for 18S). Reference based and de novo chimera detection, as well as OTUs clustering were performed using VSEARCH (version 2.14.2) [206] and the adequate reference databases (SILVA' representative set of sequences version 138.1/2020 [207] for 16S rRNA and the PR2 sequence database version 4.11.1 [208] for 18S rRNA). The identity thresholds were set at 94% for 16S based on replicate sequencing of a bacterial mock community [188] and 97% for 18S. Representative sequences for each OTU were aligned using Infernal (version 1.1.3) [209] and phylogenetic trees were construct using FastTree (version 2.1.11) [210]. Taxonomy was assigned using UCLUST (from USEARCH version 11) [211] and the SILVA database (version 138.1/2020) [207] and the PR2 database (version 4.11.1) for the 16S and 18S rRNA sequences, respectively. Raw sequences were deposited at the NCBI under the accession number PRJNA763056 for 16S rRNA sequences and PRJNA763098 for the 18S rRNA sequences.

Quantification of Microbial Communities. The abundances of total bacterial and fungal microbial communities, as well as those of N-cycle microbial guilds, were estimated by real-time quantitative PCR (qPCR) assays. For each treatment, we used five equimolar mixtures prepared from pairs of the 10 DNA extracts. Total bacterial and fungal communities were quantified using 16S rRNA and ITS primers as described by Muyzer *et al.* [212] and White *et al.* [213], respectively. Abundances of N-cycle microbial guilds were estimated using the *amoA* gene to quantify bacterial (AOB) and archaeal (AOA) ammonia-oxidizers, the *nirK* and *nirS* genes to quantify denitrifiers [214] and the *nifH* gene for the diazotrophs [215]. Real-time qPCR assays were carried out in a ViiA7 (Life Technologies, USA) with a Takyon Master Mix (Eurogentec, France) as previously described [214]. PCR efficiency for the different assays, each one performed in two independent runs, ranged from 79.32 to 104.68%. No template

controls gave null or negligible values. PCR inhibitor presence was tested by mixing soil DNA extracts with either control plasmid DNA (pGEM-T Easy Vector, Promega, France) or water. No inhibition was detected in any case.

Statistical Analysis. Statistical analyses were conducted using R statistical software [216] (version 4.0.3) and all code and data are available on gitlab at the following link: https://gitlab.com/micro_bio_info_sarah/huet_2021. Differences between treatments in gene copy abundances (*16S rRNA*, *ITS*, *amoA*, *nirK*, *nirS* and *nifH*), ammonium and nitrate concentrations, pH, microbial respiration measurements (n=5) and the microbial α -diversity indices (n=10) were tested using ANOVA followed by Tukey's honestly significant difference test (HSD; p-value ≤ 0.05) using the *agricolae* package [217] (version 1.3-5). Normality and homogeneity of the residual distribution were verified and log-transformations were performed for gene copy abundances. Prokaryotic and eukaryotic α -diversity metrics (i.e., observed species, Simpson's reciprocal, Shannon and Faith's Phylogenetic Diversity PD [218]) and Weighted Unifrac distance between samples [219] were calculated based on rarefied OTU tables (9000 sequences and 8000 sequences per sample for 16S rRNA and 18S rRNA, respectively). We also performed principal components analyses (PCoA) and permuted analysis of variance using the *ordin* and *adonis* function of the *vegan* package (version 2.6-2)[220], respectively, based on Weighted Unifrac distance matrix to detect changes in the microbial community structure. We implemented pairwise comparisons between treatment using the *pairwise.adonis* function from the *pairwiseAdonis* package [221] (version 0.4). As Weighted Unifrac distances range from zero for similar samples to one for dissimilar samples, we calculated the similarity as one minus the distance.

Identification of OTUs Differentially Abundant in Treatments. Low-abundance OTUs were filtered out by keeping OTUs that (i) represented $> 0.5\%$ of the sequences in at least one sample and (ii) were found in at least 60% of replicates for any given treatment, which resulted in 515 and 439 OTUs for the 16S rRNA and the 18S rRNA, respectively. These most abundant OTUs were used to build pruned trees using the *ape* package [222] and visualised using the Interactive Tree of Life (iTOL) Webserver [223]. To estimate the effect of each treatment on each OTU abundance, we used a generalised linear mixed model for each of the experiment steps. Considering that an OTU of abundance Y , in any k replicates of any i Step 1 treatment or ij Step 2 treatment, follows a Poisson law of parameter Λ as $Y \sim P(\Lambda)$, we used the following models for Step 1 and Step 2, respectively:

$$\log(\Lambda_{ik}) = o_{ik} + \mu + \alpha_i + Z_{ik}, Z_{ik_{1 \leq k \leq 10}} \text{ iid} \sim N(0, \sigma^2) \quad (2.1)$$

$$\log(\Lambda_{ijk}) = o_{ijk} + \mu + \beta_{ij} + C_{ij} + Z_{ijk}, Z_{ijk_{1 \leq k \leq 10}} \text{ iid} \sim N(0, \sigma^2) \quad (2.2)$$

where $i = \{1, \dots, 19\}$ represents the Step 1 treatments, $j = \{1, 2\}$ represents the Step 2 self-mixed or coalescence treatment respectively, $k = \{1, \dots, 10\}$ represents the replicates, o is the offset for each sample calculated as the log of the sample read sum, α is the effect of the Step 1 treatments, Z is the random sampling effect modelling the data overdispersion, β is the effect of the Step 2 treatment and C is the mixed effect modelling the degree of kinship between the Step 2 samples. The analysis was performed using the *glmer* function of the *lme4* package (version 1.1-27). Subsequently, we performed multiple pairwise comparisons with the *emmeans* function of the *emmeans* package (version 1.6.1) and post-hoc Tukey tests for p-value adjustment. We selected pairwise comparisons: (i) between each removal treatment and the Step 1 control (R versus C in Step 1), (ii) between each self-mixed removal treatment and the Step 2 control (R+R versus C+C in Step 2), (iii) between each coalescence treatments and its respective self-mixed treatment (R+C versus R+R in Step 2) and iv) between each coalescence

treatment and the Step 2 control (R+C versus C+C in Step 2). Significant comparisons resulted from Tukey test p-value ($p\text{-value} \leq 0.05$). A loglikelihood ratio test was applied when the OTU had a null abundance in one treatment and a median abundance higher or equal to 5 in the other treatment (see code available online).

Inference of Co-Occurrence Networks. Networks were constructed based on the most abundant OTU count data (low-abundance OTUs filtered out) using all samples from the cleaned dataset except original soil samples (389 and 386 samples for 16S and 18S, respectively). Networks were inferred using a sparse multivariate Poisson lognormal (PLN) model with a latent Gaussian layer and an observed Poisson layer using the *PLNmodels* package [224] with an offset corresponding to the number of reads in each sample. The best network was selected using a Stability Approach to Regularisation Selection (StARS) [225]. For visualisation purposes, only partial correlations with $|\rho| > 0.08$ were considered and visualised using Cytoscape [226].

Multivariate Integration to Identify Correlation Between OTUs and Variables. To identify significantly correlated variables (Pearson's correlation $|r| > 0.6$) among 16S rRNA sequences (low-abundant OTUs filtered out), gene copy abundances (*16S rRNA*, *amoA*, *nirK*, *nirS*, *nifH* and *ITS*), inorganic N-pools, microbial respiration rates and soil pH, we used DIABLO (Data Integration Analysis for Biomarker discovery using a Latent component method for Omics studies) from the *mixOmics* package [227], [228]. This approach is a supervised analysis for the integration of multiple data sets based on a multiblock sparse partial least square discriminant analysis (Multiblock sPLS-DA). The training set used is described in the code available online.

2.3 Results

Alteration in Soil Microbiome Diversity and Composition Following the Removal Treatments. In Step 1, we used 18 different removal treatments (Table 2.1) with antibiotic, fungicide, protistocide and filtration being selective removal treatments while the others were more general. Quantification of *16S rRNA* and *ITS* gene copy numbers indicated that after the 18 removal treatments, the inoculated microbial communities reached the same densities as in the control soil, except for the heat shock and pH2 treatments for both bacteria and fungi and the F3 filtration treatment for the fungi only (post-hoc Tukey $p\text{-value} \leq 0.05$; Supplementary Figure A.1a,b). Ten to 17 of the 18 depletion treatments led to a significant decrease in prokaryotic α -diversity compared to the control, depending on the indices (post-hoc Tukey $p\text{-value} \leq 0.05$; Figure 2.2a and Supplementary Figure A.2a,b). Overall, the ramoplanin, F2 filtration, heat shock, pH2 and pH11 treatments caused the largest declines in prokaryotic diversity as illustrated by losses up to 50.77% of the observed species compared to the control community (post-hoc Tukey $p\text{-value} \leq 0.05$; Figure 2.2a). The depleted microbial taxa differed between treatments with, for example, the ramoplanin treatment affecting mostly Actinobacteria, Bacilli and Clostridia while mostly γ -Proteobacteria were depleted by the heat shock treatment (Figure 2.2b). In contrast, the F2 and F3 filtration, heat shock and pH11 treatments had the strongest effect on the eukaryotic community diversity and composition (post-hoc Tukey $p\text{-value} \leq 0.05$; Supplementary Figure A.2c,d and Supplementary Figure A.3a,b). Changes in similarity between manipulated and control communities were consistent with the α -diversity results (Adonis pairwise comparison, Benjamini-Hochberg corrected $p\text{-value} \leq 0.05$; Figure 2.2c, Supplementary Figure A.3c and Supplementary Figure A.4).

Next, we used a generalized linear mixed model to identify among the dominant OTUs those exhibiting a differential abundance between the removal treatments (R) and the control (C) (Equation 2.1). We hypothesized that depletion of some taxa would allow their competitors

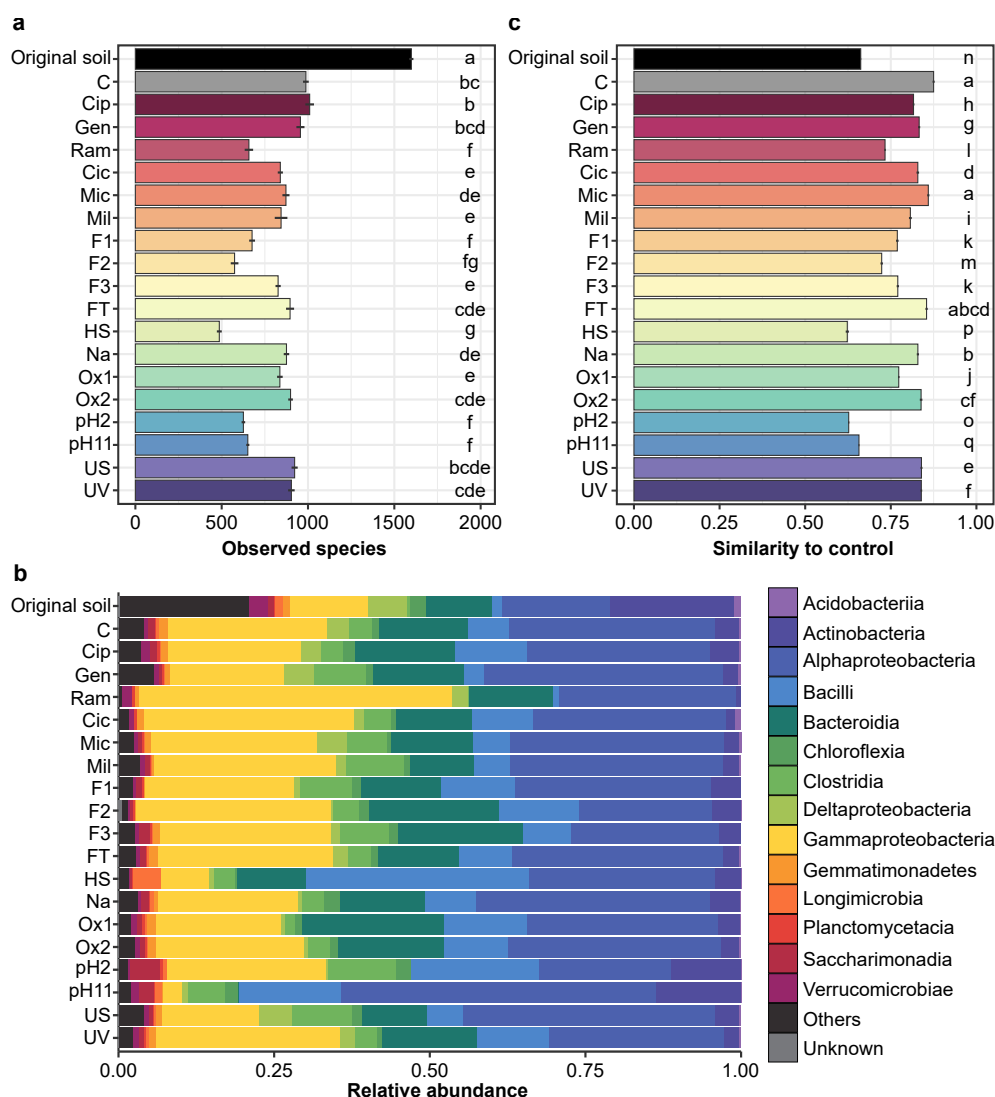


Figure 2.2: Structure and composition of the prokaryotic communities in the original soil and after Step 1. (a) Number of observed species (mean \pm s.e.). Letters indicate significantly different statistical groups (Tukey's test, p -value ≤ 0.05). (b) Relative abundances of the fourteen most abundant classes of prokaryotic community. (c) Similarity between the control samples and between the control and either the original soil or the removal treatment, based on the Weighted UniFrac distances (mean \pm s.e.). Letters indicate significantly different statistical groups (Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05).

to thrive, therefore increasing their relative abundance. Accordingly, we found that depletion of various microbial taxa positively affected the relative fitness of 245 prokaryotic and 90 eukaryotic OTUs across treatments ($R > C$, i.e. significantly higher increase in relative abundance of a given OTU in the manipulated community after the removal treatment than expected simply due to the compositional nature of the data; post-hoc Tukey p -value ≤ 0.05 ; Supplementary Table A.1). Among the prokaryotic OTUs with an increased relative abundance, 28.2%, 15.5% and 13.9% were associated with α -Proteobacteria, γ -Proteobacteria and Bacilli, respectively (Supplementary Figure A.5a). Among the eukaryotic OTUs with an increased relative abundance, 56.6% belonged to the Ascomycota class, which were mainly stimulated in the pH2 treatment (Supplementary Figure A.5b).

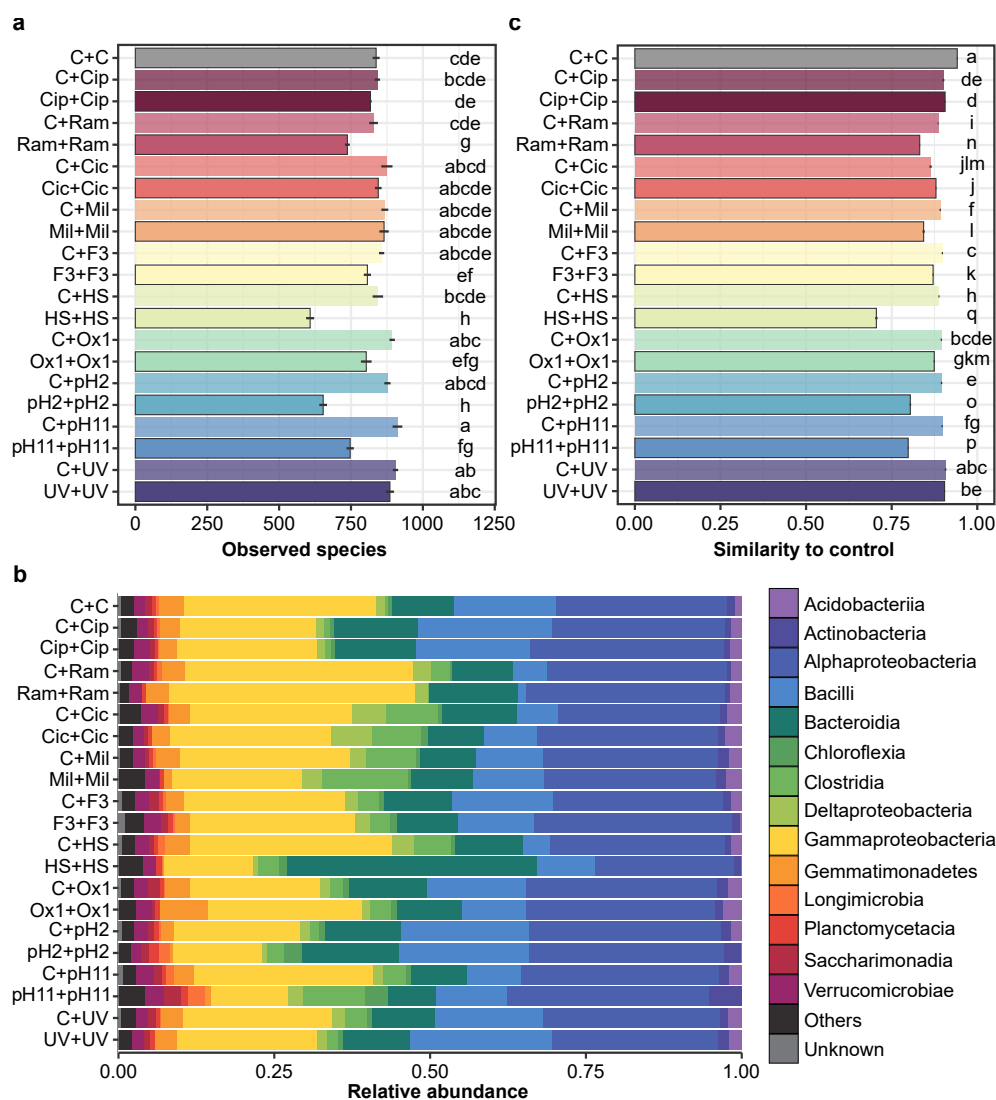


Figure 2.3: Structure and composition of the prokaryotic communities after Step 2. (a) Number of observed species (mean \pm s.e.). Letters indicate significantly different statistical groups (Tukey's test, p -value ≤ 0.05). (b) Relative abundances of the fourteen most abundant classes of prokaryotic community. (c) Similarity between the control samples and either the original soil or the removal treatment, based on the Weighted UniFrac distances (mean \pm s.e.). Letters indicate significantly different statistical groups (Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05).

Influence of Coalescence on Interactions and Community Diversity. In Step 2, the soils from 10 removal treatments were mixed into new sterile soil microcosms either by themselves (self-mixed removal treatment, R+R) or with the soil from the Step 1 control (coalescence treatment, R+C). The soil from the Step 1 control was also mixed by itself (C+C) as a new control for this Step 2 (Figure 2.1). We consider that coalescence led (i) to full recovery when no significant difference was observed between the coalescence treatment and the Step 2 control and (ii) to partial recovery when a significant difference was observed between the coalescence treatment and the self-mixed removal treatment as well as between the coalescence treatment and the Step 2 control. In 72% of the cases, a higher α -diversity was observed in the coalescence treatments compared to the impacted self-mixed removal treatments including for the communities which were the most impacted by the removal step (post-hoc Tukey p -value ≤ 0.05 ; Figure 2.3a, Supplementary Figure A.6a and Supplementary Figure A.7). Mixing the depleted

and control communities also resulted in 75% of the coalesced communities being more similar to the self-mixed control than their corresponding self-mixed removal treatments (Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05 ; Figure 2.3b,c and Supplementary Figure A.6b,c and Supplementary Figure A.8).

To test if coalescence restored the relative fitness of the affected OTUs, we used the generalized linear mixed model (Equation 2.2). Among the OTUs previously affected by the Step 1 removal treatments (R vs C, where vs means $>$ or $<$), we distinguished those still exhibiting the same differences between the self-mixed removal treatment and the self-mixed control (R vs C \cap R+R vs C+C, where \cap means intersection) from the others (R vs C \notin R+R vs C+C, where \notin means excluding). Among the latter, we found that 121 prokaryotic and 58 eukaryotic OTUs showed a significant increased relative abundance in the removal treatment from Step 1 but not in the self-mixed removal treatment from Step 2, compared to their respective controls (R>C \notin R+R>C+C; post-hoc Tukey p -value ≤ 0.05 ; Supplementary Table A.1). Alongside, 107 prokaryotic and 31 eukaryotic OTUs showed a decreased relative abundance in the removal treatment from Step 1 but not in the self-mixed removal treatment from Step 2 (R<C \notin R+R<C+C; post-hoc Tukey p -value ≤ 0.05 ; Supplementary Table A.1).

Conversely, we found 124 prokaryotic OTUs exhibiting a higher relative abundance in both the removal and the self-mixed removal treatments compared to their respective controls (R>C \cap R+R>C+C) and among them 79 no longer showed a significant difference in relative abundance after the coalescence treatments (R>C \cap R+R>C+C \cap R+C=C+C; post-hoc Tukey p -value ≤ 0.05 ; Figure 2.4a, Supplementary Table A.1, Supplementary Figure A.9a and Supplementary Figure A.10a). These OTUs belong mainly to α -Proteobacteria, Bacilli, and Actinobacteria (Figure 2.4a). Fewer eukaryote OTUs exhibited the same affected pattern in both steps (i.e., 32 OTUs R>C \cap R+R>C+C) and 20 of them no longer showed any significant difference in relative abundance after the coalescence treatments (R>C \cap R+R>C+C \cap R+C=C+C; post-hoc Tukey p -value ≤ 0.05 ; Figure 2.4b, Supplementary Table A.1, Supplementary Figure A.9b and Supplementary Figure A.10b). Similarly, among the 133 prokaryotic and 72 eukaryotic OTUs that were depleted in removal treatments from both steps (R<C \cap R+R<C+C), 113 and 61 completely recovered in the coalescence treatments, respectively (R<C \cap R+R<C+C \cap R+C=C+C; post-hoc Tukey p -value ≤ 0.05 ; Figure 2.4, Supplementary Table A.1, Supplementary Figure A.9 and Supplementary Figure A.10). Therefore, among the OTUs that were affected by the removal treatment in both steps, a total 176 prokaryotic and 80 eukaryotic OTUs fully recovered in the coalescence treatments (R vs C \cap R+R vs C+C \cap R+C=C+C; post-hoc Tukey p -value ≤ 0.05 ; Supplementary Table A.1).

Interestingly, an unexpected pattern emerged from coalescence, as 73 prokaryotic and 48 eukaryotic OTUs were significantly higher or lower in at least one of the coalescence treatments compared to each self-mixed source community separately (R+C>C+C \cap R+C>R+R or R+C<C+C \cap R+C<R+R, respectively; post-hoc Tukey p -value ≤ 0.05 ; Supplementary Table A.1, Supplementary Figure A.11 and Supplementary Figure A.12). These OTUs mostly belong to the bacterial classes γ -Proteobacteria and Bacteroidia and to the protist classes Filosa-Sarcomonadea and Colpodea (Supplementary Figure A.12).

Network Inference Deciphers Biotic Interactions. To identify antagonistic OTUs, we inferred co-occurrence networks across all samples using a sparse multivariate Poisson log-normal model [224]. Among the negative links inferred in the prokaryotic network, 46.2% involved a γ -Proteobacteria and a Bacilli (partial correlation $|\rho| > 0.08$; Figure 2.5). The number of negative links was much lower for the eukaryotic network with two negative partial-correlation between four protist OTUs (Supplementary Figure A.13). Only one positive partial-correlation between a γ -Proteobacteria and a Chrysophyceae was observed across domains (partial correlation $|\rho| > 0.08$; Supplementary Figure A.14).

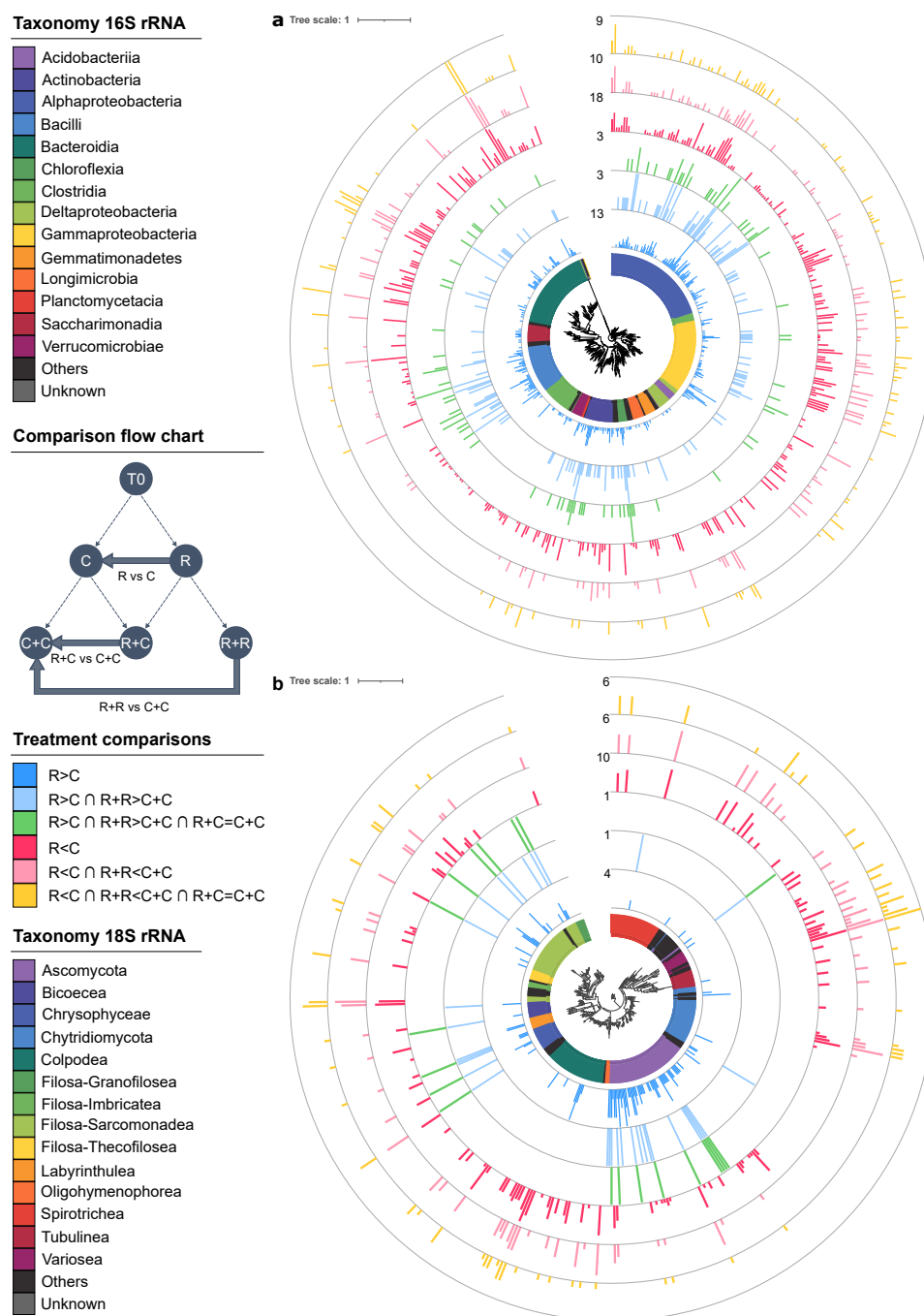


Figure 2.4: Taxonomic relationships and distribution of significantly differentially abundant OTUs across treatments. Outer rings show prokaryotic (a) and eukaryotic (b) OTUs significantly affected by the removal treatments (R vs C, where vs means > or <), OTUs significantly affected by both the removal treatments and the self-mixed removal treatments ($R vs C \cap R+R vs C+C$, where \cap means intersection) and OTUs recovering in the coalescence treatments ($R vs C \cap R+R vs C+C \cap R+C=C+C$). Bar scale is proportional to the number of treatment where the OTU is significantly differentially abundant, with the maximum indicated for each ring. The OTU class is indicated by different colors on the innermost ring.

Responses of Soil Properties and Functions to Microbial Community Manipulations. We determined treatment-induced changes in soil properties and functions by measuring soil pH, soil respiration rate as proxies of C-cycling, inorganic nitrogen pools and abundance of

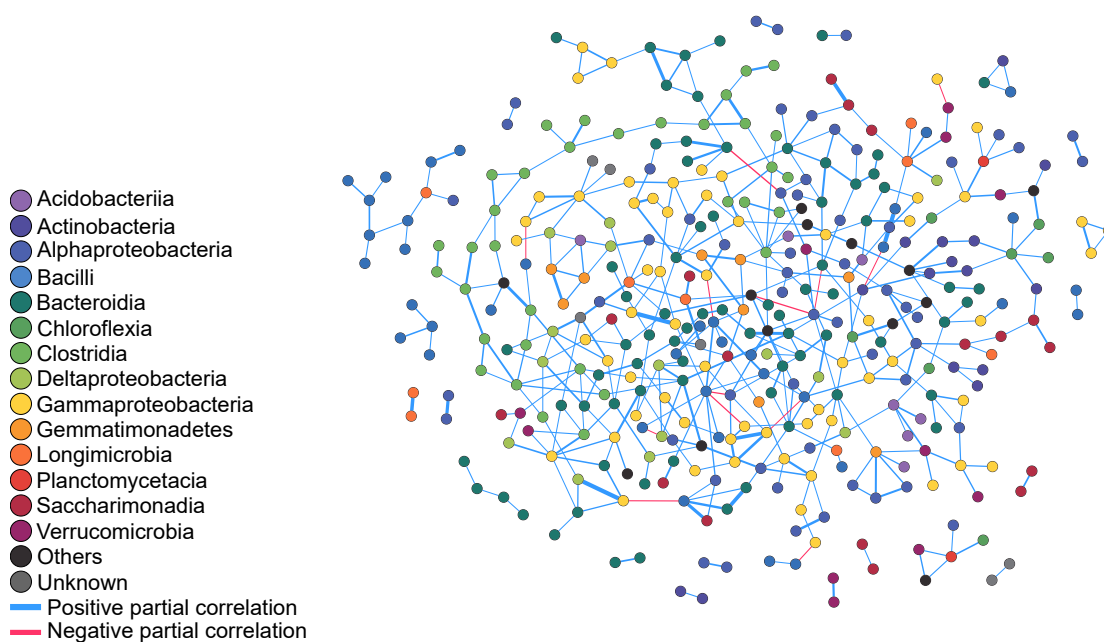
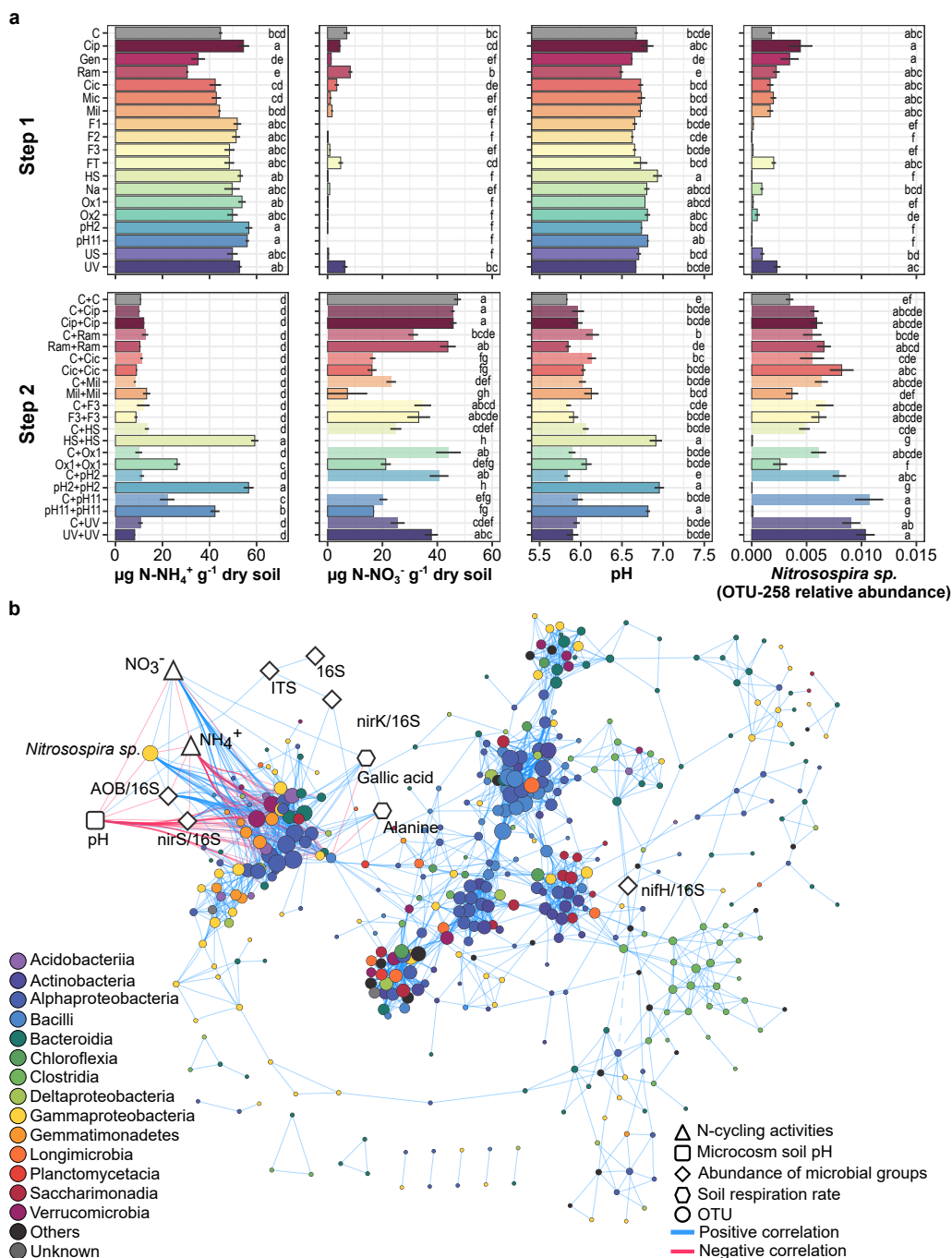


Figure 2.5: Global prokaryotic network inferred from all samples across both experimental steps. Nodes represent OTUs and they are colored according to the OTU taxonomic class. Links represent partial correlations ρ and they are colored blue if $\rho > 0$ and red if $\rho < 0$. Link width is proportional to $|\rho|$.

ammonia-oxidizers, denitrifiers and diazotrophs as proxies of N-cycling (Figure 2.6, Supplementary Figure A.15 and Supplementary Figure A.16). The depletion treatments did not significantly affect community respiration rates whatever the substrate (post-hoc Tukey p-value ≤ 0.05 ; Supplementary Figure A.15). In contrast, almost all depletion treatments lowered the nitrate content compared to the control and 8 out of 18 treatments resulted in a significant decrease in the abundance of ammonia-oxidizing bacteria (AOB; post-hoc Tukey p-value ≤ 0.05 ; Supplementary Figure A.16a). Only the pH2 and heat-shock treatments affected the abundance of all the studied N-cycling communities (post-hoc Tukey p-value ≤ 0.05 ; Supplementary Figure A.16a,b,c,d). While only the heat shock treatment affected the soil pH in Step 1, three self-mixed removal treatments (HS+HS, pH2+pH2, pH11+pH11) had a significantly much higher pH than other treatments (post-hoc Tukey p-value ≤ 0.05 ; Figure 2.6a). These same three self-mixed treatments also displayed a higher ammonium content as well as lower nitrate content and AOB abundances than the control (post-hoc Tukey p-value ≤ 0.05 ; Figure 2.6a and Supplementary Figure A.16e). Meanwhile, the three corresponding coalescence treatments (C+HS, C+pH2, C+pH11) displayed partly or fully restored pH, ammonium pool and AOB community abundance compared to the Step 2 control (post-hoc Tukey p-value ≤ 0.05 ; Figure 2.6a and Supplementary Figure A.16e). To statistically infer correlations between these proxies and microbial OTUs, we used a multi-omics integrative approach based on Projection to Latent Structure [228] (Pearson's correlation $|r| > 0.6$; Figure 2.6b). We found that pH was negatively correlated with the nitrate concentration as well as the AOB to 16S ratio while being positively correlated with the ammonium concentration. The latter was itself negatively correlated with the nitrate concentration and the AOB to 16S ratio. We found that an OTU belonging to a well-known group of ammonia-oxidizers - *Nitrosospora* sp. - was also positively correlated with the AOB to 16S ratio and negatively correlated with the ammonium concentration and the soil pH (Pearson's correlation $|r| > 0.6$; Figure 2.6).



2.4 Discussion

While microbial communities are recognized as key drivers of several ecosystem functions, a clear understanding of the factors shaping their assembly is still missing. The influence of soil abiotic properties on microorganisms has been reported in a large body of literature. However, the importance of biotic interactions between microorganisms has been overlooked and is still unclear [54], [188]. To assess to which extent microbe-microbe interactions can contribute to microbiome assembly and processes, we combined targeted removal and coalescence approaches to manipulate soil microbial communities [192].

Our removal manipulation experiment showed that about 47.6% of the dominant prokaryotic OTUs exhibited a significant relative fitness benefit after the depletion treatments. This result is consistent with our previous findings based on another community from a soil with contrasted physico-chemical properties [188]. The number of 18S OTUs showing differential relative abundance between the removal treatments and the control was much lower, which does not necessarily suggest that biotic interactions involving eukaryotes are rarer. Rather, the loss of a much higher number of eukaryotic than prokaryotic OTUs, even without applying any removal treatment, indicate that our experimental approach is more suitable for prokaryotic communities and therefore we will here mostly focus on this domain.

To establish a causative relationship between the depletion of some taxa and the increased relative fitness of others, we performed a targeted coalescence experiment to reunite potentially interacting OTUs. Such coalescence approach has previously been successfully used to assess how interactions within natural bacterial communities mediate functions in rainwater pools [229]. Here, we selected 10 depleted communities and mixed them with the control communities into a larger volume of the same sterilized soil (Figure 2.1) to alleviate environmental filtering and priority effects that can also influence the outcome of coalescence [196]. We postulated that the OTUs with increased relative fitness after the removal treatment would be detrimentally affected during soil colonization only when mixed with the control community that still contains the OTUs exerting antagonistic interactions. Integrated differential abundance analysis of both steps revealed that 79 prokaryotic OTUs followed this pattern ($R > C \cap R+R > C+C \cap R+C = C+C$; post-hoc Tukey p -value ≤ 0.05 ; Figure 2.4a and Supplementary Table A.1), which suggests that 15% (79/515 OTUs) of the dominant bacterial taxa were engaged in negative interactions during soil recolonization. However, we also found that even without coalescence events, several OTUs with increased relative abundance after the removal treatments in Step 1 ($R > C$) no longer showed significant increased relative abundance in Step 2 ($R > C \notin R+R > C+C$; Supplementary Table A.1). This can be explained by the re-growth of depleted antagonists in removal treatments during the Step 2; which is supported by the relative abundance of 107 OTUs that were no longer depleted in the self-mixed removal treatments while they were in the Step 1 ($R < C \notin R+R < C+C$; Supplementary Table A.1). Alternatively, these variations in relative abundances between Step 1 and 2 without coalescence events could also be due to changes in soil abiotic properties induced by differences in microbial community composition during Step 1 and by the mixing of soils from Step 1 with sterile soil in Step 2. These OTUs were therefore discarded when estimating the importance of the interactions between microorganisms since both abiotic and biotic effects could explain this pattern. Nevertheless, when considering only the 229 prokaryotic and 99 eukaryotic OTUs that still exhibited an affected fitness in the self-mix removal treatments compared to the self-mixed control ($R > C \cap R+R > C+C$ and $R < C \cap R+R < C+C$), we found that 76.9% and 80.8% of them, respectively, no longer exhibit a difference in relative fitness between the coalescence treatments and the self-mixed control ($R > C \cap R+R > C+C \cap R+C = C+C$ and $R < C \cap R+R < C+C \cap R+C = C+C$; Figure 2.4 and Supplementary Table A.1). This suggests that the coalescence treatments allowed re-establishing the original interactions for a large majority of the OTUs affected by the depletion treatments in both steps.

The coalescence experiment alone also revealed emergent interactions that could not be predicted from the source communities (Supplementary Table A.1 and Supplementary Figure A.12). Thus, we identified a total of 73 prokaryotic and 48 eukaryotic OTUs that exhibited significantly higher or lower relative abundances when mixing communities from the control and removal treatments (i.e., R+C) than when mixing each source community separately (i.e., C+C and R+R). Since all species are present in the control community, these non-additive changes in relative fitness are likely not due to new higher-order interactions, which are arising from the introduction of additional species [75], [230]. This pattern, however, could be explained by differences in the relative abundances of interacting OTUs between the source communities. Consistent with this view, density dependence was reported as a key feature characterizing interspecific interactions [231] and pairwise competition experiments demonstrated that species interactions can be influenced by the initial microbial species abundances [179]. Our results based on complex communities complement and extend these previous findings by suggesting that not only such density-dependent interactions may affect the outcome of coalescence but could also account for an important fraction of the observed interactions. About 83.6 and 54.2% of these non-additive changes in the prokaryotic and eukaryotic communities, respectively, resulted in an increase rather than a decrease of the relative fitness in the coalesced communities compared to the self-mixed source communities, which further suggests that cooperation between microorganisms might not be as rare as previously reported [59].

Next, we used co-occurrence network inference to identify which OTUs were interacting across the different treatments. While Russel *et al.* [181] reported that antagonism was most likely among closely related species, we found that almost 50% of the negative links in the prokaryotic network were between members of the Proteobacteria and Bacillales. However, the two sets of data are not necessarily contradictory since 5500 prokaryotic OTUs belonging to more than 300 families that were coexisting in the same soil were used here for network inference, while Russel *et al.* used 65 strains from 8 distinct environments such as soil, freshwater, maize leaf and marine algae. Our findings indicate that some members of the Firmicutes may be outcompeted by γ -Proteobacteria are of importance for understanding community assembly rules in soil. Consistent with our results, Romdhane *et al.* [188] showed that the relative fitness of Firmicutes benefited from a decrease in γ -Proteobacteria. Interestingly, this assembly rule might hold true in other environments. For example, *in vitro* and *in vivo* pairwise competition assays between phyllosphere strains revealed directional antibiosis with Firmicutes being strongly inhibited and outcompeted by a subset of Proteobacteria [232]. However, our experimental approach does not explain the nature of the observed antagonistic interactions and therefore additional work would be needed to identify the underlying mechanisms. Nevertheless, the link between γ -Proteobacteria and Chrysophyceae, which was the only one observed across domains, is consistent with previous findings reporting that members of the latter could be mixotrophic bacterivores feeding on Proteobacteria [233].

Until recently, soil microbes have seldom been considered as important players for ecological restoration of degraded ecosystems [199]. Our coalescence approach consisting in mixing the depleted and control microbial communities allowed partly or fully restoring the biodiversity of both eukaryotic and prokaryotic communities as well as their functions even in some of the most impaired treatments as compared to the control treatment from Step 2. In line with our results, Wubs *et al.* [185] recently reported that soil inocula could steer plant communities and promote ecosystem restoration in the field. In contrast, previous studies using inoculation of microbial communities often failed to prove consistent effectiveness, which was attributed to unfavorable biotic or abiotic conditions in the receptor soils [186], [234], [235]. Here, the integration of the different data sets in a supervised analysis (Figure 2.6) revealed that changes in the ammonium pools were due to impaired nitrification, which was partly or fully restored after increasing the AOB in the coalesced communities. That slow growing nitrifying bacteria

were not outcompeted during coalescence and range expansion for recolonization of the sterile soil shows promise in the possibility to steer even fastidious microorganisms for the recovery of degraded ecosystems. Although in our work impaired nitrification was due to the depletion of nitrifiers through artificial manipulation of the soil microbiome, recent work showed that N- and C-cycling in natural ecosystems such as permafrost soils could also be limited by the absence or the low abundance of the corresponding microbial guilds [236]. Another interesting feature emerging from this analysis is that manipulation of microbial community composition can lead to changes in soil pH only within a few weeks. This brings a new dimension to studies investigating the relationships between soil properties and microbiome composition. Thus, soil pH is mostly considered as a major driver of soil microbial communities [52] while the opposite has seldom been addressed [237].

In conclusion, our top-down approach combining removal and coalescence manipulation of soil microbial communities not only enabled exploration of interactions between soil microorganisms but also allowed linking community structure and ecosystem functions. Our data also highlight the importance of density-dependent interactions for soil bacterial community assembly. Coalescence between manipulated and non-manipulated communities re-established the original interactions and restored -at least partly- both microbial community diversity and functions, which open up new perspectives to steer microbial communities for ecosystem restoration. Finally, our findings that shifts in microbial community composition can lead to significant changes in soil pH warrant further studies to determine the importance of the linkages as well as of the feedback effects between soil biotic and abiotic properties.

3

Insights into the Biotic Factors Driving the Outcome of Coalescence Events Between Bacterial Communities

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Abstract

Coalescence is a widespread phenomenon that occurs in nature or owing to human activities and has recently received further attention as a promising way to test ecological theories and engineer microbial communities. Communities that coalesce can have different properties and the encounter of their respective members can trigger new interactions between them. However, the relative influence of community properties on coalescence outcome has not been clearly established. Here, we sought to assess the relative importance of the properties of the coalescing communities (i.e., diversity, composition, and density) for coalescence outcomes and for biotic interactions between community members. We performed community manipulation to implement twelve different coalescence treatments by mixing manipulated and native bacterial communities into microcosm containing their native but sterilized soil. We found that the diversity of the coalesced community is linked to that of the source communities, while its structure is mainly related to the source community composition and density. In addition, we found that 40% of the most abundant OTUs were impacted by coalescence, mostly in a density-dependent manner. However, no dominant OTU was detected as involved in biotic interactions. Altogether, our results suggest that the properties of the source communities have distinct effects on the diversity and structure of the resulting communities, mediated by biotic interactions between community members. Generalizing such assessment of how the properties of different coalescing communities influence the diversity and structure of their coalescence outcome could allow building predictive models and potentially help designing microbe engineering strategies based on coalescence.

Keywords: Microbial interactions; community manipulation; coalescence; community properties; community diversity; community composition; bacterial density

3.1 Introduction

In microbial ecology, coalescence is defined as the encounter and interchange of entire communities [196], [200]. This widespread phenomenon occurring in nature or owing to human activities lately received further attention as a promising way to both test ecological theories and engineer microbial communities. Communities that coalesce can have different properties in terms of diversity, composition and density, and the encounter of their respective members can trigger new interactions between them. A better understanding of how the relative contribution of each coalescing community and their corresponding properties will determine the composition of the coalescence outcome is needed if ecological engineering of microbial communities is considered [238], [239].

Depending on the relative contribution of each coalescing community, the coalescence outcome can be symmetrical, if the coalescing communities contribute equally to the outcome, or asymmetrical, if one community dominates the coalescence and contributes more to the outcome than the other. Mostly asymmetrical outcomes have been described in both theoretical and empirical works [187], [239]–[242]. The dominance of one community over another will be depending on their properties such as their diversity, composition, and density. Indeed, more diverse community will better resist invasion from another community, but will also have a better invasion success over another community [195], [243]. The density of a community will also contribute to its invasion success but this effect will likely be modulated by the composition of the invaded community [243], [244]. However, the relative importance of each of those properties (i.e., diversity, composition, and density) on the outcome of a coalescence event has not yet been properly evaluated.

The effect of community properties on coalescence outcome are mediated by biotic interactions occurring between and within the communities [195], [243], [244]. The nature and the number of biotic interactions occurring between the community members influences the coalescence outcome and may be under the influence of communities' properties. Indeed, communities assembled through a history of competition and trophic interactions, such as natural communities, tend to coalesce asymmetrically [240], [241], [245]. The coalescing community dominating the coalescence is predicted to be the community exhibiting more cooperative interactions as estimated using a general consumer-resource model to simulate coalescence between pairs of communities harboring sixty different species [246]. The nature of an interaction can also switch from facilitation among community members to inhibition towards each other during a coalescence event [195]. Moreover, when they encounter, the members of the different coalescing communities can also engage in new interactions which will also influence the coalescence outcome [239]. Indeed, the presence of one species in a community can impede the establishment of a species from another community, even if inoculated at a high density, because of a strong competition between them [244]. Thus, while the interactions occurring both between and within the coalescing communities steer the coalescence outcome, the influence of the coalescing community properties on those biotic interactions has not been clearly established.

Here, we sought to assess the relative importance of the diversity, the composition and the density of microbial communities during a coalescence event. For this purpose, we generated manipulated communities by altering the diversity, the composition and the density of a native soil microbial community. Then, we performed twelve different coalescence events by mixing the original community with each manipulated community into microcosm containing their native, but sterilized, soil. We hypothesized that the altered community properties (i.e., diversity, composition, and density) drive the outcome of a coalescence event and influence the biotic interactions occurring between the community members. We used linear and generalized linear mixed models as well as co-occurrence network inference based on 16S rRNA metabarcoding data to test our hypothesis and to evaluate the relative importance of the

properties of the coalescing communities for coalescence outcomes and for biotic interactions between community members.

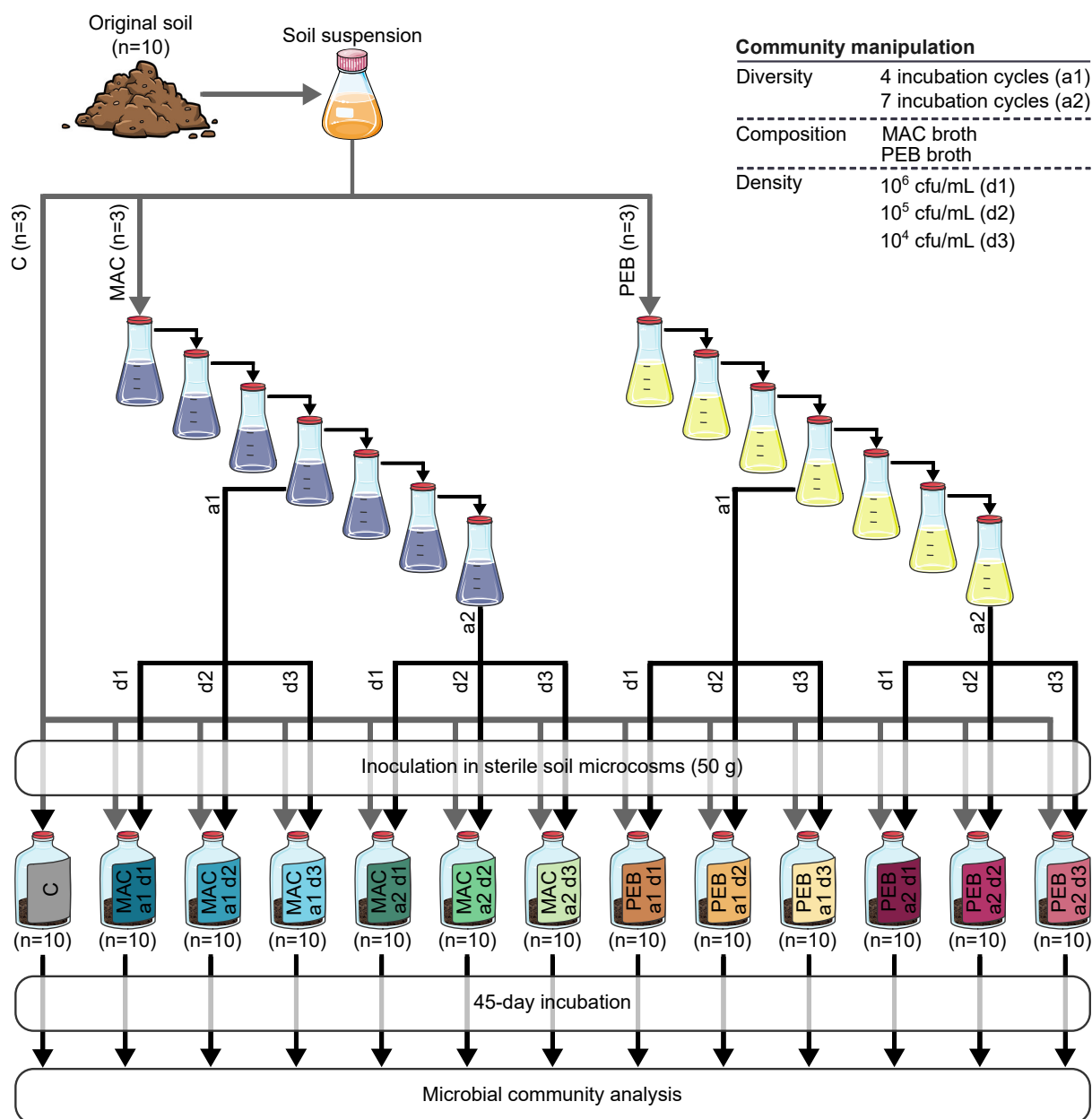


Figure 3.1: Schematic illustration of the experimental design. The soil microbial community was manipulated using a top-down approach in two incubation series. Soil suspensions were either subjected to a selective treatment consisting in an incubation series in one out of two selective broth (i.e., MAC or PEB broth, $n=10$) or were not treated (control, C, $n=10$). Incubation series consisted in either 4 or 7 incubation row to obtain two different diversity level. The same volume of the obtained modified suspensions was then diluted into increasing volume of water to obtain three different densities. Values in parentheses indicate the number of biological replicates.

3.2 Material & Methods

Soil Sampling and Experimental Design. A sandy loam soil (6.9% clay, 19% loam, and 74.1% sand, pH 5.5, and C and N content 14.7 and 1.19 $\text{g}\cdot\text{kg}^{-1}$ dry soil, respectively) was col-

lected at the research station CEREEP, France (N48°16'59.5", E2°40'18.5") and sieved through 4 mm. Part of this soil was γ -sterilized (>60 kGy; Conservatome, Dagneux, France) to set up sterilized soil microcosms. The remaining unsterilized soil was conserved at +4°C as the original soil. This experiment is based on 1:10 suspensions of this original soil. To produce communities with different compositions (Figure 3.1), we applied two different selective treatments by inoculating 750 μ L of the original soil suspension into 15 mL of two different selective broths: Mac Conkey broth (MAC) and Phenylethyl alcohol broth (PEB). Each incubation cycle lasts 3.5 days. At the end of each cycle, 300 μ L of the incubated suspension was inoculated into 15 mL of fresh sterile broth. To produce communities with different diversities, the suspensions were subjected to either 4 (a1) or 7 (a2) incubation cycles before being washed three times. We verified that the four washed suspensions (i.e., MAC a1, MAC a2, PEB a1, and PEB a2) had similar densities ranging from 7.76×10^8 to 1.84×10^9 16S *rRNA* gene copy number (Supplementary Table B.1). To obtain control and manipulated suspensions with similar densities (i.e., 10^6 gene copies; Supplementary Table B.1), we diluted the washed manipulated suspensions as follows. Each washed suspension was diluted to $1/200^{th}$ in sterile water resulting in the d1 density. This d1 density was then diluted either to $1/100^{th}$ or to $1/1000^{th}$ to obtain the d2 and d3 densities, respectively. Thereby, soil suspensions were subjected to two selective treatments, two incubation cycle numbers and three dilutions, resulting in twelve manipulated suspensions. To produce 1:1 ratio coalescence, 75 mL of each manipulated suspension was mixed with 75 mL of original soil suspension (i.e., control suspension) into a 250 mL sterile Erlenmeyer. After a 30 sec homogenization, 13 mL of this mix was inoculated into a sterile microcosm containing 50 g of γ -sterilized soil in 147 mL plasma flasks (n=10). Microcosms were closed with sterile lids allowing gas exchange and incubated at 23°C, at soil moisture ranging between 60% and 80% of the soil water-holding capacity. After 45 days of incubation, soil microcosms, the washed suspensions and the original soil were used for subsequent analyses.

Assessment of Microbial Community Composition and Diversity. DNA was extracted from 155 samples (ten original soil samples, 130 microcosms, n=10; 15 inoculated suspensions, n=3) using the DNeasy PowerSoil-htp 96 well DNA isolation kit (Qiagen, France) according to the manufacturer's instructions. To generate amplicons, a 2-step PCR approach was used according to Berry *et al.* [203]. The V3-V4 hypervariable region of the 16S *rRNA* gene was amplified using the 341F (5'-CCTACGGGGRSGCAGCAG-3') and 805R (5'-GACTACCAGGGTATCTAAT-3'). The amplicon size was checked with 2% agarose gel and DNA concentration was estimated using the Quant-IT™ dsDNA HS Assay kit (Invitrogen™, Carlsbad, CA, USA). Final PCR products were purified and their concentration was normalized using the SequalPrep Normalization plate kit (Invitrogen™, Carlsbad, CA, USA). Sequencing was performed on MiSeq (Illumina, 2 x 250 bp) using the MiSeq reagent kit v2. Demultiplexing and trimming of Illumina adaptors and barcodes were done with Illumina MiSeq Reporter software (version 2.5.1.3). Sequence data from the 155 samples were analyzed using an in-house developed Python pipeline (available upon request). Briefly, 16S *rRNA* gene sequences were assembled using PEAR [204] with default settings. Further quality checks were conducted using the QIIME pipeline [205] and short sequences were removed (<375 bp). Reference-based and *de novo* chimera detection, as well as OTUs clustering, were performed using VSEARCH [206] and the adequate reference databases (SILVA's representative set of sequences; see [207]). The identity thresholds were set at 94% based on replicate sequencing of a bacterial mock community [188]. Representative sequences for each OTU were aligned using Infernal [209] and a phylogenetic tree was constructed using FastTree [210]. Taxonomy was assigned using UCLUST [211] and the SILVA database (138.1/2020) [207]. Samples with less than 8000 sequences were filtered out (n=146).

Quantification of Microbial Communities. The abundances of total bacterial communities were quantified by real-time quantitative PCR (qPCR; Supplementary Table B.1) assays using 16S rRNA as described by Muyzer *et al.* [212]. Real-time qPCR assays were carried out in a ViiA7 (Life Technologies, USA) with a Takyon Master Mix (Eurogentec, France) as previously described by Bru *et al.* [214]. PCR efficiency for the different assays, each one performed in two independent runs, ranged from 98.58% to 99.29%. No template controls gave null or negligible values. PCR inhibitor presence was tested by mixing soil DNA extracts with either control plasmid DNA (pGEM-T Easy Vector, Promega, France) or water. No inhibition was detected in any case.

Statistical Analysis. Statistical analyses were conducted using R statistical software [216] (version 4.0.3) and all code and data are available on gitlab at the following link: https://gitlab.com/micro_bio_info_sarah/huet_2022.

Statistical Analysis of Diversity Metrics. Both α -diversity (i.e., observed species, Simpson's reciprocal, and Faith's Phylogenetic Diversity PD; see [218]) and β -diversity metrics (i.e., weighted Unifrac distances between samples; see [219]) were calculated based on rarefied OTU table (9000 sequences per sample). The α - and β -diversity indices of the suspensions were first analyzed by using Welch's t-tests and Bonferroni p-value correction to compare each suspension ($n=3$) to the original soil ($n=10$). Then, the manipulated suspensions were compared among each other using the same approach than for the coalesced communities described as follows (Equation 3.1).

To estimate the effect of each treatment on α -diversity metrics, we used ANOVAs based on a linear model (Equation 3.1) followed by Tukey's honestly significant difference test (HSD test; p-value ≤ 0.05) using the *agricolae* package [217] (version 1.3-5). Normality and homogeneity of the residual distribution were verified. Considering that a diversity index Y , in any j replicates of any i treatment, follows a Gaussian distribution of mean \bar{y} and variance σ^2 as $Y \sim N(\bar{y}, \sigma^2)$, we used the following model:

$$Y_{ij} = \mu + \gamma_i + \varepsilon_{ij}, \varepsilon_{ij} \underset{1 \leq j \leq 10}{i.i.d} \sim N(0, \sigma^2) \quad (3.1)$$

where $i = \{0, \dots, 12\}$ represents the non-coalesced control and the coalescence treatments, $j = \{1, \dots, 10\}$ represents the replicates, γ is the fixed effect of the treatments and ε is the model residuals. To estimate the effect of each treatment on β -diversity, we performed principal components analyses (PCoA) and permuted analysis of variance using the *ordin* and *adonis* function of the *vegan* package [220] (version 2.6-2), respectively, based on weighted Unifrac distance matrix. We also implemented pairwise comparisons between treatment using the *pairwise.adonis* function from the *pairwiseAdonis* package [221] (version 0.4).

To assess the relative contribution of the coalescence factors to each α - and β -diversity metric, we calculated the factor F-value using ANOVAs based on a linear model (Equation 3.2). Normality and homogeneity of the residual distribution were verified. Considering that a diversity index Y , in any j replicates of any coalescence treatment with a diversity, b composition and d density, follows a Gaussian distribution of mean \bar{y} and variance σ^2 as $Y \sim N(\bar{y}, \sigma^2)$, we used the following model:

$$Y_{abdj} = \mu + \alpha_a + \beta_b + \delta_d + (\alpha\beta_{ab}) + (\alpha\delta_{ad}) + (\beta\delta_{bd}) + (\alpha\beta\delta_{abd}) + \varepsilon_{abdj}, \varepsilon_{abdj} \underset{1 \leq j \leq 10}{i.i.d} \sim N(0, \sigma^2) \quad (3.2)$$

where $a = \{1, 2\}$ represents the diversity, $b = \{1, 2\}$ represents the composition, $d = \{1, 2, 3\}$ represents the density, $j = \{1, \dots, 10\}$ represents the replicates, α, β, δ are the fixed effects of the diversity, composition, and density of the manipulated communities, respectively, and their interactions, and ε is the model residuals.

Statistical Analysis of OTUs. Statistical analyses of OTUs abundances were focused on the most abundant OTUs in microcosms. Briefly, low-abundance OTUs were filtered out of the count table by keeping OTUs that (i) represented $>0.1\%$ of the sequences in at least ten samples and (ii) were found in at least 60% of replicates for any given treatment, which resulted in 258 dominant OTUs. These dominant OTUs were used to build pruned trees using the *ape* package [222] and were visualized using the Interactive Tree of Life (iTOL) webserver [223].

To estimate the effect of each treatment on each OTU abundance, we used a generalized linear mixed model (Equation 3.3). Considering that an OTU of abundance Y , in any j replicates of any i treatment, follows a Poisson distribution of parameter Λ as $Y \sim P(\Lambda)$, we used the following model:

$$\log(\Lambda_{ij}) = o_{ij} + \mu + \gamma_i + Z_{ij}, Z_{ij}_{1 \leq j \leq 10} \text{ iid} \sim N(0, \sigma^2) \quad (3.3)$$

where $i = \{0, \dots, 12\}$ represents the non-coalesced control and the coalescence treatments, $j = \{1, \dots, 10\}$ represents the replicates, γ is the fixed effect of the treatments, o is the offset for each sample calculated as the log of the sample read sum and Z is the random sampling effect modelling the data overdispersion. The analysis was performed using the *glmer* function of the *lme4* package (version 1.1-27). Each model was tested against a null model (i.e., a model without the effect of the treatments) using likelihood-ratio test and p-value were corrected using a Bonferroni correction (adjusted Chi square p-value ≤ 0.05). Subsequently, we performed a post-hoc Tukey test on significant models with the *emmeans* function of the *emmeans* package (version 1.6.1) implementing multiple comparisons and p-value were corrected using a Bonferroni correction (p-value ≤ 0.05).

To estimate the effect of the coalescence factor on each OTU abundance, we calculated the factor F-value using ANOVAs based on the following generalized linear mixed model (Equation 3.4). Considering that an OTU of abundance Y , in any j replicates of any coalescence treatment with a diversity, b composition and d density, follows a Poisson distribution of parameter Λ as $Y \sim P(\Lambda)$, we used the following model:

$$\log(\Lambda_{abdj}) = o_{ij} + \mu + \alpha_a + \beta_b + \delta_d + (\alpha\beta_{ab}) + (\alpha\delta_{ad}) + (\beta\delta_{bd}) + (\alpha\beta\delta_{abd}) + Z_{abdj}, Z_{abdj}_{1 \leq j \leq 10} \text{ iid} \sim N(0, \sigma^2) \quad (3.4)$$

where $a = \{1, 2\}$ represents the diversity, $b = \{1, 2\}$ represents the composition, $d = \{1, 2, 3\}$ represents the density, $j = \{1, \dots, 10\}$ represents the replicates, α, β, δ are the fixed effects of the diversity, composition, and density of the manipulated communities, respectively, and their interactions, o is the offset for each sample calculated as the log of the sample read sum and Z is the random sampling effect modelling the data overdispersion. The analysis was performed using the *glmer* function of the *lme4* package (version 1.1-27). Each model was tested against a null model (i.e., a model without the effect of the treatments) using likelihood-ratio test and p-value were corrected using a Bonferroni correction (adjusted Chi square p-value ≤ 0.05).

Inference of Co-Occurrence Networks. Co-occurrence networks were constructed across all microcosm samples (i.e., 130 samples) and based on the most abundant OTUs using the same filter as described above (i.e., 258 OTUs). Networks were inferred using a sparse multivariate Poisson log-normal (PLN) model with a latent Gaussian layer and an observed Poisson layer using the *PLNmodels* package [224] with an offset corresponding to the number of reads in each sample. The best network was selected using a Stability Approach to Regularization Selection (StARS) [225]. For visualization purposes, only partial correlations with $|\rho| \geq 0.06$ were considered and were visualized using the Interactive Tree of Life (iTOL) webserver [223].

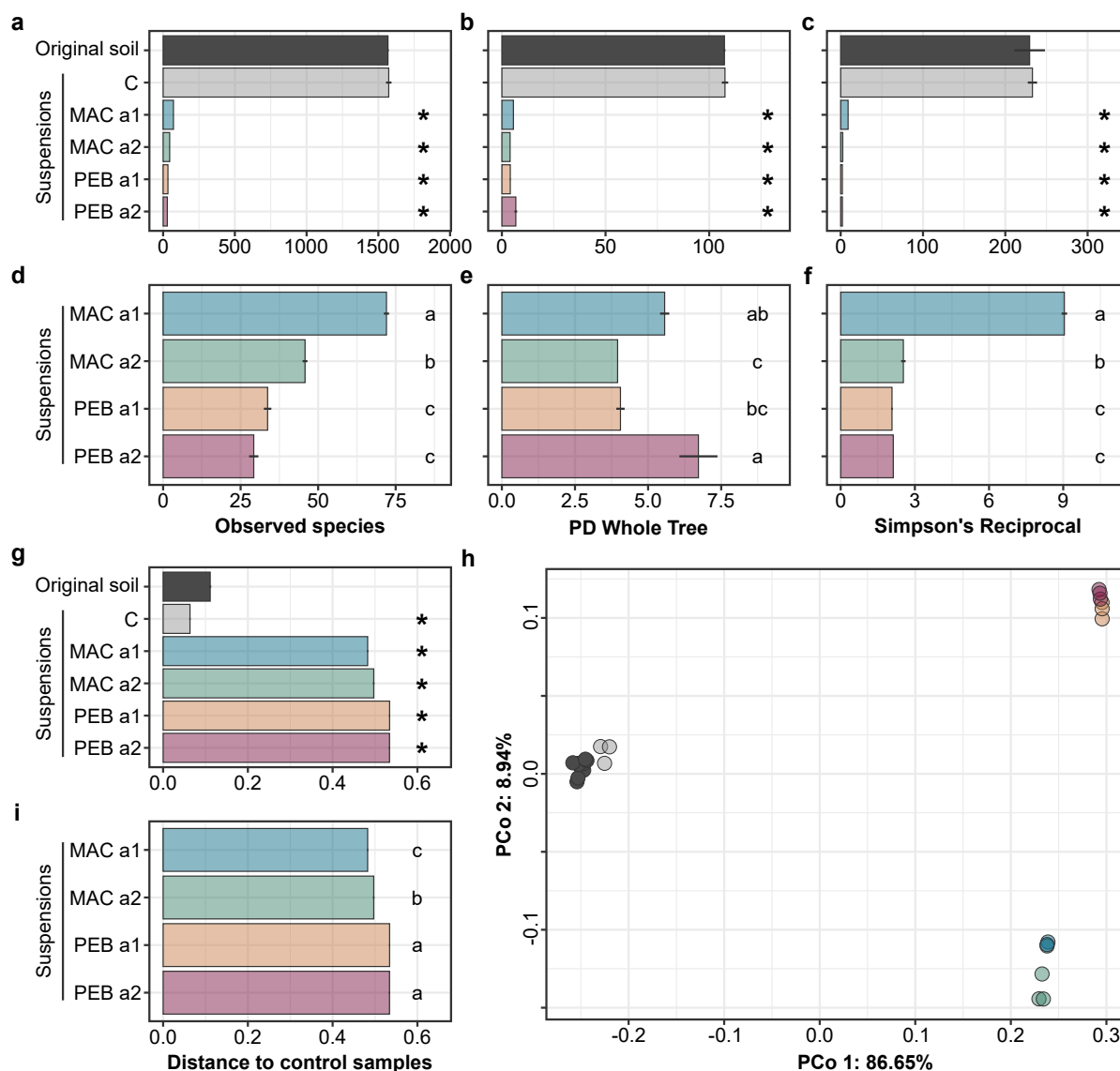


Figure 3.2: Diversity and structure of the communities in the original soil, the non-treated control suspension and the manipulated suspensions. (a to c) The number of observed species (a), the Faith's phylogenetic diversity (b) and Simpson's reciprocal (c) indices are shown (mean \pm s.e.) in the original soil, the non-treated control suspension and the manipulated suspensions. Asterisks indicate suspensions significantly different than the original soil (Welch's t-test p-value \leq 0.05). (d to f) The number of observed species (d), the Faith's phylogenetic diversity (e) and Simpson's reciprocal (f) indices are shown (mean \pm s.e.) in the manipulated suspensions. Letters indicate significantly different statistical groups (Tukey's test, p-value \leq 0.05). (g) Weighted UniFrac distances between the control suspension samples and between the control suspension and either the original soil or the manipulated suspensions (mean \pm s.e.). Asterisks indicate suspensions significantly different than the original soil (Welch's t-test p-value \leq 0.05). (h) Principal coordinate analysis (PCoA) based on the weighted UniFrac distance matrix showing the original soil, the non-treated control suspension and the manipulated suspension samples. The dot colours correspond to the bar colours in a to g. (i) Weighted UniFrac distances between the control suspension and the manipulated suspensions (mean \pm s.e.). Letters indicate significantly different statistical groups (Tukey's test, p-value \leq 0.05).

3.3 Results

Manipulating a Native Soil Community to Alter Its Diversity, Composition and Density. Inoculation of soil suspensions into MAC and PEB media and incubation for either 4 (a1) or

7 (a2) cycles, respectively (Figure 3.1), led to a severe decrease in all measured α -diversity metrics (Figure 3.2a,b,c; Welch's t-test p-value ≤ 0.05). Overall, the manipulated communities lost on average 97.1% and 95.3% of their richness and 98.3% of their evenness compared to the control suspension, as estimated by the observed species, the PD whole tree and the Simpson's reciprocal indices, respectively. Among the manipulated communities, the MAC a1 was the most diverse followed by the MAC a2 and PEB a1 (Figure 3.2d,e,f). The PEB a2 had the lowest observed species and evenness while it had the highest phylogenetic diversity (i.e., PD whole tree).

Incubation in MAC and PEB media for several cycles also led to a severe shift in the composition of the manipulated communities. While the distance between the control suspension and the original soil community was low, the Weighted Unifrac distances increased significantly between the manipulated communities and the control samples (Figure 3.2g,h; Welch's t-test p-value ≤ 0.05), with the PEB suspensions being the most different (i.e., PEB a1 and PEB a2; Figure 3.2i). Moreover, the incubation in MAC and PEB broths led to a simplification of the community composition as taxa belonging to only three bacterial classes represented 99.8% of the OTU relative abundance in manipulated suspensions (Supplementary Figure B.1). The γ -Proteobacteria represented 87.33% and 64.63% of the relative abundance of the MAC (i.e., MAC a1 and MAC a2) and PEB (i.e., PEB a1 and PEB a2) suspensions, respectively. The α -Proteobacteria represented between 3.00% and 8.91% and the Bacteroidetes between 10.90% and 2.48% of the Mac a1 and the MAC a2 suspensions, respectively, while Bacteroidetes represented 35.08% of the PEB suspensions. Thereby, suspension manipulations led to four communities with different diversities and compositions (Figure 3.2d,e,f,h,i; Tukey's test, p-value ≤ 0.05). We then diluted the manipulated suspensions to obtain three different densities (Supplementary Table B.1), in order to test the effect of three factors on the coalescence outcome (i.e., diversity, composition, and density).

Impact of Microbial Diversity, Composition, and Density on Coalescence Outcome. To implement coalescence treatments, 75 mL of a suspension containing a manipulated community at one density was mixed with the same volume of the control suspension and inoculated into sterile microcosms. All the communities resulting from this microcosm incubation were different than the original soil one (Supplementary Figure B.1). Using a linear model (Equation 3.1), we estimated the effect of coalescence on the bacterial diversity of the coalesced communities compared to the non-coalesced control community (i.e., observed species, PD whole tree, and Simpson's reciprocal indices). Only three out of the twelve coalesced communities had a significantly lower richness than the non-coalesced control community (Figure 3.3a; Tukey's test, p-value ≤ 0.05) with losses up to 10.8% of the observed species for the PEB a1d1 treatment. Nonetheless, the impact of coalescence was idiosyncratic (Figure 3.3a,b,c) with differences up to a range of 40% between two coalesced communities (e.g., between PEB a1d2 and MAC a2d2 Simpson's Reciprocal values; Figure 3.3c). Therefore, to estimate the effect of each manipulated factor (i.e., diversity, composition, and density) on the α -diversity metrics of coalesced communities, we performed ANOVAs on linear models (Equation 3.2). We found that the diversity of the manipulated communities was the only factor impacting the richness and the phylogenetic diversity of the coalesced communities (Figure 3.3d; F value, Bonferroni adjusted p-value ≤ 0.05) while both the diversity and the composition of the manipulated communities impacted their evenness (i.e., Simpson's Reciprocal; Figure 3.3d). None of the 3-ways nor 2-ways interactions were significant for the α -diversity metrics, confirming that the diversity of the manipulated communities was the main factor steering the diversity of the coalesced communities.

To estimate the impact of coalescence on the bacterial community structure, we implemented a PERMANOVA on the weighted Unifrac distances between samples (Equation 3.1). All coalesced communities had structures significantly different from the control one (Fig-

ure 3.3e,f; Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05). Coalesced communities were also significantly different from one another in 75% of the pairwise comparisons between them (Figure 3.3e,f; Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05). Then, to estimate the relative effect of each manipulated factor on the β -diversity of coalesced communities, we performed ANOVAs on linear models (Equation 3.2). The manipulated community density was the main factor impacting the coalesced β -diversity, followed by the manipulated community composition while their diversity only had a slight direct impact on the outcome β -diversity (Figure 3.3d). Indeed, the impact of the coalescence significantly increased with the manipulated suspension density. For each manipulated suspension, the coalescence outcomes from the highest density (d1) had the highest degree of dissimilarity to the control, comparing to the d2 and d3 densities (Figure 3.3e). Furthermore, three out of four d1 coalesced communities were different than their respective d2 coalesced communities while only two out of four d2 coalesced communities were different than their respective d3 coalesced communities (Figure 3.3e; Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05), underlying the effect of density on the coalescence outcome was modulated by the composition of the manipulated communities. All 2-ways interactions, but not the 3-ways interaction, were significant for explaining β -diversity, highlighting that all manipulated factors interacted and steered the structure of the coalesced communities.

Identification of OTUs Impacted by Coalescence. Significant changes in relative abundance of the dominant OTUs between the coalesced and the control communities were determined using a generalized linear mixed model (Equation 3.3). The coalescence between a manipulated community and the control one had a more negative than positive impact on the OTU relative abundance in the coalesced communities compared to the non-coalesced control community. While the coalescence affected either positively or negatively, 21% and 22% of the dominant OTUs (55 and 58 out of 258 OTUs, respectively), 12 and 26 OTUs were affected in half or more of the treatments, respectively (Figure 3.4a; Bonferroni adjusted p -value ≤ 0.05). Furthermore, we found that the impact of the coalescence on the OTUs' abundances depended on the bacterial classes of the studied OTUs. Indeed, the positively affected OTUs were mostly associated with α -Proteobacteria while the negatively impacted OTUs mostly belonged to Firmicutes and γ -Proteobacteria (Figure 3.4a). Among the 55 OTUs positively affected by coalescence, only 22 were detected in the manipulated suspensions before coalescence (Supplementary Figure B.2 and Supplementary Figure B.3).

To estimate the relative effect of each manipulated factor on the OTUs' abundance, we performed ANOVA's on a generalized linear mixed model (Equation 3.4). Again, we found that the contributing factors largely depended on the bacterial classes of the studied OTUs (Figure 3.4b). Firmicutes were mostly impacted by density (Figure 3.4b; F value, Bonferroni adjusted p -value ≤ 0.05) as their abundances decreased significantly more within the highest density treatments than within the lowest (Figure 3.4a; Bonferroni adjusted p -value ≤ 0.05). Indeed, fourteen, thirteen and twelve Firmicutes OTUs lost on average 80.6%, 71.3% and 68.0% of their abundance within the d1, d2 and d3 density treatments compared to the control, respectively (Figure 3.4a). For five of those Firmicutes OTUs, this density effect was influenced by the composition effect resulting in the decrease of 79.1% and 58.5% of their abundance within the MAC and PEB composition treatments compared to the control, respectively (Figure 3.4a). This suggests that the Firmicutes OTUs engaged in competitive interactions that were more density-dependent than specific to some taxa.

In contrast, α -Proteobacteria were mainly impacted by the composition (Figure 3.4b; F value, Bonferroni adjusted p -value ≤ 0.05) with higher increase in their relative abundances within the MAC than within the PEB composition treatments (Figure 3.4a; Bonferroni adjusted p -value ≤ 0.05). The interaction between composition and diversity factors highly influenced

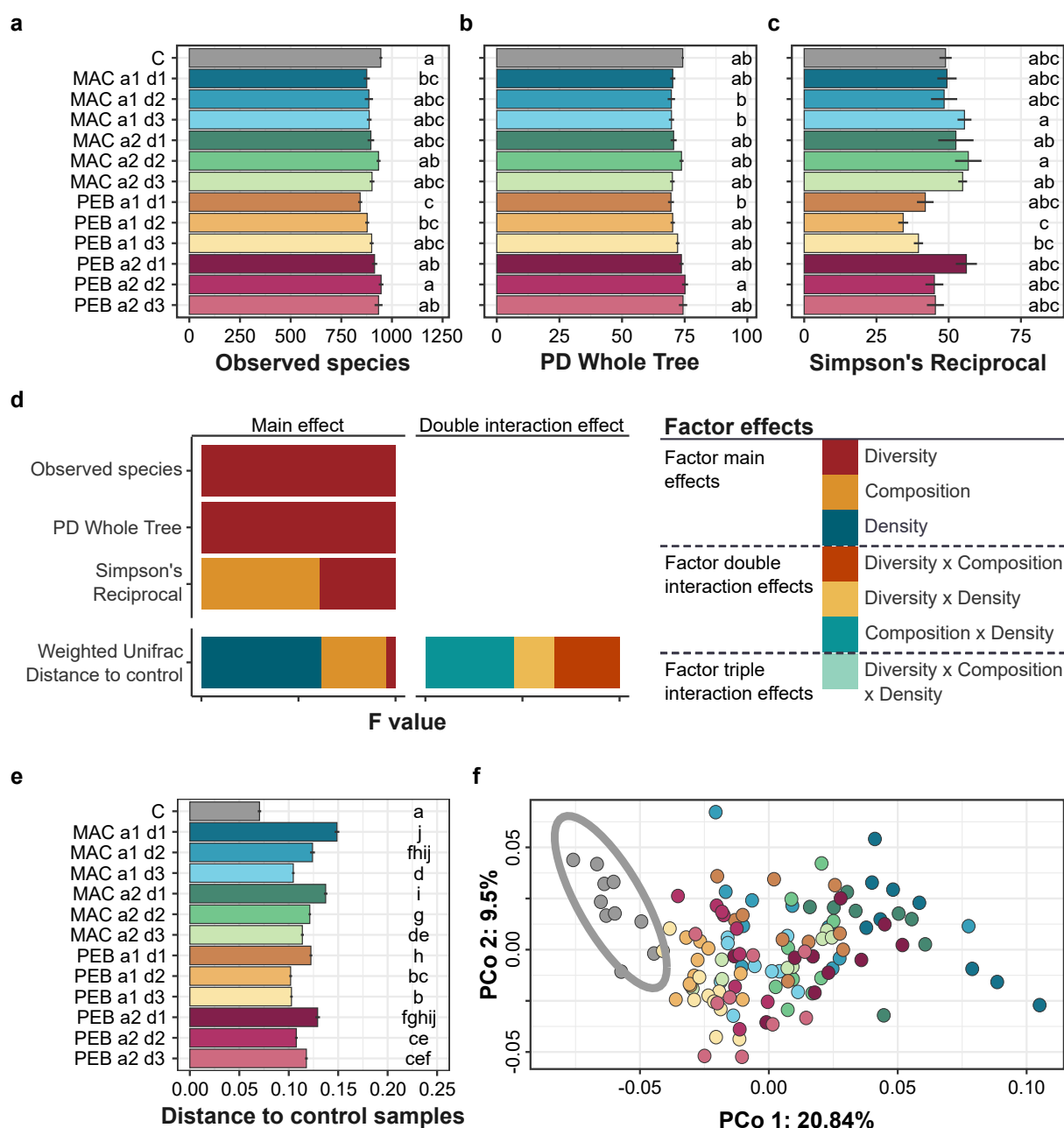


Figure 3.3: Diversity and structure of the non-coalesced control and the coalesced communities. (a to c) The number of observed species (a), the Faith's phylogenetic diversity (b) and Simpson's reciprocal (c) indices are shown (mean \pm s.e.) in the non-coalesced control and the coalesced communities. Letters indicate significantly different statistical groups (Tukey's test, p -value ≤ 0.05). (d) The effect (F value) of each manipulated factor on the diversity indices in the coalesced communities, as estimated using a linear model. (Bonferroni adjusted p -value ≤ 0.05) (e) Weighted UniFrac distances between the control suspension samples and between the control suspension and either the original soil or the manipulated suspensions (mean \pm s.e.). Letters indicate significantly different statistical groups (Adonis pairwise comparison, Benjamini-Hochberg corrected p -value ≤ 0.05). (f) Principal coordinate analysis (PCoA) based on the weighted UniFrac distance matrix showing the non-coalesced control and the coalesced community samples and the 95% joint confidence ellipse for the control suspension samples. The dot colours correspond to the bar colours in a to g.

the α -Proteobacteria abundances (Figure 3.4b) with more OTUs positively affected within the MAC a1 and PEB a2 than within the MAC a2 and PEB a1 treatments, respectively, but with a

higher mean increase of their abundances in the MAC a2 treatments than in the others (Figure 3.4a). The α -Proteobacteria OTUs were also impacted by the density (Figure 3.4b) as they had a higher mean increase of their abundance in the highest density treatments (i.e., d1; Figure 3.4a). This suggests that the α -Proteobacteria engaged in interactions with specific taxa present in the suspensions, allowing them to increase their abundance, with a higher positive effect when the specific taxa were inoculated at a higher density.

Regarding γ -Proteobacteria, two different patterns emerged from two different phylogenetic groups. A first group of OTUs belonging to *Pseudomonas sp.* was mostly impacted by the composition and its 2-way interaction with diversity (Figure 3.4b; F value, Bonferroni adjusted p-value ≤ 0.05), resulting in a negative impact more important in the MAC a2, PEB a1 and PEB a2 than in the MAC a1 treatments (Figure 3.4a; Bonferroni adjusted p-value ≤ 0.05). This suggests that those *Pseudomonas* OTUs engaged in competitive interactions with some specific taxonomic groups and that this interaction effect on their abundance was influenced by the diversity of the taxonomic group. A second group composed of *Massilia sp.* OTUs was impacted by both diversity and density (Figure 3.4b; F value, Bonferroni adjusted p-value ≤ 0.05) as their abundance was negatively affected in the a1 d1 treatment of the MAC suspension (Figure 3.4a; Bonferroni adjusted p-value ≤ 0.05). This suggests that the *Massilia sp.* engaged in a competitive interaction with a specific taxon that impacted their abundance only when inoculated at a sufficient density.

Coalescence Unraveled Biotic Interactions. To identify interacting OTUs, we inferred a co-occurrence network across bacterial communities from all microcosm samples using a sparse multivariate Poisson log-normal model (Figure 3.4c). None of the 42 OTUs present in the network was among the ten most abundant OTUs (i.e., dominant OTUs; Figure 3.5, Supplementary Figure B.3), suggesting that biotic interactions during coalescence events were not affecting the most abundant OTUs of the microbial community. However, they represented the majority of the most abundant OTUs detected in the manipulated suspensions, suggesting that the most abundant OTUs in suspension triggered biotic interactions during the coalescence (Supplementary Figure B.3).

Among the 82 links inferred in the network, 92.7% of them connected three bacterial classes: α -Proteobacteria, Firmicutes and γ -Proteobacteria. Furthermore, 58% of the network links connected 8 genera: *Brevundimonas*, *Pseudochrobactrum* and *Ochrobactrum* within the α -Proteobacteria, *Pseudomonas* and *Stenotrophomonas* within the γ -Proteobacteria and *Bacillus*, *Solibacillus* and *Tumebacillus* within the Firmicutes (Figure 3.4c). One OTU alone, belonging to the *Pseudochrobactrum sp.*, was connected with 15.8% of the network links and was negatively correlated with six OTUs belonging to the Bacilli class within the Firmicutes (Figure 3.4c). We thus looked more closely at these OTUs' abundances (Figure 3.6).

In the non-coalesced control community, the relative abundance of the Bacilli OTUs was higher than the abundance of the *Pseudochrobactrum sp.* OTU. Conversely, the *Pseudochrobactrum sp.* OTU relative abundance increased in nine out of the twelve coalescence outcomes compared to the control, overtaking the Bacilli OTUs relative abundance that decreased in most of the coalesced communities. Furthermore, both coalescence effects (i.e., positive and negative) increased with the manipulated suspension density, resulting in the Bacilli relative abundance mirroring the *Pseudochrobactrum sp.* one (Figure 3.6). This suggests that the *Pseudochrobactrum sp.* OTU, which was not dominant in the control community, was enriched by the coalescence with a manipulated suspension, allowing it to outcompete the Bacilli OTUs by shifting the outcome of the interaction between them in a density-dependent manner.

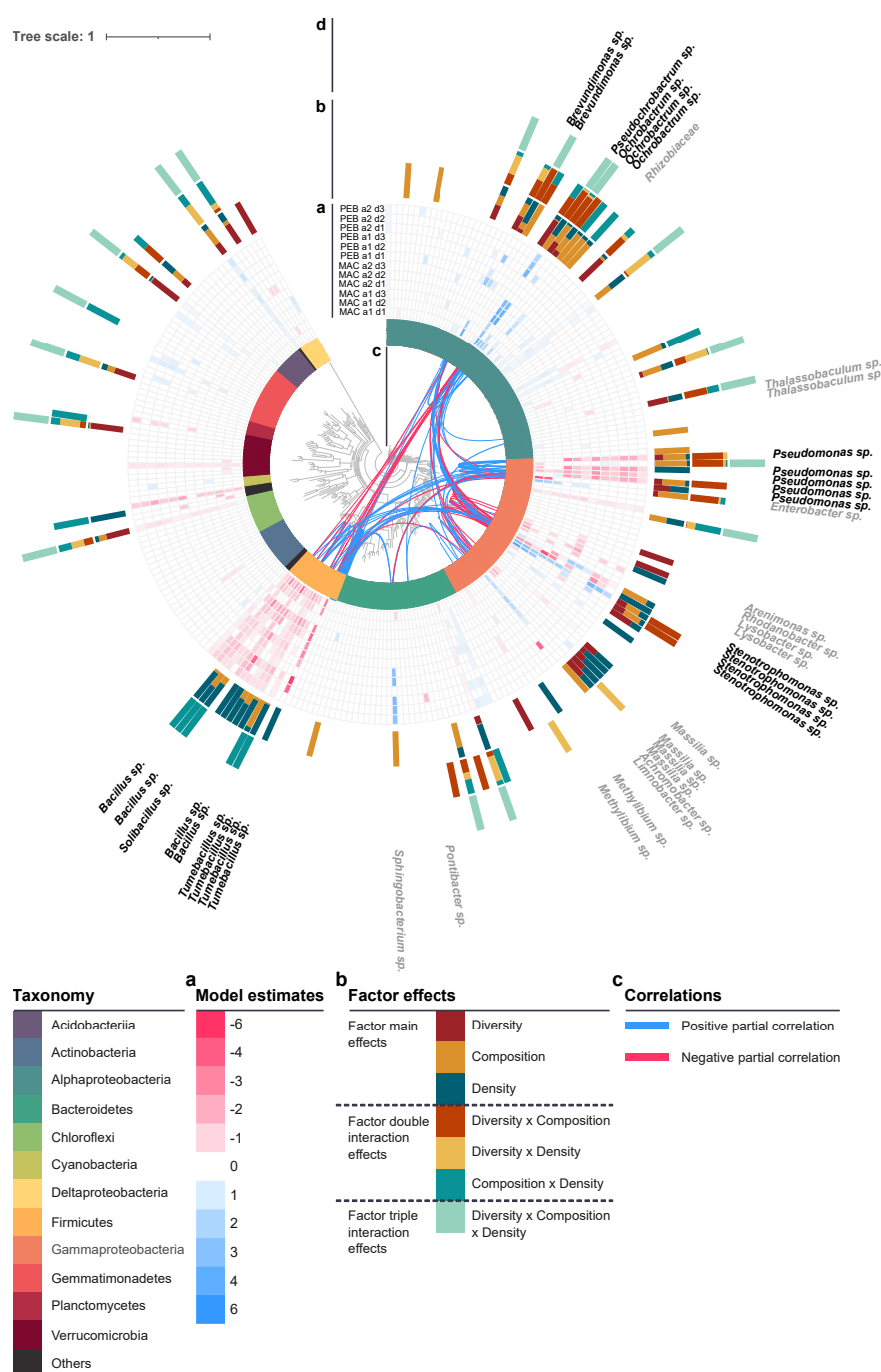


Figure 3.4: Taxonomic relationships and distribution of the 258 most abundant 16S rRNA OTUs. The OTU class level is indicated by different colours on the innermost ring. (a) The inner ring shows the coalescence treatment effects on the OTUs' relative abundances compared to the non-coalesced control, as estimated using a generalized linear mixed model. The blue and red boxes indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment (Bonferroni adjusted p -value ≤ 0.05). (b) The middle ring shows the effect (F value) of each manipulated factor on the OTUs' relative abundance in the coalesced communities, as estimated using a generalized linear mixed model (Bonferroni adjusted p -value ≤ 0.05). (c) The network, inferred from the non-coalesced control and the coalesced communities, is showed by the links displayed above the tree and representing partial correlations ρ coloured blue if $\rho > 0$ and red if $\rho < 0$. (d) The outer ring shows the genera of the OTUs involved in the network.

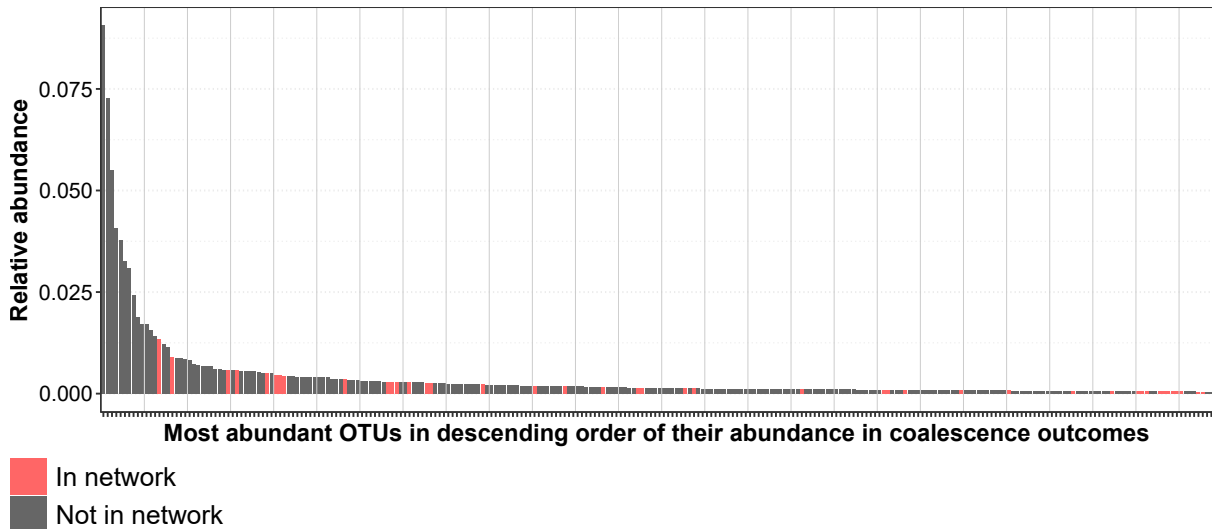


Figure 3.5: Relative abundance of the 258 dominant OTUs. OTUs are placed in descending order of their abundance and those involved as nodes in the inferred correlation network are displayed in red.

3.4 Discussion

A growing body of evidence shows that community properties, and especially their diversity, composition, and density, can influence in different ways the outcome of coalescence events [195], [196], [243], [247]. However, the relative importance of each of those properties (i.e., diversity, composition, and density) on the diversity and structure of the coalesced communities has not yet been properly evaluated. The contribution of these community properties on biotic interactions occurring during community coalescence is also unclear. Here, we used community manipulation to implement twelve different coalescence treatments by mixing a native microbial community with various manipulated communities with distinct diversity, composition, and density to assess the relative importance of these manipulated community properties for the assembly of coalesced communities and the involved biotic interactions.

Mixing the same native community with the manipulated communities resulted in coalesced communities exhibiting weak or no differences in α -diversity while large differences in β -diversity were observed with all the coalesced communities being significantly different from the non-coalesced control community (Figure 3.3e,f). We found that these differences in the diversity and structure of the coalesced communities were explained by different properties of the manipulated communities. Previous studies showed that the diversity of communities that are mixed during a coalescence event is of importance for the composition of the coalesced community [186], [195], [243]. Here, we nuanced this result by showing that the diversity of the manipulated communities was marginally directly explaining the observed changes in community structure as measured by Weighted Unifrac distance to the control (Figure 3.3d) and that this effect of diversity was actually modulated by the density and the composition of the manipulated communities. In contrast, we found that the composition of the manipulated communities influenced both the α - (Simpson's reciprocal) and β - (Weighted Unifrac) diversity of the coalesced communities, which is consistent with previous results [195], [244]. Our results also indicated that density of the manipulated communities was the main factor influencing the structure of the coalesced communities, with -as expected- higher initial density leading to larger differences in community structure. Accordingly, Jones *et al.* [244] and Vila *et al.* [243] showed that higher density of invading cells increased the invasion success therefore leading to more important changes in the community structure.

Overall, coalescence treatments affected the relative abundance of 40% of the most abun-

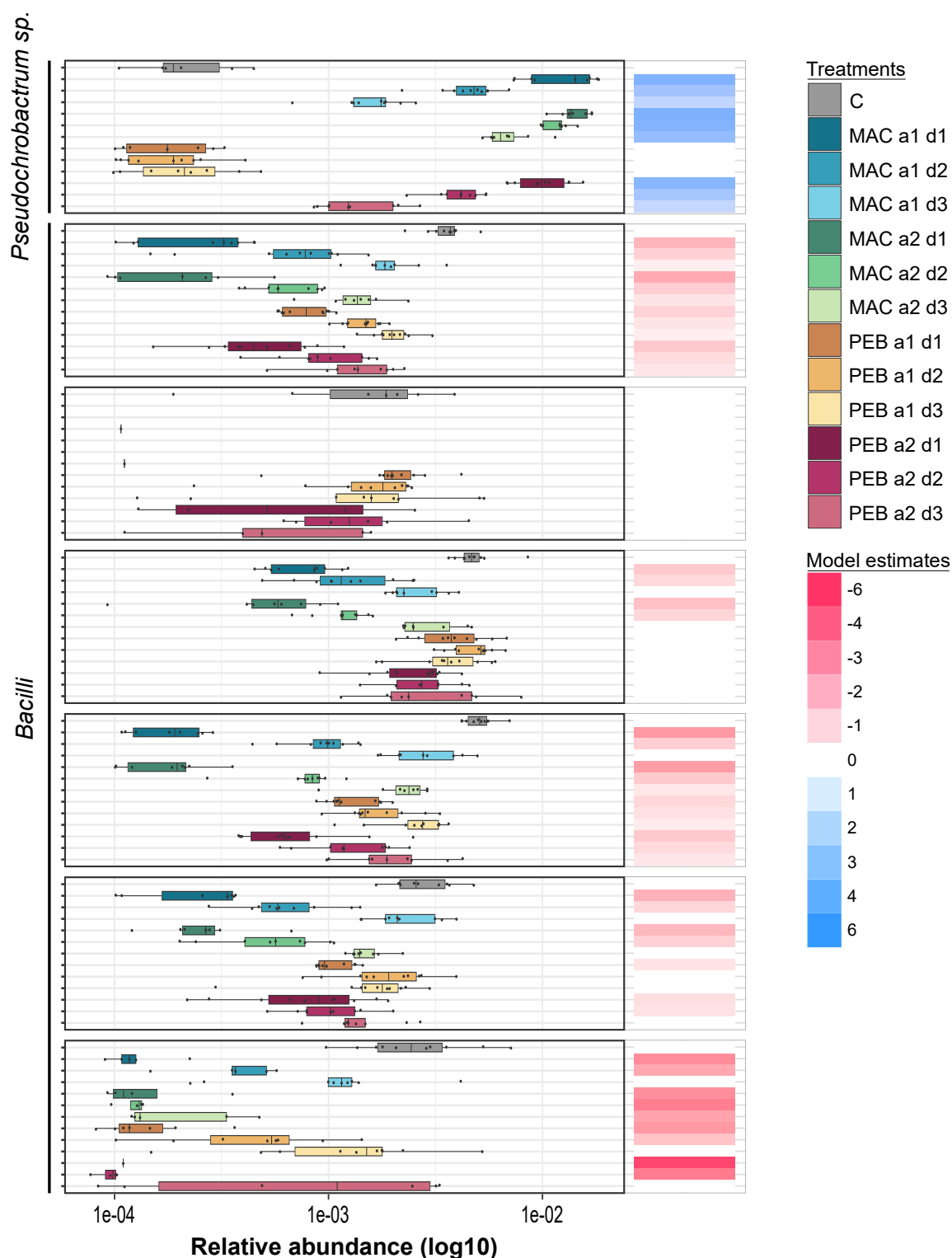


Figure 3.6: Relative abundance of the *Pseudochrobactrum* sp. OTU and connected *Bacilli* OTUs in the non-coalesced control and the coalesced communities. The blue and red boxes on the right indicate OTUs with significantly increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not significantly affected by the treatment, as estimated using a generalized linear mixed model.

dant OTUs. Among the OTUs that were affected by at least half of the treatments, the number of OTUs with decreasing abundances was more than two times higher than that of increasing

abundances (Figure 3.4), which suggests that the coalescence treatments triggered negative rather than positive interactions. Interestingly almost all the OTUs belonging to the Bacilli exhibited a decrease in relative abundance in the coalesced communities (Figure 3.4a), which was mostly explained by the effects of the density and the composition of the manipulated communities (Figure 3.4b). Six of these OTUs were linked by a negative correlation to OTUs belonging to the α -Proteobacteria, and in particular to a *Pseudochrobactrum sp.*. While these Bacilli OTUs are dominating over the *Pseudochrobactrum sp.* in the non-coalesced control (Figure 3.6), we found that they were negatively affected by the coalescence events (Figure 3.4a), and their shifts in relative abundance mirrored that of the *Pseudochrobactrum sp.* This suggests that *Pseudochrobactrum sp.* and the Bacilli were engaged in density-dependent negative interactions and that the *Pseudochrobactrum sp.* outcompeted the later during coalescence. Several studies have already described such negative interactions between members of the Proteobacteria and the Firmicutes families. For instance, phyllosphere strains belonging to Firmicutes were strongly inhibited and outcompeted by Proteobacteria strains in both *in vitro* and *in vivo* pairwise competition assays [232]. In the human gut microbiota, it is now recognized that the decrease in Firmicutes is correlated with an increase in Proteobacteria, such dysbiosis being associated with inflammatory bowel disease [248], [249]. Even without identifying the exact underlying mechanisms, our results further suggests that competition between Firmicutes and Proteobacteria may be an overall rule of microbial community assembly.

Intriguingly, the thirteen most abundant OTUs were not present in the PLN network, which suggests that they were not involved in the biotic interactions of importance for the outcome of coalescence events. Accordingly, dominant OTUs have previously been described to be more influenced by abiotic factors than rare OTUs, which may be more constrained by biotic interactions [190], [250]. Indeed, Rivett & Bell [229] showed that the abundance of the dominant taxa was positively correlated with respiration rate, cell yield, and ATP concentration, which are parameters associated to growth rate. As in our work coalescence events are comprising a range expansion phase, fast-growing taxa were likely less affected by changes in their biotic environment and could outperform their competitors during soil colonization to become dominant.

In conclusion, our results suggest that the structure rather than the diversity of coalesced communities is affected by differences in the source communities and that the composition and the density of the source communities are the main drivers of the outcome of coalescence events. Furthermore, we found that 40% of the most abundant OTUs were impacted by coalescence, mostly in a density-dependent manner. However, no dominant OTU was detected as involved in biotic interactions. Generalizing such assessment of how the different properties of source communities influence the diversity and the structure of a coalesced community could allow building predictive models and potentially help in designing microbe engineering strategies based on coalescence [200], [238], [251].

4

Manipulating the Physical Distance Between Cells During Soil Colonization Reveals the Importance of Biotic Interactions in Microbial Community Assembly

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Abstract

Microbial communities are of tremendous importance for ecosystem functioning and yet we know little about the ecological processes driving the assembly of these communities in the environment. Here, we experimentally manipulated the physical distance between neighboring cells during soil colonization by three microbial communities exhibiting some variations in membership to determine the role of bacterial interactions in soil community assembly. We show that reducing distances between cells resulted in a loss of bacterial diversity with at least 41% of the most abundant OTUs being significantly affected by physical distancing depending on community membership. Our results also indicate that high cell density may favor competitive rather than mutualistic interactions. Our study demonstrates that the competitiveness of bacteria strongly depends on cell density and community membership, therefore highlighting the fundamental role of microbial interactions in the assembly of soil communities.

Keywords: Microbial interactions; community manipulation; physical distance; soil colonization; community assembly; coalescence

4.1 Introduction

Understanding community assembly processes is one of the fundamental goals in community ecology. Although soil microbial communities play an essential role in several key ecosystem functions such as biogeochemical cycling, plant productivity, and carbon sequestration [2], [8], [252], it is unclear what process, or combinations of processes, are driving their composition. For example, much focus has been laid on the role of environmental filters in shaping microbial communities [52] while how interactions among microorganisms drive their assembly remains largely unexplored [253]. However, a growing body of evidence suggests that these biotic interactions may also play an important role in microbial community assembly [58], [59], [188].

Most microorganisms face a constant battle for resources, and a large range of interactions between microorganisms has been reported [82]. For example, microorganisms can produce tremendous diversity of compounds (e.g., antibiotics, toxins, and siderophores) to sequester resources or to harm their competitors [254]. On the opposite, positive interactions include tightly coupled mutualistic interactions such as syntrophy, in which both partners depend on each other to perform a metabolic activity [255]. Whether positive or negative, interactions mostly occur between individuals that are close in space. In some cases, a physical contact between cells is even required for the injection of secreted toxins to the rival strain as exemplified by the type VI secretion system, which mediates interactions between a broad range of Gram-negative bacteria. In soil, it has been estimated that a single bacterium has about 120 neighboring species within interaction distance [137]. Yet we still lack a clear understanding of how prevalent these interactions are, and how they affect community composition.

Theory predicts that in a new environment and without immediate neighbors, microorganisms will first colonize the empty space, which can be considered as a surrogate for resources [256]. After this initial phase of range expansion, direct interactions at the boundaries between neighboring patches of different species will emerge [32], [63]. Although models have shown that relative fitness of individuals strongly depends on the density of surrounding neighbors, i.e., the level of competition for resources [140], [257], [258], we are not aware of any study that has explicitly tested how changing physical distance between microbes in a complex environment influences the strength of species interactions.

Here we examine to which extent the distance between neighboring species in soil determine their competitiveness for a more general understanding of the role of biotic interactions in microbial community assembly. We hypothesized that (i) experimentally manipulating the physical distance between microbial cells will modify the interaction frequency leading to differences in microbial community composition with increasing distances between neighbors favoring poor competitors (Figure 4.1a), and (ii) the importance of physical distance in shaping microbial communities will be modulated by community membership. For this purpose, we first subjected a soil microbial community to two removal treatments (heat-shock and ramoplanin) in order to generate microbial community inocula with some variation in membership. We then experimentally manipulated the physical distance between microbial cells by introducing the same species pool (control, heat-shock, and ramoplanin treated microbial communities), into increasing volumes of sterilized soil. Finally, we performed a coalescence experiment with a reciprocal transplant design by mixing microbial communities that assembled with different initial distances between cells in order to assess their relative competitiveness (Figure 4.1b).

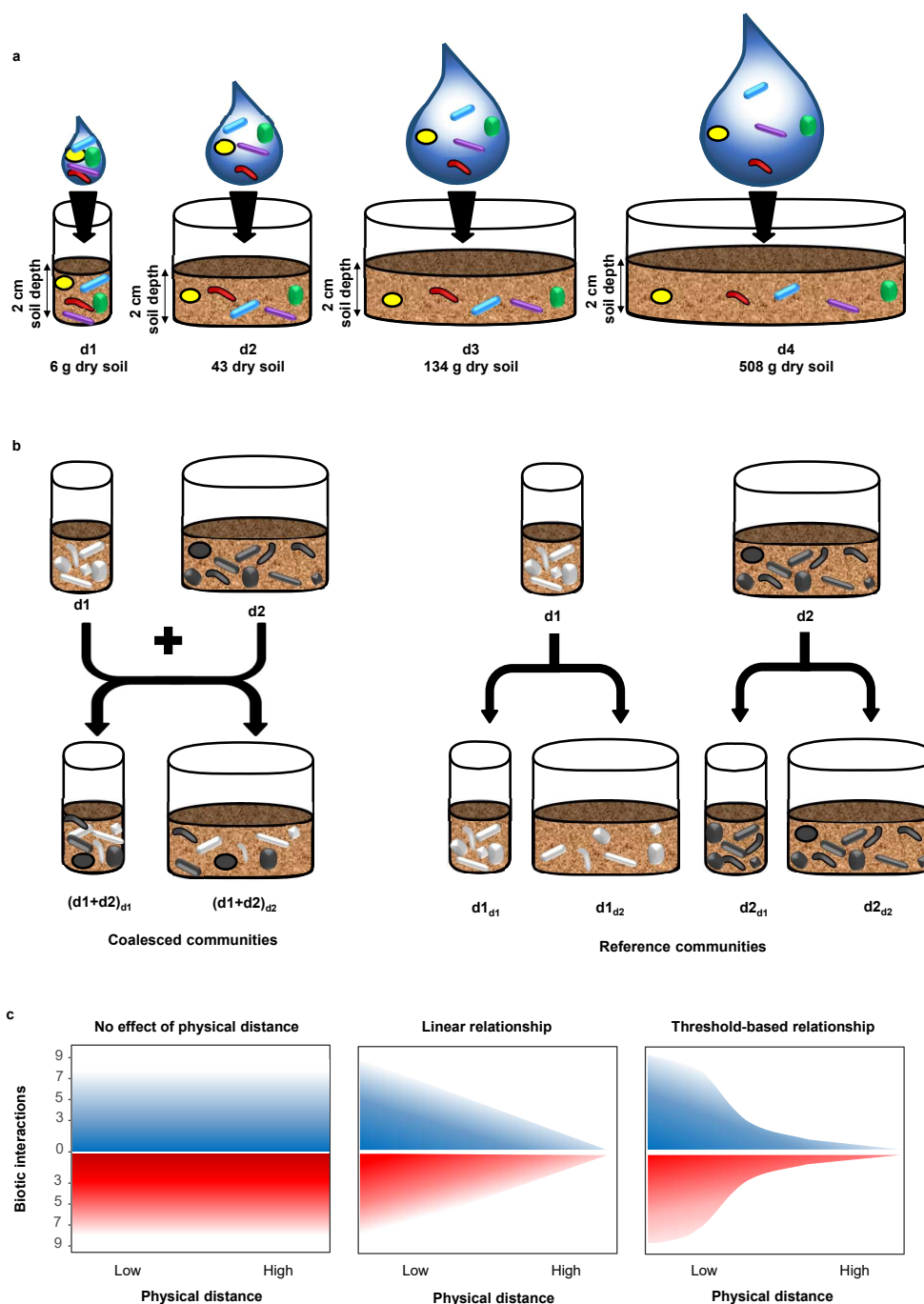


Figure 4.1: Schematic illustration of the experimental design. (a) The physical distance between microbial cells was experimentally manipulated by introducing the same species pool into increasing volumes of sterilized soil during Step 1 experiment ($n=10$) for control, heat-shock and ramoplanin communities. (b) Step 2 consisted in a coalescence experiment with a reciprocal transplant design by mixing microbial communities that assembled at low (d_1) or high (d_2) densities during Step 1 in sterile soil (coalesced communities). Soils colonized under the d_1 and the d_2 initial densities were also incubated separately with sterile soil at low (d_1) or high (d_2) densities (reference communities). (c) Summary of possible scenarios of the impact of increasing physical distance between the neighboring cells on biotic interactions (negative in red and positive in blue).

4.2 Material & Methods

Soil Sampling and Experimental Design. The soil was collected from the Epoisses experimental farm in France (N47°30'22.1832", E4°10'26.4648") in October 2019 and sieved through 4 mm. The soil properties were 41.9% clay, 51.9% silt, and 6.2% sand, pH 7.2, and C and N content 15.5 and 1.4 g.kg⁻¹ dry soil, respectively. The soil was γ -sterilized (>60 kGy at Conservatoire, Dagneux, France) and used to prepare microcosms with 4 different volumes of soil and diameters in order to obtain the same soil depth (2 cm) in all microcosms (i.e., d1=6 g, d2=43 g, d3=134 g, and d4=508 g). Each microcosm volume was replicated 10 times. Soil suspensions were then prepared by mixing 100 g equivalent dry mass of fresh soil with 150 ml sterile distilled water using a waring blender and ten times diluted under sterilized conditions. To generate microbial community inocula with some variation in membership, the soil suspensions were subjected to two different removal treatments aiming at depleting various microbial groups. The heat-shock treatment (HS) was applied as follows: 0°C for 5 min / 70°C for 15 min / 0°C for 5 min, and the biocidal antibiotic (ramoplanin, RA) was applied at a concentration of 70 $\mu\text{g}\cdot\text{mL}^{-1}$ of soil suspension. Non-treated soil suspensions were used as controls (C). Then, to manipulate physical distance between neighboring cells, the soil suspension was first diluted into increasing volume of water before inoculation of the microcosms containing different volume of sterilized soil in order to obtain the same soil moisture in all microcosms (Figure 4.1). The soil suspensions were thoroughly vortexed and equally distributed on the entire soil surface whatever the microcosms diameter to reach a soil moisture of 30%. The microcosms were then sealed with Parafilm allowing gas exchange in aseptical conditions and incubated at 20°C for 4 months. After incubation, soil microcosms and the original soils were used for subsequent analyses (n=10 for a total of 130 soil samples).

In a second step, we selected the control (C) and heat-shock (HS) communities from d1 and d2 microcosms (i.e., Cd1, Cd2, HSd1, and HSd2) for the coalescence experiment. For this purpose, 1.5 g of soil from microcosm colonized under the d1 density was thoroughly mixed with 1.5 g of soil from microcosm colonized under the d2 density into either 3 or 43 g of sterile soil microcosm to again obtain microcosms with low (d1) or high (d2) physical distancing between cells (Figure 4.1b). Soils from Step 1 selected communities (i.e., Cd1, Cd2, HSd1, and HSd2) were also mixed only with sterile soil at the d1 and d2 densities to obtain reference communities. Soil microcosms from Step 2 were replicated 5 times and incubated under the same condition as Step 1 for 90 days.

Assessment of Microbial Community Composition and Diversity. DNA was extracted from 190 samples (ten original soil samples, 120 Step 1 microcosms, and 60 Step 2 microcosms) using the DNeasy PowerSoil-htp 96 well DNA isolation kit (Qiagen, France) according to the manufacturer's instructions. To generate amplicons, a 2-step PCR approach was used according to (Berry et al., 2011). The V3-V4 hypervariable region of the *16S rRNA* gene was amplified using the 341F (5'-CCTACGGGSGCAGCAG-3') and 805R (5'-GACTACCAGGGTATCTAAT-3'). The amplicon size was checked with 2% agarose gel and DNA concentration was estimated using Quant-IT™ dsDNA HS Assay kit (Invitrogen™, Carlsbad, CA, USA). Final PCR products were purified and their concentration normalized using the SequalPrep Normalization plate kit (Invitrogen™, Carlsbad, CA, USA). Sequencing was performed on MiSeq (Illumina, 2 x 250 bp amplicons) using the MiSeq reagent kit v2. Demultiplexing and trimming of Illumina adaptors and barcodes was done with Illumina MiSeq Reporter software (version 2.5.1.3). Sequence data from soil samples were analysed using an in-house developed Python pipeline (available upon request). Briefly, *16S rRNA* gene sequences were assembled using PEAR [204] with default settings. Further quality checks were conducted using the QIIME pipeline [205] and short sequences were removed (<400 bp). Reference based and de novo chimera detection, as well as OTUs clustering were performed using VSEARCH [206] and the

adequate reference databases (SILVA' representative set of sequences from [207]). The identity thresholds were set at 94% based on replicate sequencing of a bacterial mock community [188]. Representative sequences for each OTU were aligned using Infernal [209] and phylogenetic trees were constructed using FastTree [210]. Taxonomy was assigned using UCLUST [211] and the SILVA database (138.1/2020) [207]. Raw sequences were deposited at the NCBI under the BioProject PRJNA883551.

Quantification of Microbial Communities. The abundances of the total bacterial community were estimated by real-time quantitative PCR (qPCR) assays. For each treatment, we used five equimolar mixtures prepared from pairs of the 10 DNA replicates extracts. The total bacterial community was quantified using 16S rRNA primers as described by Muyzer *et al.* [212]. Real-time qPCR assays were carried out in a ViiA7 (Life Technologies, USA) with a Takyon Master Mix (Eurogentec, France) as previously described [214]. An average PCR efficiency of 100.7% was found for the two independent runs. No template controls gave null or negligible values. PCR inhibitor presence was tested by mixing soil DNA extracts with either control plasmid DNA (pGEM-T Easy Vector, Promega, France) or water. No inhibition was detected in any case.

Statistical Analysis. Statistical analyses were conducted using R statistical software [216] (version 4.0.3). Bacterial α -diversity metrics (i.e., observed species, Simpson's reciprocal, Shannon, and Faith's Phylogenetic Diversity PD from [218]) and Weighted Unifrac distance [219] between samples were calculated based on rarefied OTU tables (12000 sequences). Differences between treatments in gene copy abundances (16S rRNA) ($n=5$) and the microbial α -diversity indices ($n=10$) were tested using ANOVAs followed by Tukey's honestly significant difference (HSD) test (p -value ≤ 0.05) using the *agricolae* package [217] (version 1.3-5). Normality and homogeneity of the residual distribution were verified, and log-transformations were performed for gene copy abundances. Differences between Weighted Unifrac distances were tested using a Kruskal-Wallis test followed by a Nemenyi's all-pairs comparison test (p -value ≤ 0.05) using the *PMCMRplus* package (version 1.9.4). We also performed principal components analyses (PCoA) based on the Weighted Unifrac distance matrix to detect changes in the microbial community structure, and a Permutational multivariate analysis of variance (PERMANOVA from [220]) to detect significant differences between treatments using the *adonis* function of the *vegan* package (version 2.5-7). Pairwise post-hoc tests were conducted using the function *pairwise.adonis* from the *pairwiseAdonis* package with Benjamini-Hochberg corrections [221].

Identification of Differentially Abundant OTUs in Treatments. Low-abundance OTUs were filtered out by keeping OTUs that (i) represented $>0.01\%$ of the sequences in at least one sample, and (ii) were found in at least 60% of replicates, which resulted in 792 OTUs. Due to differences in community composition between control and removal treatments, OTUs with low prevalence (i.e., present in less than 50% of replicates within each removal treatment or control) were removed which resulted in the Step 1 experiment in 529, 306, and 468 OTUs for C, HS, and RA communities, respectively, and in the Step 2 experiment in 495 and 323 OTUs for C and HS communities, respectively. To estimate differences in OTUs abundances between treatments, we used a generalized linear mixed model. We considered that an OTU of abundance Y follows a Poisson law of parameter Λ as $Y \sim P(\Lambda)$ in any j replicates of any i treatment. Thus, we used the following model (Equation 4.1):

$$\log(\Lambda_{ij}) = o_{ij} + \mu + \alpha_i + Z_{ij}, Z_{ij}, Z_{ij, 1 \leq j \leq 10} \text{ iid} \sim N(0, \sigma^2) \quad (4.1)$$

where o is the offset for each sample calculated as the log of the sample read sum, α is the effect of the treatments, and Z is the random sampling effect modelling the data overdispersion.

For the Step 1 experiment, $i = \{1, \dots, 4\}$ represents the density treatments of either one removal treatment or control, and $j = \{1, \dots, 10\}$ represents the replicates. For the Step 2 experiment, $i = \{1, \dots, 6\}$ represents the coalescence and self-mixed treatments of either one removal treatment or control, and $i = \{1, \dots, 5\}$ represents the replicates. The analysis was performed using the *glmer* function of the *lme4* package (version 1.1-27). Subsequently, we performed a post-hoc Tukey test with the *emmeans* function of the *emmeans* package (version 1.6.1). Thereby, we implemented multiple pairwise comparisons for each OTU (i) between density treatments within each Step 1 removal treatment or control, and (ii) between each coalesced community and its references communities within each Step 2 removal treatment or control. Only comparison with a Tukey test p-value below or equal to 0.05, and with coefficient estimates higher or equal to 0.5 were considered significant.

Inference of Co-Occurrence Networks. Networks were constructed based on the most abundant OTU count data (low-abundance OTUs filtered out) using samples from either one removal treatment or control from the Step 1 experiment (n=40). For each sample set, we used one model with (Equation 4.1 as the full model, M1), and one model without the physical distance (Equation 4.2 as null Model, M0) as a covariate to identify nodes and links specific to the density effect.

$$\log(\Lambda_{ij}) = o_{ij} + \mu + Z_{ij}, Z_{ij}_{1 \leq j \leq 10} \text{ iid} \sim N(0, \sigma^2) \quad (4.2)$$

Networks were inferred from each model using a sparse multivariate Poisson log-normal (PLN) model with a latent Gaussian layer and an observed Poisson layer using the *PLNmodels* package [224] with an offset corresponding to the number of reads in each sample. For each model, the best network was selected using a Stability Approach to Regularization Selection (StARS) [225].

4.3 Results

Manipulating Cell Density Alters the Diversity and Composition of the Bacterial Community. The initial gradient in physical distance between cells resulted in differences in α -diversity after 120 days of incubation especially for the control community (C) and the community subjected to ramoplanin (RA) with the lowest diversity indices observed in the smallest microcosms d1 (Supplementary Figure C.1; Tukey's test, p-value < 0.05). The impact of physical distancing was weaker for the community exposed to heat-shock (HS) with significant differences observed only for the Shannon and Simpson's Reciprocal diversity indices. As expected, Principal Coordinates Analysis (PCoA) of the weighted Unifrac distances revealed differences in structure between the C, HS, and RA communities due to the removal treatments (PERMANOVA, $P < 0.001$, $R^2=0.69$), but also a clear clustering according to the density gradient (PERMANOVA, $P < 0.001$, $R^2=0.09$) (Figure 4.2a and Supplementary Table C.1). Thus, significant differences were observed between d1 and all the other initial distances for the C, RA, and HS communities, and also between d2 and d4 for the C community only (Supplementary Table C.2). Clostridia, Gemmatimonadetes, and γ -proteobacteria were mostly affected in the C and RA communities, while the largest changes were observed for the Clostridia, α -Proteobacteria, and Actinobacteria in the HS community (Figure 4.2b). We also quantified the *16S rRNA* gene copy numbers using qPCRs as a proxy for bacterial abundance and found the highest number of bacteria at the lowest initial physical distance (d1) in the C community, while no differences were observed between d2, d3, and d4. By contrast, the abundances of bacterial communities subjected to heat-shock and ramoplanin were not affected by physical distancing (Supplementary Figure C.2). These similar numbers of *16S rRNA* gene copy per gram of soil, which were in the same range than in the natural soil, also indicate that inoculated communities had completely colonized the microcosms whatever their volumes.

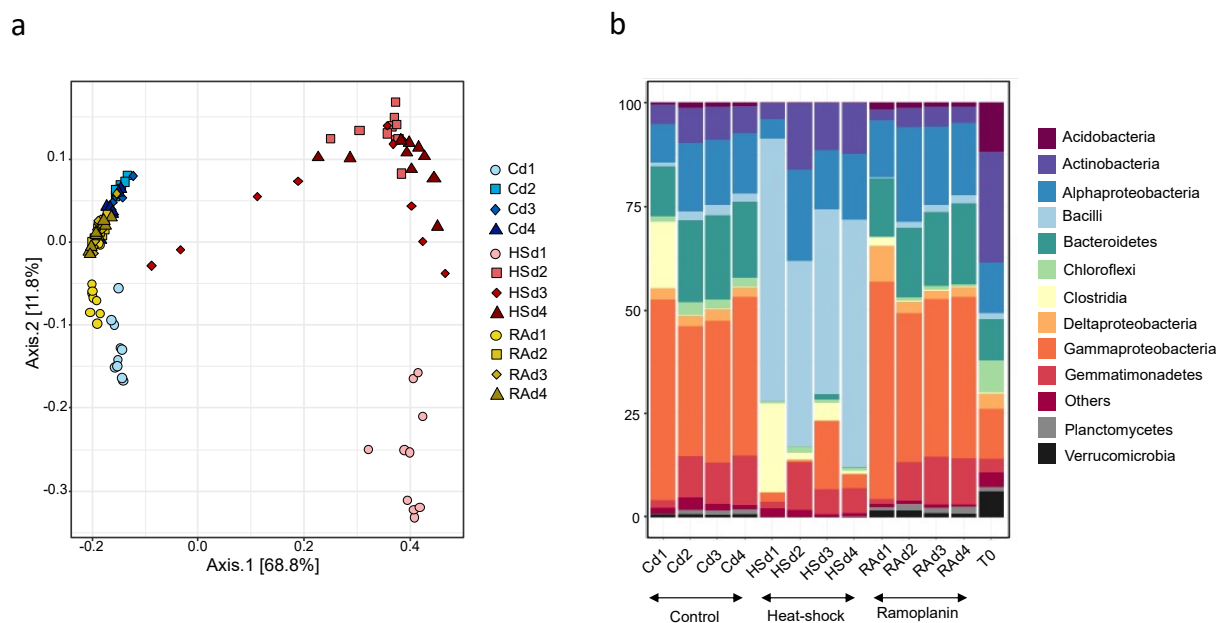
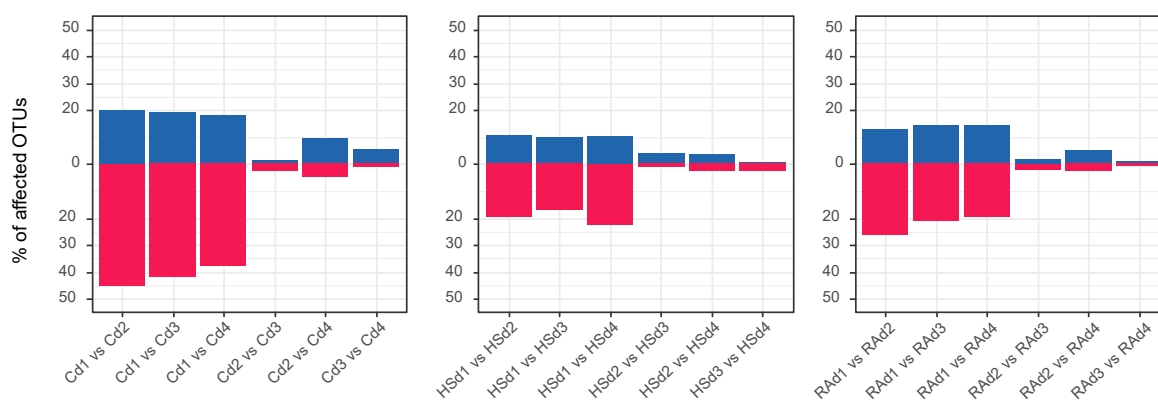


Figure 4.2: Differences in bacterial community structure and composition across removal and density treatments for the Step 1 experiment. (a) Principal Coordinates Analysis (PCoA) of the weighted UniFrac distance matrix of 16S rRNA gene amplicons showing shifts in the structures of the control (C), heat-shock (HS), and ramoplanin (RA) communities within the density gradient (d1, d2, d3, and d4). The different treatments are represented by different colors and symbols as specified in the legend. (b) Bacterial community composition in the removal treatments and control. Relative abundances are shown at the phylum and class levels and expressed as a percentage of the total number of OTUs.

We expected the shifts in biotic interactions along the gradient in physical distance to be mirrored by changes in OTUs relative abundances. Thus, more OTUs should be affected at high initial cell density because higher interaction frequency is predicted to result in increased relative abundances of the taxa that are positively interacting, and a decreased relative abundances of the poor competitors. To identify the OTUs affected by our cell physical distancing approach within the C, HS, and R communities, we used a generalized linear mixed model estimating significant shifts in the relative abundance of each of the most abundant OTUs between density treatments (Equation 4.1; Figure 4.3). Our analysis showed that in total 73%, 41%, and 52% of the most abundant OTUs were significantly affected by the density treatment for the C, HS, and RA communities, respectively. These differences were mostly observed between the lowest cell density (d1) and all other densities. We also found that the number of OTUs with decreasing relative abundance between densities was about two time higher than the number of OTUs with increasing relative abundances, whatever the community (Figure 4.3a). OTUs belonging to γ -Proteobacteria and Clostridia were mostly positively affected by lower physical distance, while members of Bacilli, Actinobacteria, and α -Proteobacteria were negatively affected (Supplementary Figure C.3). In overall, the magnitude of changes in the relative abundances of significantly affected OTUs was also the highest when comparing d1 to all the other densities. The impact of physical distancing on the magnitude of changes in relative fitness was also different between communities with a stronger negative impact for the C and RA communities, and a stronger positive effect for the HS community (Figure 4.3b).

Co-Occurrence Networks. To further explore to which extent interactions between bacterial OTUs were influenced by cell density within each community (i.e., control, heat-shock, and ramoplanin), we compared microbial co-occurrence networks inferred without covariate (Model 0) or with the initial physical distance as a qualitative covariate (i.e., the covariation

a



b

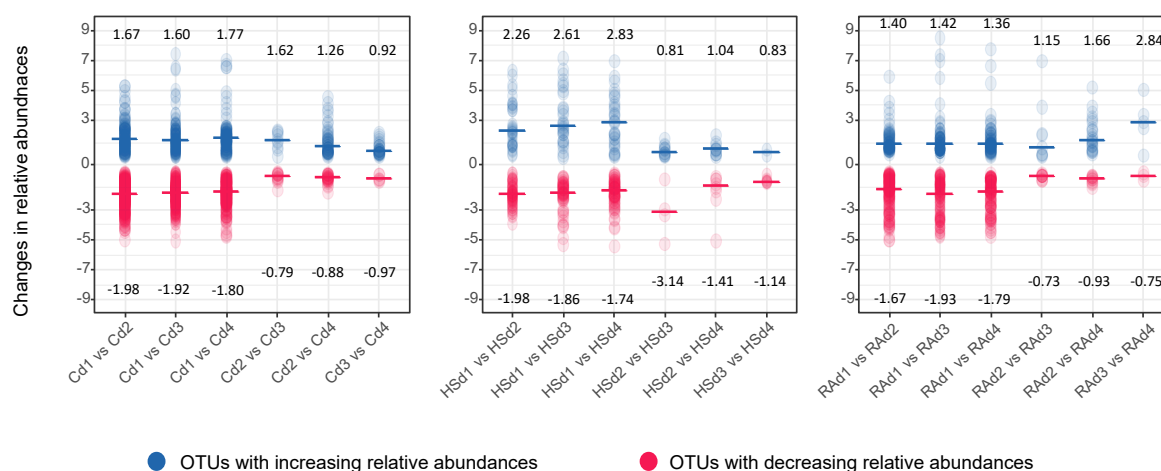


Figure 4.3: Significantly differential abundant OTUs between density treatments (d1, d2, d3, and d4) as identified using a generalized linear mixed model for the control (C), heat-shock (HS), and ramoplanin (RA) communities. (a) Percentage of OTUs exhibiting significant increasing/decreasing relative abundances for each pairwise comparison between density treatments (where vs means > or <). (b) Changes in the relative abundances of significantly affected OTUs as represented by the coefficient estimates obtained by the generalized linear mixed model for each OTU within each comparison and used as a measure of the effect size. Median of the coefficient estimates are indicated for each comparison.

between OTUs were not caused by the effects of physical distancing; Model 1; Equation 4.2) [224]. We found that 20.8% of nodes and 51.8% of links were specifically related to the effect of the physical distancing for the C community, and 24.5% of nodes and 43.3% of links for the HS community. In contrast, 87% of nodes and 97.3% of links were related to the initial physical distance for the RA community (Figure 4.4). Negative links were also more dependent on physical distancing in the networks inferred from the C (M0: 17.68% vs M1: 2.98%) and RA communities (M0: 9.27% vs M1: 0%), compared to the HS community (M0: 12.87% vs M1: 12.53%). Among the negative links, 90% were connecting Clostridia with either Proteobacteria, Longimicrobia or Bacteroidia in the C network while 72% of negative links in the HS community network were between Clostridia and Bacilli, and 13% between Clostridia and Actinobacteria (Supplementary Figure C.4). The depletion of Clostridia in the RA community resulted in shifts in the taxa associations with the highest percentage of negative links (53%) connecting δ -Proteobacteria with mainly α -Proteobacteria, γ -Proteobacteria, Bacilli, and Bacteroidia. By identifying nodes that were both related to physical distancing in co-occurrence

networks and exhibiting significant changes in relative abundances as determined by the differential abundance analysis, we found that the effect of our physical distancing approach was clearer at a family-level than at a phylum or class-level (Supplementary Figure C.5). Thus, while both positive and negative links were observed between Clostridia and Bacilli in the heat-shock communities for example (Supplementary Figure C.4b), we found only positive links between Ruminococcaceae and Bacillaceae, and only negative links between Ruminococcaceae and Paenibacillaceae (Supplementary Figure C.5b).

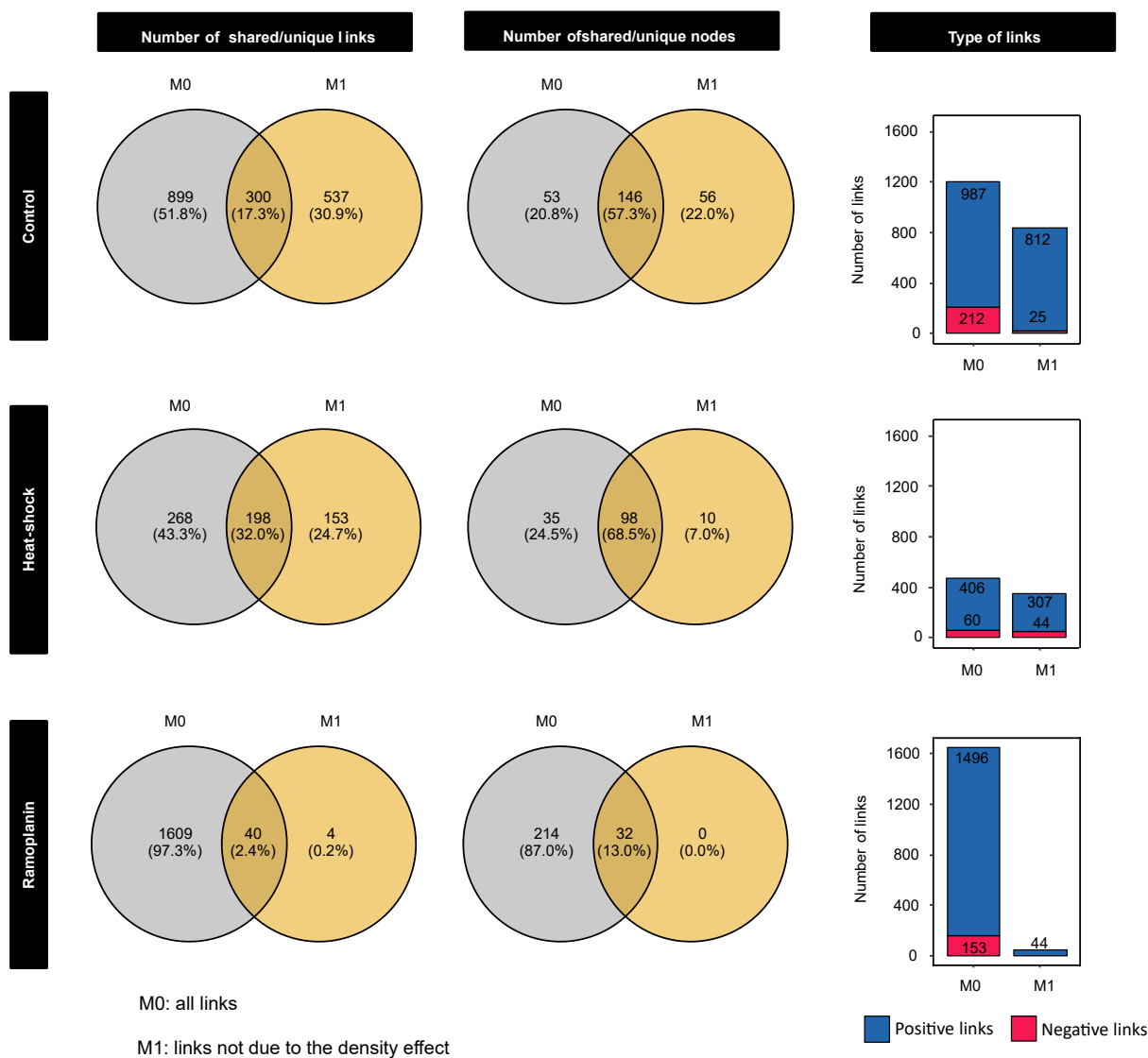


Figure 4.4: Effects of the physical distancing approach on the microbial co-occurrence networks. The Venn diagrams show the number of shared/unique links or nodes between co-occurrence networks inferred without covariate (M0) or with the initial physical distance as covariate (M1) to estimate links not due to the density treatment. For each network model and community, the number of positive (blue) and negative (red) links are represented. Links represent partial correlations ρ and they are colored blue if $\rho > 0$ and red if $\rho < 0$.

Coalescence Outcomes Between Communities Assembled Under Different Initial Physical Distances. To determine the extent to which the initial gradient in physical distance selected OTUs with different competitive abilities, we focused on the C and HS communities as well as the d1 and d2 densities for performing a coalescence experiment. The same volumes of soil

colonized under the d1 and d2 initial densities were mixed together with two different volumes of sterile soil to again obtain microcosms with low (d1) or high (d2) physical distancing between cells (Figure 4.1b). As reference communities, we also used the soils colonized under the d1 and the d2 initial densities but mixed separately with sterile soil at low (d1) or high (d2) densities. After 90 days of soil recolonization, we quantified the outcome of community coalescence by comparing similarities between each reference community and the coalesced community for both densities using weighted Unifrac distances (Supplementary Figure C.6 and Supplementary Table C.3). For the C communities, we found that the coalesced communities (Cd1+Cd2) were more similar to the Cd2 than to the Cd1 reference community whatever the density (Figure 4.5a). This coalescence asymmetry was confirmed by a higher proportion of OTUs originating from the Cd2 reference community in the coalesced community at both densities (Figure 4.5b). Differential abundance analysis between coalesced and reference communities also showed that the percentage of OTUs with significantly affected relative abundances in the C community was higher when comparing the coalesced community Cd1+Cd2 to the reference community Cd1 (30.90% and 23.43% in d1 and d2, respectively; post-hoc Tukey p -value ≤ 0.05) than to the reference community Cd2 (13.93% and 12.92% in d1 and d2, respectively; post-hoc Tukey p -value ≤ 0.05) whatever the density (Supplementary Table C.4). In contrast, mixing HS communities that had colonized the soil under the d1 and d2 densities resulted in coalesced communities that were equally similar to the HSd1 and HSd2 reference communities regardless of the density (Figure 4.5c). The similar percentage of OTUs shared between the reference and coalesced HS communities also indicated a symmetric coalescence with no predominant community (Figure 4.5d). No effect of the reference community was observed by differential abundance analysis on the outcome of coalescence events between the HS communities (13% and 15.17% of affected OTUs for HSd1 and HSd2, respectively in d1; 6.81% and 7.43% for HSd1 and HSd2, respectively in d2; post-hoc Tukey, p -value ≤ 0.05 ; Supplementary Table C.4). However, we found an effect of the physical distance on the outcome of coalescence events between the HSd1 and HSd2 communities only, with the coalesced community being more similar to the reference communities in d2 than in d1. This importance of physical distancing for the HS community during coalescence was supported by the differential abundance analysis showing that more OTUs were significantly affected at high (average of 14.08% in d1; post-hoc Tukey, p -value ≤ 0.05) than at low densities (7.12% in d2; post-hoc Tukey, p -value ≤ 0.05) whatever the reference community (Supplementary Table C.4).

Interestingly, the generalized linear mixed model also revealed non additive-effects with a few OTUs exhibiting significantly higher or lower relative abundances in the coalesced communities than in both reference communities (Supplementary Figure C.7 and Supplementary Figure C.8). Thus, among the 343 OTUs that exhibited significant different relative abundances in the coalesced compared to reference communities, we found 30 OTUs showing either synergistic or antagonistic effects in the coalesced community at high cell density (d1) and only 9 OTUs at low cell density (d2).

4.4 Discussion

Although mathematical models have shown that the relative fitness of individuals strongly depends on the density of surrounding neighbors [140], [257], little is known about how biotic interactions are influenced by physical distance between cells in complex environments and their role in microbial community assembly. Here, using removal treatments [188], we generated three microbial inocula differing in community membership that were then subjected to a physical distancing approach in order to assess to what extent microbial communities are shaped by biotic interactions between neighboring cells. Inoculation of the same microbial pools in microcosms containing different volumes of the same sterilized soil but with the same soil depth and humidity allowed us to control for potential confounding abiotic factors that

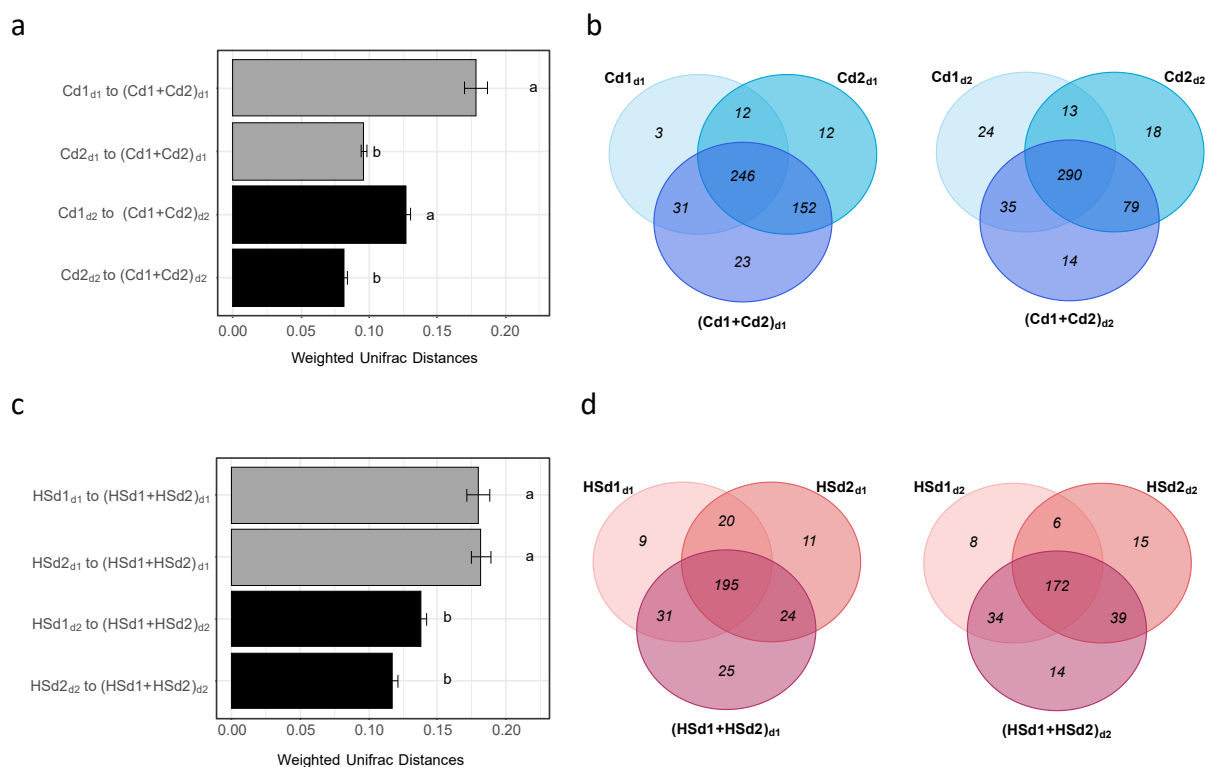


Figure 4.5: Differences in bacterial community structure and composition between coalesced and reference communities during the Step 2 experiment. Weighted UniFrac distances between coalesced and reference communities are represented for the control (a) and heat-shock (c) (mean \pm s.e.). Different letters above the bars indicate significant differences according to Tukey's test (p -value ≤ 0.05). The Venn diagrams show the number of shared/unique OTUs between the coalesced and reference communities for the control (b) and heat-shock (d) at low (d1) and high densities (d2).

could interfere with the assessment of the effects of spatial distancing.

Manipulation of the initial physical distances between bacterial cells successfully highlighted the importance of biotic interactions for bacterial community assembly with at least 41% of the most abundant OTUs being affected by cell density. Thus, physical distancing modified the outcome of soil colonization with significant differences both in community diversity and composition that were related to the initial densities. We found a lower bacterial diversity when the initial physical distance was the highest (d1), which is in agreement with the classical competition theory predicting that more competitive environments are less species-rich [259]. The identification by differential abundance analysis of a higher percentage of most abundant OTUs with decreasing (28%) than increasing relative abundance (14%) at high cell density suggests that reducing the physical distance during soil recolonization increased negative rather than positive interactions. Although cooperation is thought to be a common interaction between species [106], our results support previous studies based on experimental approaches highlighting the importance of competitive interactions in shaping microbial communities [59], [60], [66]. The effect of spatial distancing was not linear but threshold based with a stronger effect in d1 versus the other densities and to a lesser extent, in d2 versus d4. This could be explained by the ability of bacteria to detect local cell density through quorum sensing, which can for example repress bacterial competition systems including secretion systems until a threshold density has been reached. It has also been showed that at lower densities, bacteria have more opportunities during range expansion to form established clonal patches, which are more protected from competitors [92], [260]. Inferring microbial networks with and without the initial density gradient as covariate allowed us to identify the

links that were explained by physical distancing. In overall, the higher proportion of negative links explained by the initial gradient in the inferred microbial co-occurrence networks further supported that competitive interactions were dominating over mutualistic interactions at the boundaries between neighboring patches of different species. Among the co-occurrence networks, we found that Clostridia were often involved in negative interactions with Proteobacteria and Bacilli that were specifically related to physical distancing. This is consistent with previous findings reporting that, in soil, members of Clostridia could produce antimicrobial compounds which negatively affected the growth of species belonging to *Pseudomonas* and *Bacillus* [261].

The generation of three different microbial communities using removal treatments allowed us to characterize to which extent the effect of physical distancing was dependent on community composition. Specifically, we found a higher percentage of OTUs with decreasing fitness in the C and RA communities compared to the HS community, which was concomitant with a higher number of negative links related to the physical distancing in C and RA bacterial networks. Inferring network with or without the initial physical distance as a qualitative covariate also revealed that RA community network was the most responsive to the initial neighboring cell density. Ramoplanin is an antibiotic with bactericidal activity against Gram-positive bacteria while many secretion systems involving cell contact or cell-cell communication through quorum sensing and having a pivotal role in bacterial competition were described only in Gram-negative bacteria [262]. Exposure to ramoplanin may therefore have led to an enrichment of Gram-negative bacteria for which biotic interactions are cell-density dependent. Taken together, our results suggests that physical distancing could differentially modulate competitiveness between surrounding species depending on community membership.

To further explore how physical distancing affects interactions within microbial communities, we used a coalescence experiment based on the mixing of communities that assembled at high (d1) and low (d2) cell densities. We found that coalescence events resulted in distinct patterns for C and HS communities with the physical distance being more important for the assembly of the HS coalesced communities while the source community was more important for that of the C coalesced communities. While we hypothesized that increasing spatial distance will favor poor competitors, the asymmetric outcome of coalescence between C coalesced communities assembled at high and low densities was due to the fact that the Cd2 source community was dominant over the Cd1 community. This scenario can be explained with the findings of Lechón-Alonso *et al.* [246] who showed that the less competitive parent communities can dominate after coalescence when they are more cooperative because of their superior ability to deplete resources. Conversely, we found a symmetric coalescence for the HS communities indicating that the HSd1 and HSd2 communities were equally competitive, which suggests that physical distancing during Step 1 experiment had little effect on their competitiveness. This is supported by the much weaker effect of physical distancing on the HS than on the C communities during the Step 1 experiment with about 36% and 69% of significantly affected OTUs between d1 and d2, respectively. This lack of “home field advantage” during coalescence with the communities selected at the d2 density being equally or more competitive even in the d1 microcosms suggests that the observed differences in competitiveness were due to biotic rather than abiotic filtering which occurred during the first step of the physical distancing approach.

Using the coalescence approach, we also identified OTUs with significantly higher or lower relative abundances in the coalesced communities compared to the reference communities. These antagonistic and synergistic effects resulting from the mixing of partly different communities could be due to shifts in the initial abundance of the interacting cells in the coalesced communities [179]. Alternatively, the introduction during the coalescence of new species present only in one of the parent communities may have modified the existing interaction

in the other parent community. Accordingly, the importance of such higher-order interactions is increasingly recognized in microbial community assembly [184]. Interestingly, we found that these antagonistic and synergistic interactions also occur more often under low than high physical distancing, which further supports the importance of neighboring cell density for biotic interaction frequency.

In summary, by experimentally manipulating the physical distance between neighboring cells, our study showed the importance of biotic interactions in microbial community assembly. However, the differential effect of spatial distancing observed between the generated inocula suggests that community membership either modulates the importance of biotic interactions in community assembly or the extent to which biotic interactions are dependent on neighboring cell density. Further studies are therefore needed to resolve microbe-microbe interactions at the community level, which is crucial for steering microbial communities in the environment.

5

Through three top-down native community manipulation experiments, we studied interactions occurring between soil microorganisms and assessed their contribution to community assembly. The general purpose of this general discussion chapter is to provide an overall synthesis of our results and to discuss the potential of our experimental approaches to decipher biotic interactions determining soil microbial community assembly and to steer microbial communities.

5.1 Biotic Interactions in Soil and Their Role in the Community Assembly

5.1.1 Biotic Interactions Steer Community Assembly

In the three community manipulation experiments performed, we estimated that between 15, 22 and 28% of the most abundant bacterial OTUs were subjected to negative interactions during community assembly, in Chapter II, III, and IV, respectively. In a previous work using community manipulation with the same soil than in Chapter IV, 39% of the most abundant OTUs were subjected to competitive interactions during soil recolonization [188]. Our results extend previous evidence of the generic occurrence of negative interactions within microbial communities obtained through bottom-up approaches [59], [60], [181]. In these studies, comparison of monoculture versus coculture of two strains selected among up to 72 bacterial species revealed a highly variable proportion of inhibitions of one strain on another, ranging from 3.4% to 63%. The use of top-down community manipulation could help better assess interactions within complex microbial communities and, moreover, their contribution to the assembly of the entire community, which is not possible with pairwise coculture.

In the three chapters, we found recurrent negative correlations between the relative abundance of species belonging to Proteobacteria and Firmicutes. More precisely, we found negative correlations between γ -Proteobacteria and Bacilli, between α -Proteobacteria and Bacilli, and between Clostridia and γ -Proteobacteria, in Chapters II, III, and IV, respectively. These recurrent patterns have also been observed in two different soils [186] and in the same soil as Chapter IV in another study [188]. A negative correlation was even found as a checkerboard patterns in the distribution of Firmicutes and γ -Proteobacteria throughout France [263] and in other habitats such as in plant phyllosphere [232] or the human gut microbiota [248], [249]. Some studies strongly suggest that members of these two phyla could be engaged in both interference and exploitative competition. For instance, in the human gut microbiota, it is now recognized that the decrease in Firmicutes is correlated with an increase in Proteobacteria, such dysbiosis being associated with inflammatory bowel disease [248], [249]. In the leaf microbiota, phyllosphere strains belonging to Firmicutes were strongly inhibited and outcom-

peted by Proteobacteria strains in both in vitro and in vivo pairwise competition assays [232]. Besides, Proteobacteria have a greater potential than Firmicute to utilize manure-derived carbohydrates in the soil, illustrating exploitative competition [264]. Even without identifying the exact underlying mechanisms, our results are strongly suggesting that competition between Firmicutes and Proteobacteria may be an overall rule of microbial community assembly.

5.1.2 Biotic Interactions are Influenced by Community Properties and Physical Distance Between Cells

In Chapters II and IV, our coalescence approach revealed that between 8 and 10% of the most abundant OTUs exhibited significantly higher or lower relative abundances in the coalesced communities than in both source communities, revealing non-additive effects during coalescence. In Chapter II, coalescence was performed by mixing the control and manipulated communities. Therefore, no new species was introduced during coalescence since all species were present in the control community. Thus, the non-additive effects observed here were likely not due to new higher-order interactions [75], [230] but could be explained by differences in the relative abundances of interacting OTUs between control and manipulated communities, revealing density-dependent interactions [179]. Density dependence was reported as a key feature characterising interspecific interactions [231], and pairwise competition experiments demonstrated that species interactions can be influenced by initial abundances of microbial species [179]. Here, we further suggest that changing the relative abundance of the interacting taxa not only impacts an important fraction of the observed interactions in a complex community but also affects the outcome of coalescence. In contrast, in Chapter IV, coalescence was performed by mixing communities that could harbour different species, allowing the introduction of species present in only one of the source communities. The encounter of species present in only one of the two source communities may have triggered higher-order interactions by modifying existing interactions in each source community [184]. Interestingly, these non-additive effects occurred more often under high density than low density, suggesting also density-dependent mechanisms. Thus, in both chapters, the non-additive effects further supported the importance of density-dependent interactions in microbial communities and affected OTUs mostly belonged to Bacteroidetes, α - and γ -Proteobacteria.

In Chapter III, we manipulated the diversity, composition, and density of a native community to assess the relative importance of these community properties on biotic interactions and on the outcome of coalescence between native and manipulated communities. We found that the properties of the manipulated community affected OTU differently depending on their taxonomical affiliation, with α -Proteobacteria being mainly affected by the composition of the manipulated community, while Bacilli were mainly affected by density. Moreover, while previous studies showed that the diversity of a community is correlated with its capacity to invade and change the composition of another one [186], [195], [243], we nuanced these results. Indeed, we found that the diversity of the coalesced communities was mainly related to that of the source communities, but that its structure was mostly influenced by the composition and density of the manipulated communities. Together, this suggests that community properties will affect biotic interactions during a coalescence event, thereby impacting the coalesced community assembly.

In Chapter IV, we manipulated the physical distance between cells to identify interactions and assess their contribution to community assembly during soil colonization. Manipulation of the physical distance affected on average 55% of the bacterial community with significant differences between the communities assembled at different initial densities. Furthermore, the number of interactions decreased in a threshold-dependent manner with increasing physical distance between cells. As bacteria often interact at a distance ranging from 1-20 μm [137]–[139], increasing the physical distance between cells could keep more bacteria out of reach, thus decreasing the number of interactions. Indeed, the number of cell-to-cell interactions in-

creases with cell density [265] and, for AHL-mediated communication, the maximum effective calling distance was less than $37\ \mu\text{m}$ from the focal cell with the highest intensity around 4 to $5\ \mu\text{m}$ [133], suggesting that closer cells experience stronger AHL-mediated interaction. Thus, reducing the physical distance between cells could result in an increase of both the number and strength of interactions. However, the spatial distribution of individuals could be as decisive as the overall density to trigger biotic interactions, especially in a complex environment such as soil [146], [265], [266]. Indeed, soil is an heterogeneous habitat organized of patchy and piled-up arrangements of mineral-associated organic matter entrapped in micro-aggregates which compartmentalize soil and create a variety of micro-habitats [146], [267], [268]. Thus, in a heterogeneous structured habitat such as soil, cell aggregates can be compartmentalized and exhibit a clustered spatial distribution, preventing their potential encounter and, thereby, the interactions between them. Increasing the density in a spatially structured environment could increase the probability of cell encounter and interaction less than in a homogeneous environment (e.g., well-mixed liquid broth).

5.1.3 Limits of Our Approach

In our approach, we hypothesized that interactions would be enhanced when microbes undergo an exponential growth phase, such as range expansion [20], [186]. Thereby we purposefully use a sterile soil with non-limited nutrients and plenty of empty niches to inoculate microbial communities at a density lower than the soil carrying capacity, allowing them to experience a range expansion phase during the recolonization of their native, but sterilized soil. However, we could have overestimated the number of taxa involved in interactions compared to what occurs in natural communities. Indeed, most of natural community members are inactive and/or dormant microorganisms [269], and thus, do not interact with other microorganisms. When the amount of available nutrients increases, such as when we inoculate a microbial suspension into a sterilized soil microcosm, inactive microorganisms become active [269], and thus, could start interacting with other community members. Furthermore, range expansion promotes the intermixing of primary and secondary populations (i.e., fast and slow growers, respectively) by dividing cell patches into small sub-populations, increasing interactions between them (Figure 5.1) [270]. Range expansion could also have changed the balance between the interaction types, which determine community assembly [76]. Indeed, range expansion promotes cooperation by increasing the number of cooperative individuals among a patch and by decreasing the impact of cheaters among this cooperative population [271]. In addition, inoculating the microbial community at a density lower than the carrying capacity could have changed the prevalence of the competition strategy. While interference competition dominates at high density, competition for space is prevalent in lower initial density [272]. This relates to the competition-colonization trade-off, a theory suggesting that while some individuals are better competitors, others are better colonizers [273], [274]: range expansion at low density would favour colonizers over competitors. To restrain range expansion, future studies could assess the relative importance of community properties for steering a microbial community *in situ*. To that purpose, manipulated communities could be inoculated into microcosm containing soil with an already established community, such as a native one, in contrast with Chapter III in which we inoculated both manipulated and native community at the same time into a sterile soil microcosm.

Microbial communities are not static systems, but dynamic ones that reflect the temporal scale of the interactions occurring between their members [275]. For example, predation and parasitism are defined as interactions that have a positive impact on the predator and a negative impact on the prey. But this effect on abundance is observable only in a tiny momentum of the Lotka-Volterra dynamic of the interaction (the purple momentum in Figure 5.2), while other momentum displays very different patterns on the abundances of both partners. Competition and cooperation are also subjected to such temporal dynamics [83], [276]. For

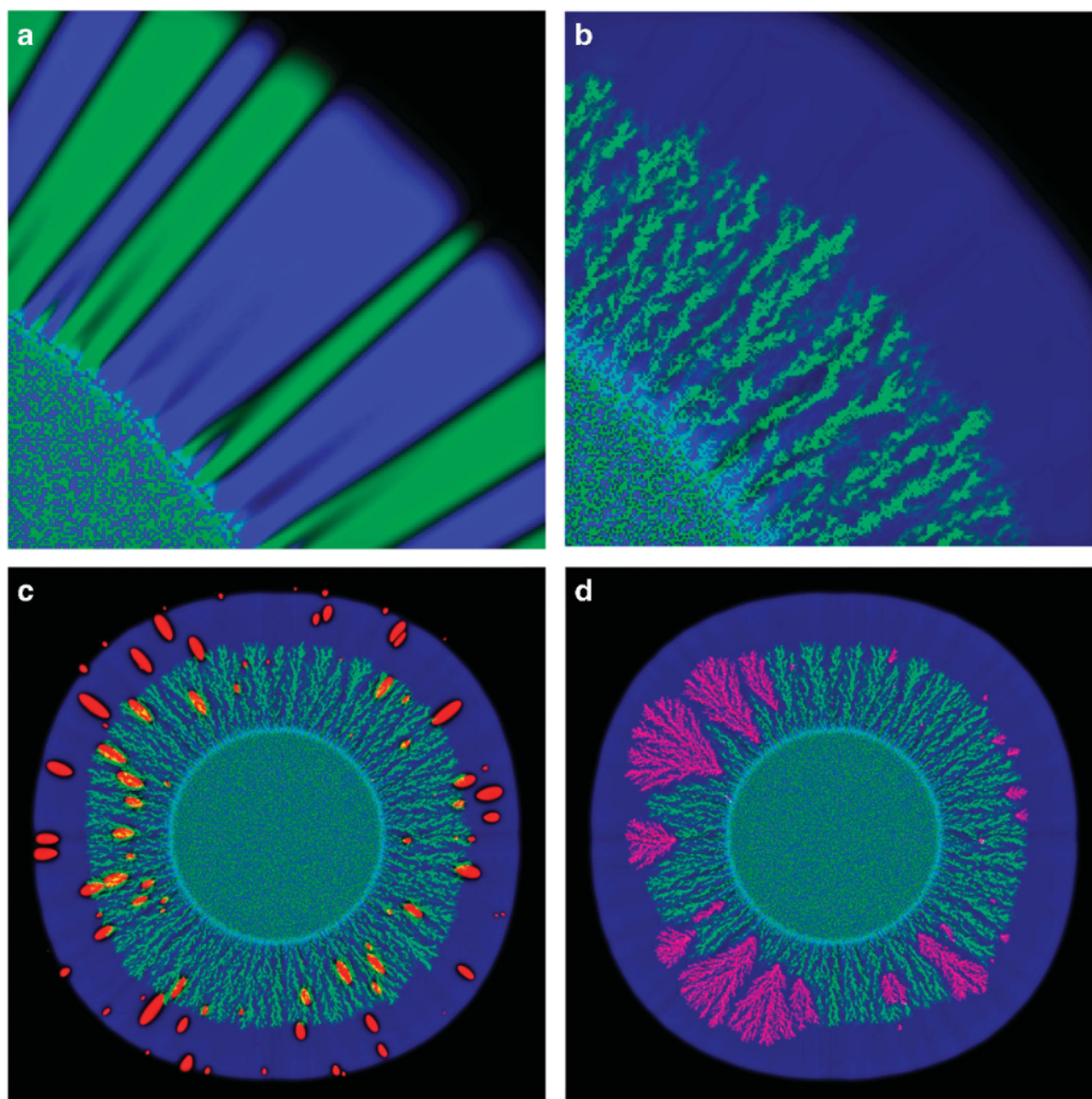


Figure 5.1: Modeling results and predictions of primary and secondary expansions. (a) Two completely degrading populations rapidly segregated into sectors. (b) The secondary expansion of the consumer produced fractal dendrites (magnifications of sections of the modeled circular colonies are shown). In the second row the model output with mutants that have a 1.4 times higher maximum growth rate than the ancestor. Blue is the primary and green the secondary population. In panel (c), the red areas indicate mutants in the primary population. It can be seen that they form patches that go extinct relatively rapidly. In panel (d), the mutants (also red) are introduced in the secondary population. In contrast to the mutants in the primary population in panel (c), they can take over dendrites and establish locally. From Goldschmidt *et al.* (2017) [270]

instance, in exploitative competition with competitors consuming the same limiting resource, the resource availability will decrease over time, affecting both competitor abundance (Figure 5.3) [277]. Here, we sampled communities at a single time point, at the end of each incubation, therefore overlooking the dynamics of biotic interactions during and after soil recolonization.

To decipher interactions occurring during the community assembly, we did not measure interactions directly but rather used OTUs relative abundance. To assess the proportion of OTUs involved in biotic interactions, we estimated shifts in the relative abundance of OTUs

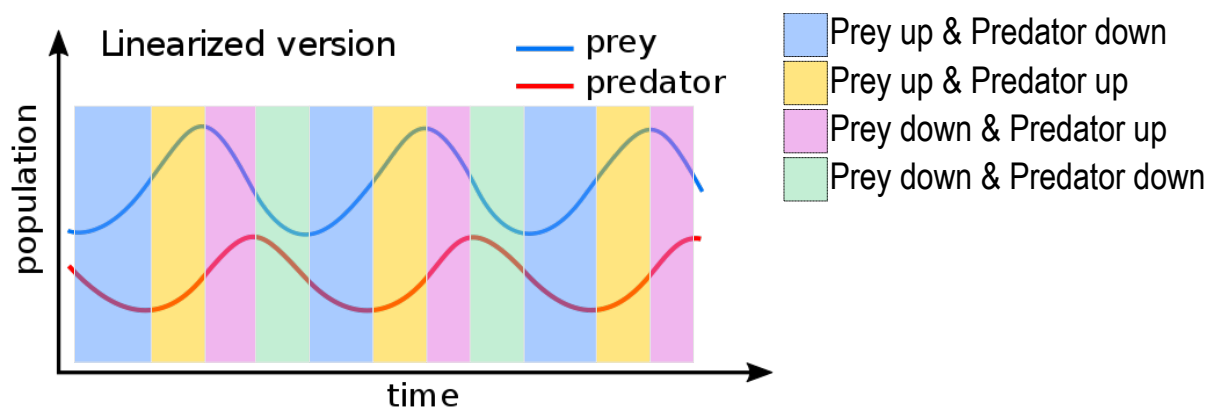


Figure 5.2: Schematic illustration of different momenta across a linearized solution of the non-linear Lotka-Volterra equations.

as a proxy of the effect of the interaction on their relative fitness. Then, to identify OTUs that may interact, we inferred co-occurrence networks. However, inferring ecological interactions based on OTUs abundance still presents several well-known and long debated pitfalls [83], among which two I would like to emphasize here. First, we cannot conclude on true direct ecological interactions between two taxa from a co-occurrence correlation. For instance, a correlation between two taxa can reflect that they are both affected by a third species, but without directly interacting. Secondly, correlations based on co-occurrence patterns do not provide any insight into potential interactions' underlying mechanisms. However, in Chapter II, we could establish a causative relationship between the depletion of some taxa and the increased relative fitness of others, by performing a targeted coalescence experiment to reunite potentially interacting OTUs. For understanding the underlying interactions, future studies could include metatranscriptomics approaches or RT-qPCR to relate shifts in gene expression to shifts in the OTUs abundances. For example, many bacteria use Type VI secretion system to directly inject toxins into their competitors. This injection triggers the expression of several genes in the targeted cells [278]. The expression level of these genes could be quantified through metatranscriptomics approaches or RT-qPCR, allowing to assess if an increase in OTUs negatively affected in their abundance is correlated with an increase of the expression of such genes. Monitoring microbial traits involved in interactions alongside assessing taxonomic group abundance could help decipher the “who and how” of biotic interactions in complex communities [190].

Finally, we estimated the biotic interactions based on the abundance of only the most abundant OTUs (i.e., OTUs with a relative abundance higher than 0.5% in any sample), representing ca. 10% of the total number of OTUs. We thus completely overlooked what happened for the rare OTUs. However, we cannot include such rare taxa in the network inference model due to their sparse counts [83]. The sparsity of rare OTUs also implies that their counts are uncertain, as they may be in the limit of the sequencing detection ability in samples [279]. Literature tells us that dominant OTUs are more affected by abiotic environment than rare OTUs [250] while rare OTUs could be more constrained by biotic interactions [190]. Taxa may become and remain rare under the influence of deterministic processes [280], such as competitive exclusion by dominant taxa [281]. Also, in Chapter III, no dominant taxa (i.e., the ten most abundant OTUs) but only subdominant taxa were detected as involved in the co-occurrence network. Thus, by overlooking rare OTUs in the three chapters, we may have underestimated the proportion of the community subjected to biotic interactions during the community assembly.

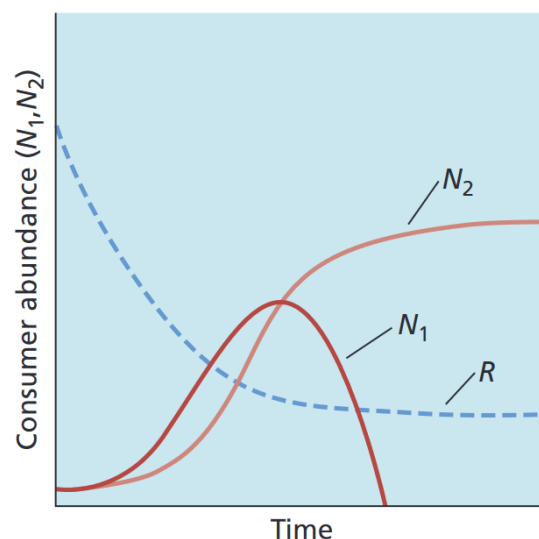


Figure 5.3: Change in consumer and resource abundances over time. Consumer species N_1 and N_2 and resource availability (R). Note that consumer species N_2 can maintain its population on a lower resource level than can consumer species N_1 , thus consumer species N_1 is driven extinct by competition with consumer species N_2 . From Mittelbach & McGill (2019) [277]

5.2 Using Community Manipulation as a Tool

5.2.1 A Tool to Decipher Biotic Interactions

Historically, biotic interactions have been deciphered through empirical observation of natural communities, allowing the detection of significant patterns from which assembly rules were inferred, and later through bottom-up approaches, using coculture of a handful of strains. While these approaches laid the basis of modern microbial ecology, they present several pitfalls: (i) the correlation inferred from the observed co-occurrence networks needs experimental validation for an ecological interpretation [83], and (ii) while synthetic communities with a few strains can directly test interactions between them, they cannot decipher their role in the assembly and functioning of complex native microbial communities [191], [192].

In this thesis and other studies, manipulation of native microbial communities was used to obtain communities with hundreds of OTUs, representing most of the taxonomic groups present in their respective environment (Chapters II, III and IV, [186], [188]). We could thereby implement three hypothesis-driven experiments with highly complex communities, overcoming the limits highlighted above. By modulating diversity, composition, and density, we were able to identify the interactions occurring between the community members from which we could infer the competition between Proteobacteria and Firmicutes as a potential assembly rule. Future work could directly test this rule, for example, by inoculating a community enriched in Firmicutes with a community enriched in Proteobacteria. Combining such an approach with a microbial-trait approach based on other ‘Omics’ techniques could also help decipher the underlying mechanisms of biotic interactions [190]. Moreover, identifying biotic interactions in complex communities allowed us to assess their contribution to the community assembly.

While bottom-up approaches were mostly carried out with simple communities in simple environments, (e.g., liquid broth or agar plates), community manipulation allows to inoculate complex communities in a complex environment such as soil, getting closer to natural conditions. Using complex environment to decipher biotic interactions in complex communities is

of tremendous importance as the complexity and heterogeneity (e.g., spatial structure) of an environment can directly influence biotic interactions. For instance, changing carbon sources in an environment can drastically change interactions [60], [181]. Environments with high concentrations of available nutrients will lead to more negative interactions and reduce overall community diversity [58]. In contrast, the lack of some nutrients can trigger interactions, such as siderophore production triggered by an iron deficiency [282]. Future experiments could use community manipulation to assess the importance of environment complexity and heterogeneity on biotic interactions and the assembly of complex communities.

5.2.2 A Tool for Microbiome Engineering

One of the main goals of microbiome engineering is to steer microbial communities to promote the services they support or to restore communities after a disturbance [200], [251], [283]. Microbial consortia and microbiome-based solutions are already used to harness microbial communities in agriculture, food processes and even human health [284]. However, steering a resident microbial community by inoculating microbial strain or consortium *in situ* remains one of the main challenges facing microbiome engineering. In fact, inoculating microbes *in situ* has often inconsistent efficiency and reliability. One reason of this inconsistency is that the inoculated microorganisms fail to invade the resident communities, notably due to strong priority effects. Two different mechanisms explain priority effects: niche preemption and niche modification [131], [285]. In niche preemption, early-arriving species (e.g., members of a resident community) deplete resources, thereby preventing the establishment of late-arriving species (e.g., a microbial inoculum) or impeding their growth due to exploitative competition. On the other hand, niche modification can be negative or positive. Niche modification is also due to biotic interactions: early-arriving species modify the environment which changes the available niches and thereby either impedes or facilitates the establishment of late-arriving species [131], [285]. In niche inhibitory modification, an early-arriving species prevents the establishment of a late-arriving species. Early arriving species can secrete bacteriocins with antimicrobial activity in the environment, impeding the establishment of sensitive strains (i.e., interference competition) [285] or can produce acid that reduces pH (i.e., amensalism), preventing the colonization of more alkaliphile species. Conversely, a decrease in pH could be a niche facilitative modification for species that grow better in more acidic environment. In niche facilitative modification, an early-arriving species facilitates the establishment of a late-arriving species. The early-arriving species can break down large organic molecules into more easily accessible nutrients or produce extracellular polysaccharides that retain water (i.e., commensalism or cooperation) [285]. Thereby, as priority effects are due to biotic interactions between the resident-community members and the late-arriving species, biotic interactions could be used to overcome such priority effect and allow to steer microbial communities. For instance, introducing a strain that outcompetes the dominant strain of the resident community can free niches, allowing subdominant strain to access now vacant niches [286]. In contrast, late-arriving species could also establish syntrophic cooperation with some members of the native community, stimulating these indigenous organisms [287]. Priority effects can also be reduced by disturbances, as less diverse and disturbed communities are more prone to invasion than diverse and undisturbed communities [274]. In this thesis, we purposely inoculated native and manipulated communities at the same time to avoid such a priority effect, allowing identification of biotic interactions occurring between community members. Future work could use community manipulations to identify biotic interactions occurring within a native community and use these identified interactions to inoculate into the targeted environment a manipulated community whose members will interact with previously identified indigenous microorganisms to steer the community towards a desirable state.

The deliberate introduction of non-indigenous microorganisms into the environment raises ethical issues and can have potential side effects. For instance, non-native species can invade

the environment they were introduced in and may thereby threaten resident species to extinction and affect ecosystem services in unpredictable ways [288]. A set of practices has been suggested to prevent such side effects. One could be to use microorganisms native from the targeted environment, preventing invasions of non-endemic species [289]. In this thesis, we successfully shifted the assembly of native communities without adding new species, only using community manipulation to shift the biotic interactions that occur between members of the same native community. Thus, our work highlights the potential of top-down manipulation of native microbial community for obtaining simplified communities. Such manipulated communities could serve as microbial inoculants for steering microbial communities *in situ* without introducing non-endemic species into the targeted environment, by using the biotic interactions occurring between the native community members.

Community manipulation and, in particular, community coalescence represents a promising avenue to engineer microbial communities [200], [238], [251]. Recent studies even used coalescence to restore altered soil microbial communities. For instance, Wubs *et al.* [185] applied soil inocula from two different biomes (i.e., grassland and heathland) on an ex-arable land (i.e., a disturbed ecosystem), with or without removing the topsoil layer. They show that replacing the topsoil layer by a soil inoculum from another biome promoted the ecosystem restoration and even steered the plant community towards this other biome vegetation. This effect was lighter, but still significant, when the soil inoculum was spread in a thin layer over the untouched topsoil layer. In another work, Calderón *et al.* [186] generated communities altered in their diversity and functioning by using a removal-by-dilution approach. Then, they attempted to rescue soil diversity and functioning by re-inoculating diluted native microbial communities from the same soils into microcosms containing soil with the altered communities. They successfully overridden the priority effect of the resident altered communities, steering the community diversity and structure towards the newly introduced one, but only when the newly introduced community were obtained from a less-diluted suspension than the altered resident one and without successfully restoring the soil community functions. Here, in Chapter II, our coalescence approach restored -at least partly- both microbial community diversity and functions and even re-established most of the original interactions between the community members. Altogether, these results further support the use of microbial community coalescence to restore soil biodiversity and ecosystem functioning.

6

Conclusions & Perspectives

Microorganisms are the second major component of biomass on Earth after plants. They live in communities that not only drive major biogeochemical cycles, but also influence plant, animal, and human welfare. These communities are assembled through different processes commonly referred to as abiotic and biotic filters. In terrestrial ecosystems, an emphasis has been placed on the role of abiotic filters for understanding microbial community assembly. However, a growing body of evidence suggests that interactions between microorganisms could have a pervasive and important role in community assembly. The contribution of these interactions to the assembly of the microbial community and the factors influencing them have not been clearly established. Moreover, most attempts to identify and/or characterize biotic interactions between microorganisms have been based on coculture of a handful of strains, completely overlooking the high complexity of microbial communities in the environment.

In this thesis, our objectives were to decipher biotic interactions occurring between microbial community members and to assess the role of these interactions in the assembly of complex communities. To address these objectives, we used three top-down experiments based on the manipulation of soil microbial communities. In Chapter II, we used a two-step top-down approach combining removal manipulation (i.e., taxa depletion in the community) and coalescence (i.e., mixing manipulated and control communities). In Chapter III, we used community manipulation to modulate the diversity, composition, and density of a native soil microbial community and implement twelve different coalescence treatments by mixing manipulated and native bacterial communities. In Chapter IV, we used a removal approach to modulate community membership, and then we manipulated the physical distance between neighbouring cells by inoculating the manipulated communities into increasing volume of soil. Then, we performed a coalescence experiment by mixing microbial communities that assembled with different initial distances between cells to assess their relative competitiveness. In all our experiments, the microbial suspensions (or incubated soils in Chapters II and IV coalescence steps) were inoculated in microcosms containing their native, but sterilised, soil to allow them to reassemble during soil recolonisation.

Together, our results suggest that between 15% and 28% of the bacterial community are subjected to negative interactions during soil recolonization. We found a recurrent negative correlation between Proteobacteria and Firmicutes, suggesting that competition between these taxa could be a rule governing microbial community assembly. Our approach also uncovered the importance of density-dependent interactions in microbial community assembly. By manipulating the physical distance between microbial cells, we confirmed the importance of biotic interactions for microbial community assembly and showed that the competitiveness of individuals strongly depends on the density and identity of surrounding neighbours. Furthermore, we also investigated the role of biotic interactions in community assembly using coalescence. We showed that coalescence can re-establish impaired interactions between mi-

croorganisms by reuniting potentially interacting OTUs and can also restore -at least partly- community diversity and soil functions. We also untangled the respective effects of the properties of the source communities on the diversity and structure of the coalesced communities. We show that the diversity of the source communities is not the most important community property influencing a coalescence outcome but that the density and the composition of the source communities had the most impact on the structure of the coalesced communities.

Nonetheless, our approach may have enhanced the interactions occurring between community members compared to what occurs in an already established community. In fact, all our experiments were based on the inoculation of microbial suspension in microcosms containing sterile soil. Thus, the communities experienced a range expansion phase during soil recolonization. To address such pitfalls, future work could use community manipulation to inoculate microbial suspensions into microcosms containing already established communities, such as in an original soil. Furthermore, such experiment could aim to harness or restore microbial community functions in situ by combining community manipulation and microbial-trait approach. First, community manipulation could be used to generate different manipulated communities originating from the same native one with specific functional traits, that could serve as microbial inoculants. Then, community manipulation could be used to identify windows of opportunity which are momenta in the community dynamics when microbial inoculants would be the most efficient to steer the targeted native community and promote specific functional traits. Windows of opportunity can open after seasonal stress such as drought or at different stages of a crop life cycle. Microcosm as well as field experiment could be implemented to identify such momenta by inoculating microbial inoculants into an original soil containing a native community after different seasonal disturbance or at different stage of a crop life cycle. While such an experiment could produce actionable knowledge for agricultural systems, it could also provide insights into the relationship between community structure and function.

Altogether, we bring here additional evidence that community manipulation could become a standard tool to test ecological theories. Community manipulation with native communities allow the assessment of microbial interactions in complex communities, their contribution to community assembly, and the links between community structure and function. Furthermore, our work highlights the potential of using community manipulation to design new microbial engineering strategies allowing to steer microbial communities in situ. By using the interactions occurring between community members, these strategies could overrun priority effect of the resident communities without adding non-endemic species. Our work highlights the potential of community manipulations, especially coalescence, to restore soil biodiversity and ecosystem functioning and to harness microbial communities so that they provide the ecosystem services needed by humankind.

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A

Chapter 2 Supplementary Materials

Table A.1: Number of OTUs significantly differentially abundant among all treatments as estimated using a generalized linear mixed model, among the 515 most abundant 16S rRNA OTUs and the 439 most abundant 18S rRNA OTUs. (pages 26, 28, 32 and 33)

Comparison	16S OTU count		18S OTU count	
		<i>Total</i>		<i>Total</i>
R>C	245		90	
R<C	240		103	
<i>R vs C</i>		375		164
R>C \notin R+R>C+C	121		58	
R<C \notin R+R<C+C	107		31	
<i>R vs C</i> \notin R+R <i>vs</i> C+C		228		89
R>C \cap R+R>C+C	124		32	
R<C \cap R+R<C+C	133		72	
<i>R vs C</i> \cap R+R <i>vs</i> C+C		229		99
R>C \cap R+R>C+C \cap R+C=C+C	79		20	
R<C \cap R+R<C+C \cap R+C=C+C	113		61	
<i>R vs C</i> \cap R+R <i>vs</i> C+C \cap R+C=C+C		176		80
R+C>C+C \cap R+C>R+R	61		26	
R+C<C+C \cap R+C<R+R	16		22	
<i>R+C vs C+C</i> \cap <i>R+C vs R+R</i>		73		48

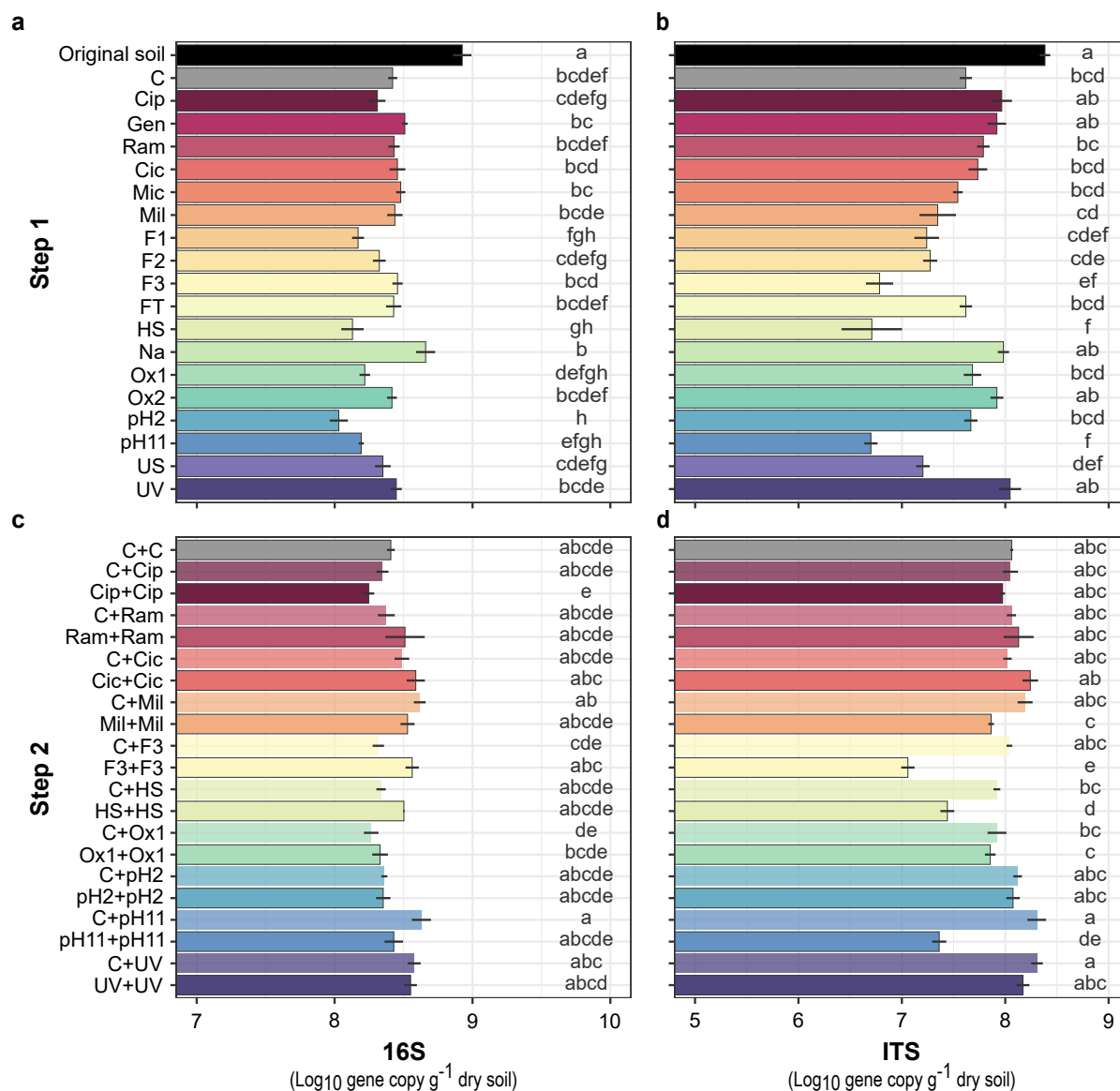


Figure A.1: Abundances of total bacteria and total fungi. Quantification of 16S rRNA (a and c) and ITS (b and d) gene copy numbers in the original soil, the removal treatments and the control (Step 1; a and b) and in the coalescence treatment, the self-mixed removal treatment and the control samples (Step 2; c and d) (mean ± s.e. of log-transformed data expressed as gene copy g⁻¹ dry soil). Letters indicate significantly different statistical groups (Tukey's test, p-value ≤ 0.05). (page 25)

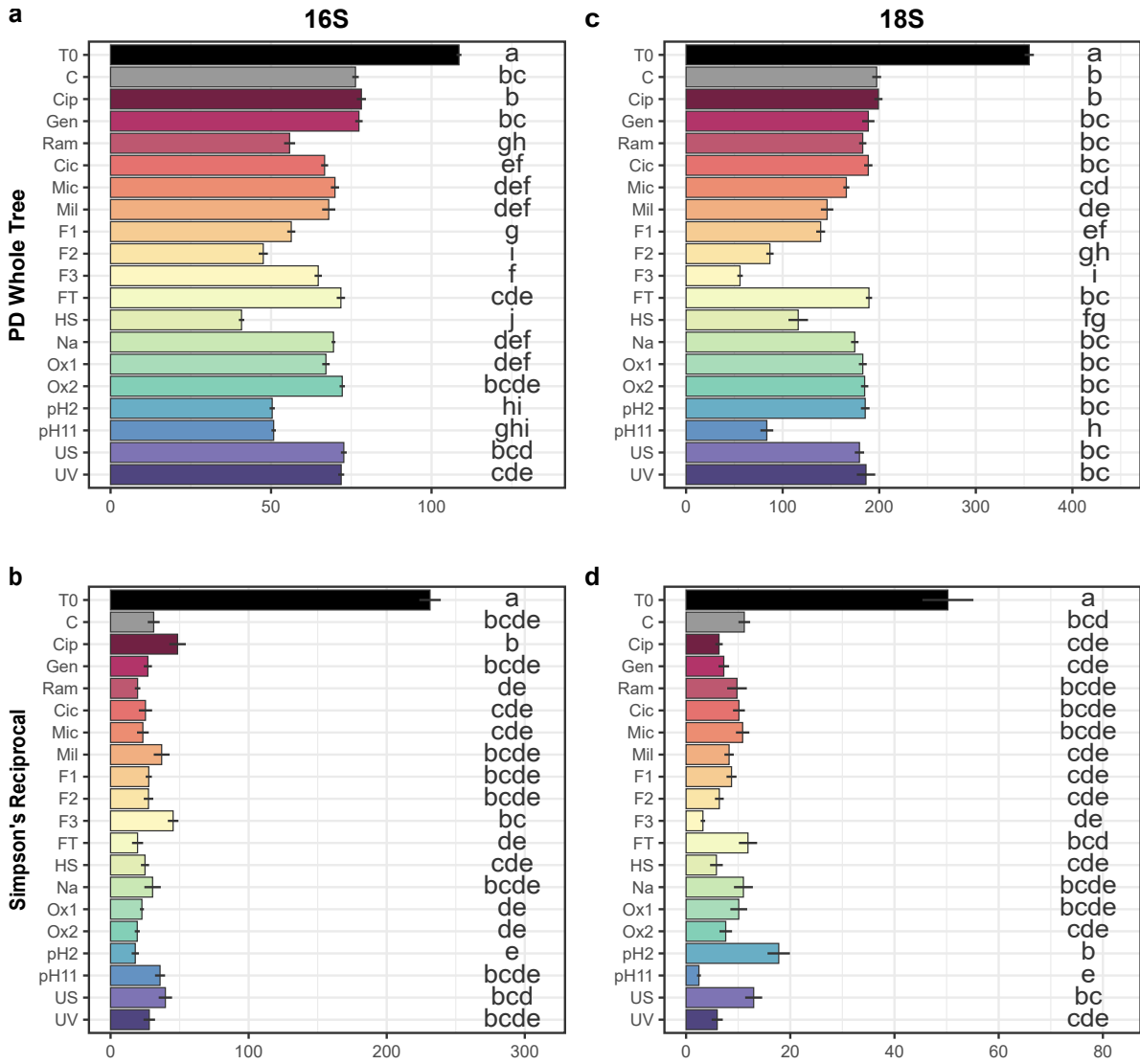


Figure A.2: Diversity of prokaryotic and eukaryotic communities after Step 1. The Faith's phylogenetic diversity (a and c) and Simpson's reciprocal (b and d) indices are shown (mean \pm s.e.) in the original soil, the removal treatments and the control (Step 1) for the 16S rRNA OTUs (a and b) and the 18S rRNA OTUs (c and d). Letters indicate significantly different statistical groups (Tukey's test, p-value \leq 0.05). (page 25)

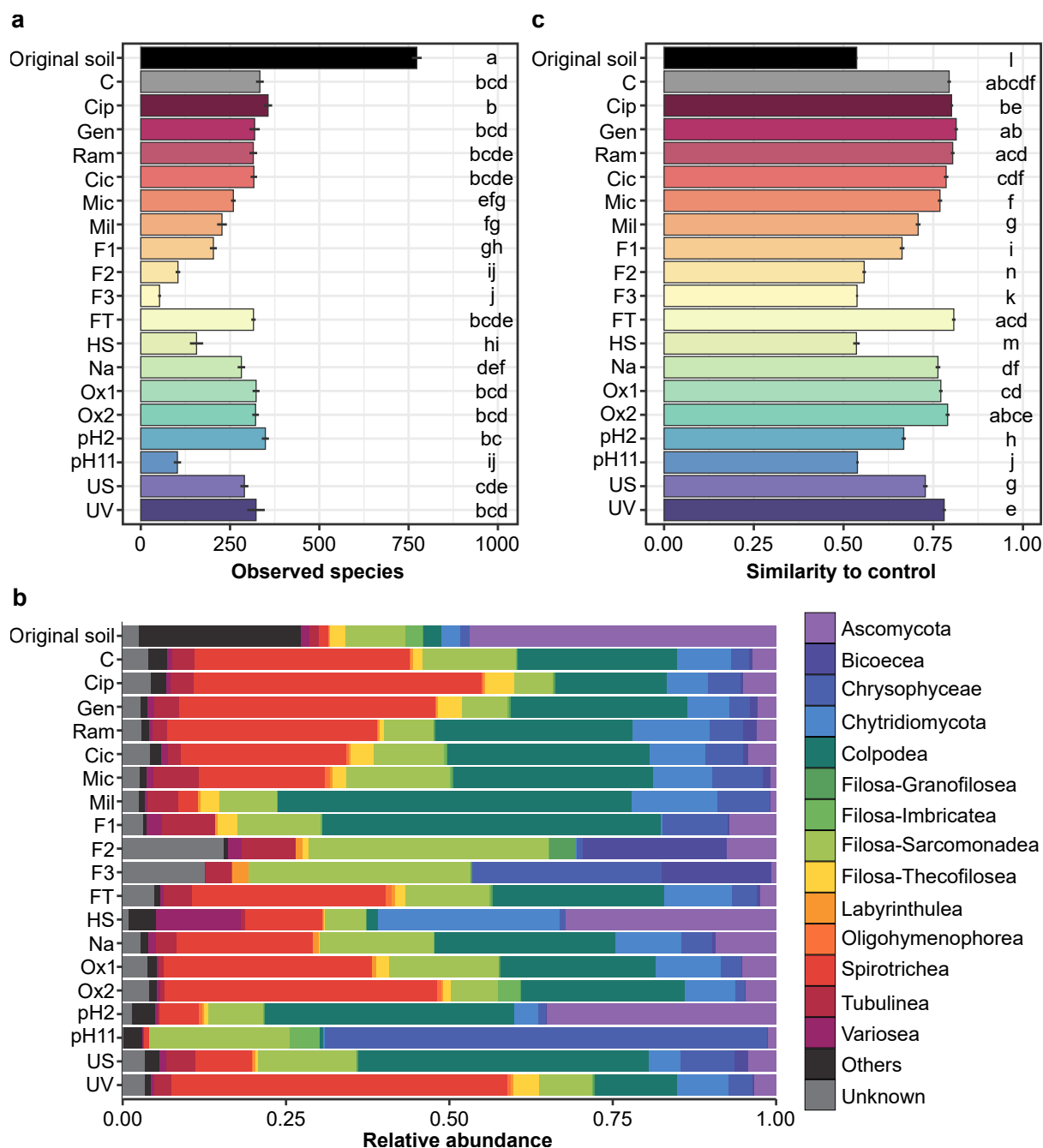


Figure A.3: Structure and composition of the eukaryotic communities in the original soil and after Step 1. (a) Number of observed species (mean ± s.e.). Letters indicate significantly different statistical groups (Tukey's test, p-value ≤ 0.05). (b) Relative abundances of the fourteen most abundant classes of eukaryotic community. (c) Similarity between the control samples and between the control and either the original soil or the removal treatments, based on the Weighted UniFrac distances (mean ± s.e.). Letters indicate significantly different statistical groups (Adonis pairwise comparison, Benjamini-Hochberg corrected p-value ≤ 0.05). (page 25)

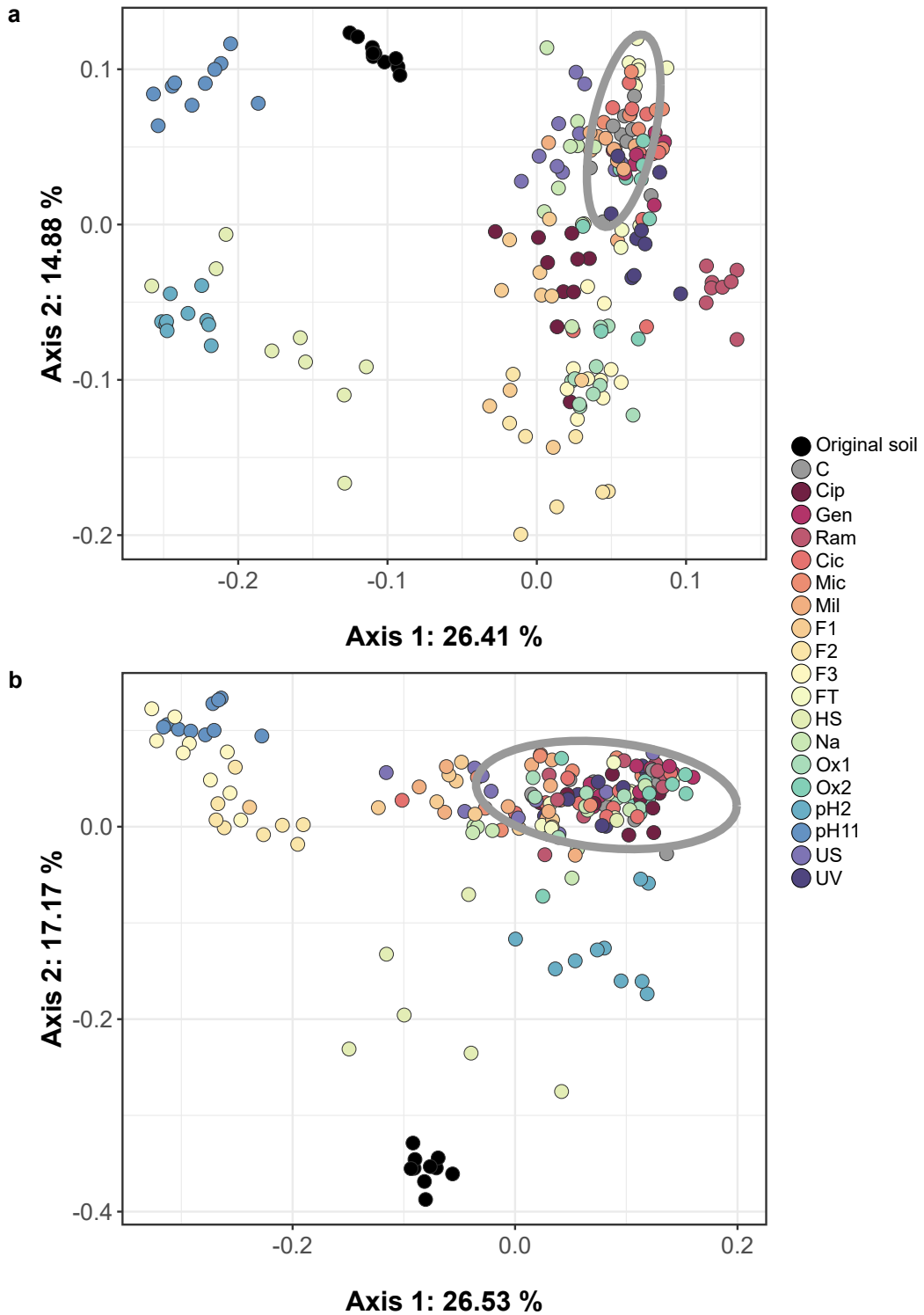


Figure A.4: Principal coordinate analysis (PCoA) of the communities in the original soil and after Step 1. PCoA of the prokaryotic (a) and eukaryotic (b) communities, based on the weighted UniFrac distance matrix showing the original soil, the removal treatment and the control samples and the 95 % joint confidence ellipse for the control samples. (page 25)

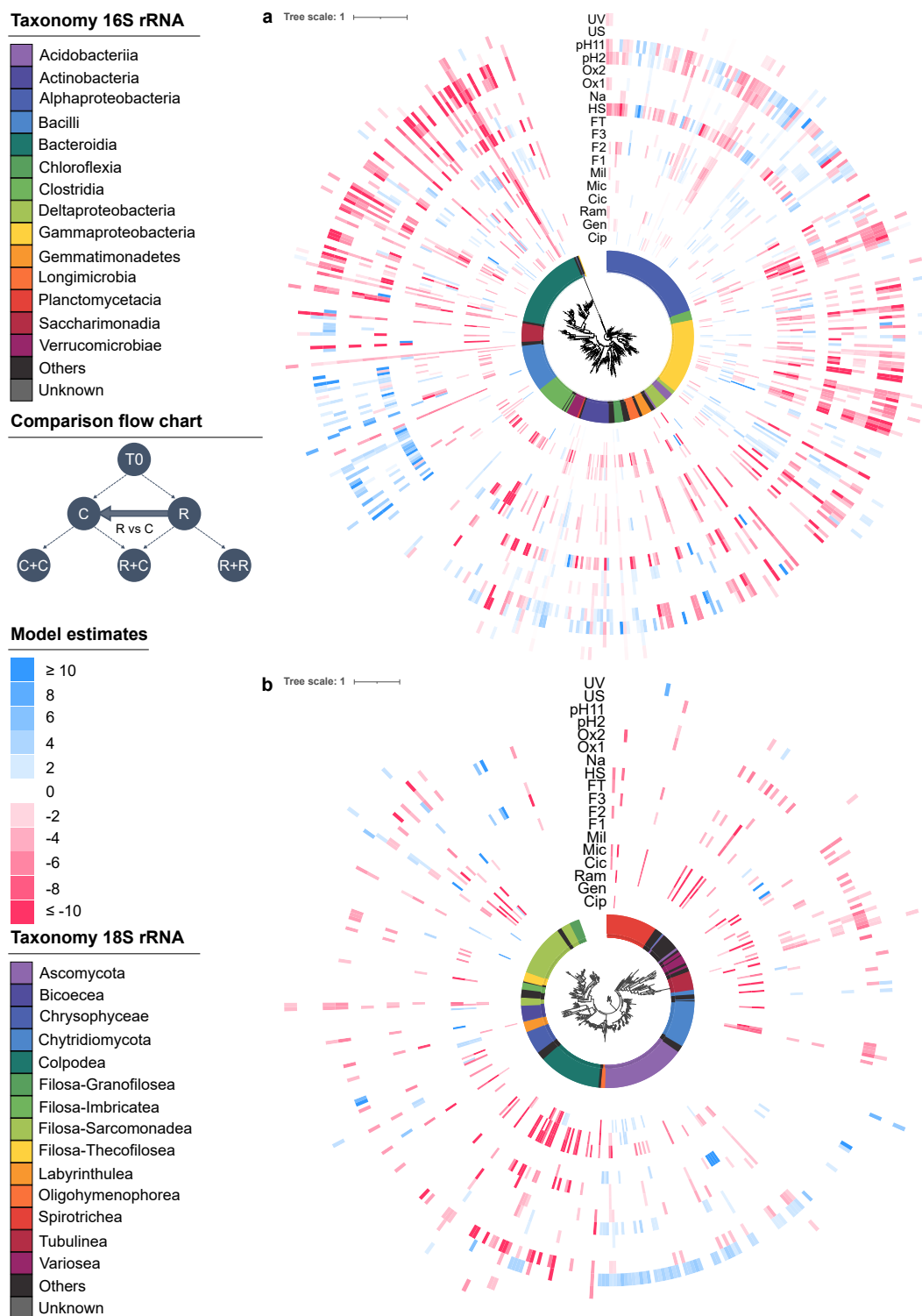


Figure A.5: Taxonomic relationships and distribution of OTUs significantly affected by the Step 1 removal treatments compared to the Step 1 control. The outer rings show the effect of each removal treatment on the relative abundances of the 515 most abundant 16S rRNA OTUs (a) and the 439 most abundant 18S rRNA OTUs (b) compared to the control (R vs C), as estimated using a generalized linear mixed model. The blue and red boxes in the outer rings indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment. The OTU class level is indicated by different colors on the innermost ring. (page 26)

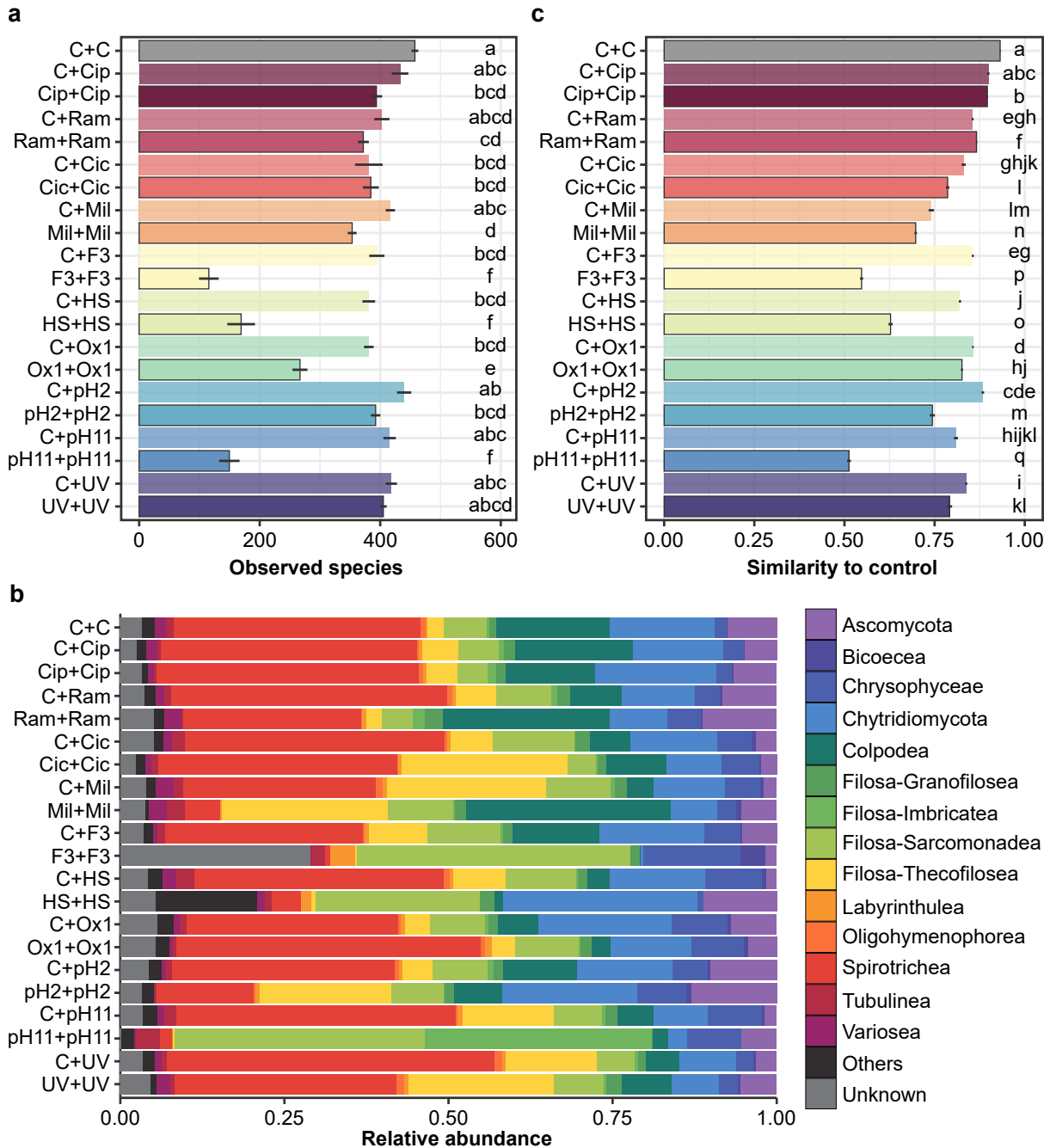


Figure A.6: Structure and composition of the eukaryotic communities after Step 2. (a) Number of observed species (mean ± s.e.). Letters indicate significantly different statistical groups (Tukey’s test, p-value ≤ 0.05). (b) Relative abundances of the fourteen most abundant class of eukaryotic community. (c) Similarity between the control samples and between the control and either the self-mixed removal treatment or the coalescence treatments based on the Weighted UniFrac distances (mean ± s.e.). Letters indicate significantly different statistical groups (Adonis pairwise comparison, Benjamini-Hochberg corrected p-value ≤ 0.05). (page 27)

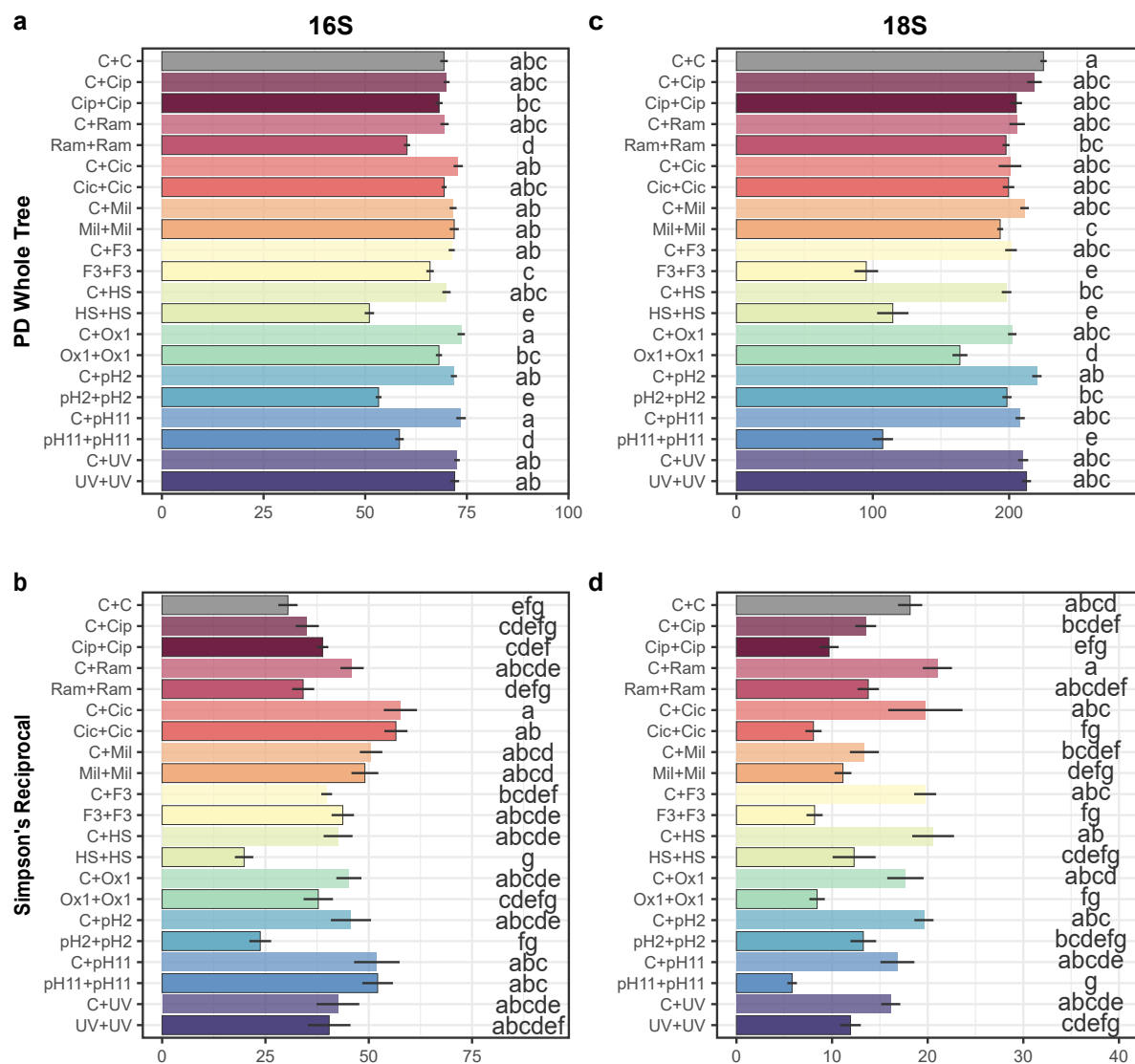


Figure A.7: Diversity levels of prokaryotic and eukaryotic communities after Step 2. The Faith's phylogenetic diversity (a and b) and Simpson's reciprocal (c and d) indices are shown (mean \pm s.e.) in the coalescence treatment, the self-mixed removal treatments and the control samples (Step 2) for the 16S rRNA OTUs (a and c) and the 18S rRNA OTUs (b and d). Letters indicate significantly different statistical groups (Tukey's test, p-value \leq 0.05). (page 27)

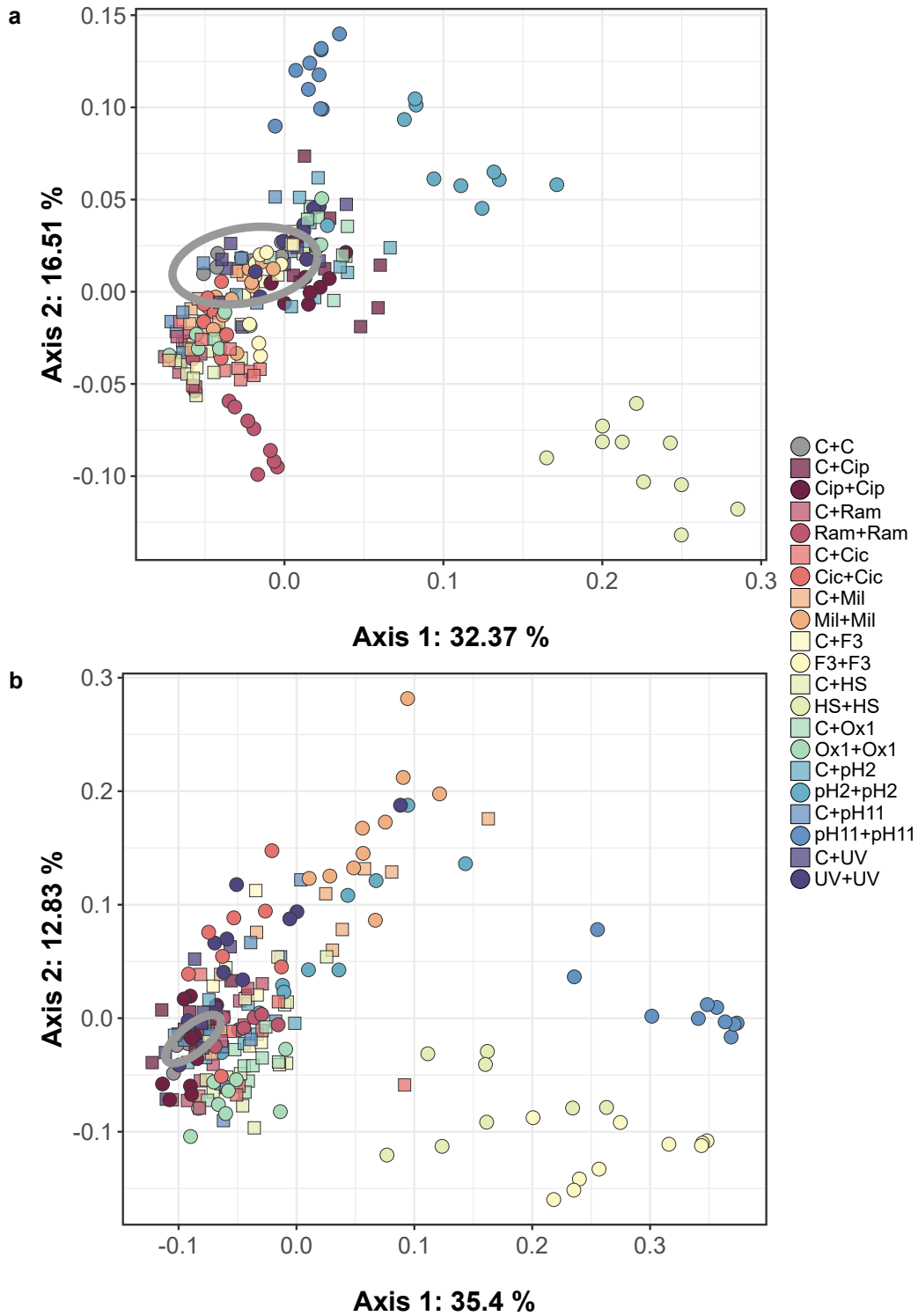


Figure A.8: Principal coordinate analysis (PCoA) of the communities after Step 2. PCoA of the prokaryotic (a) and eukaryotic (b) communities, based on the weighted UniFrac distance matrix showing the original soil, the removal treatment and the control samples and the 95 % joint confidence ellipse for the control samples. (page 28)

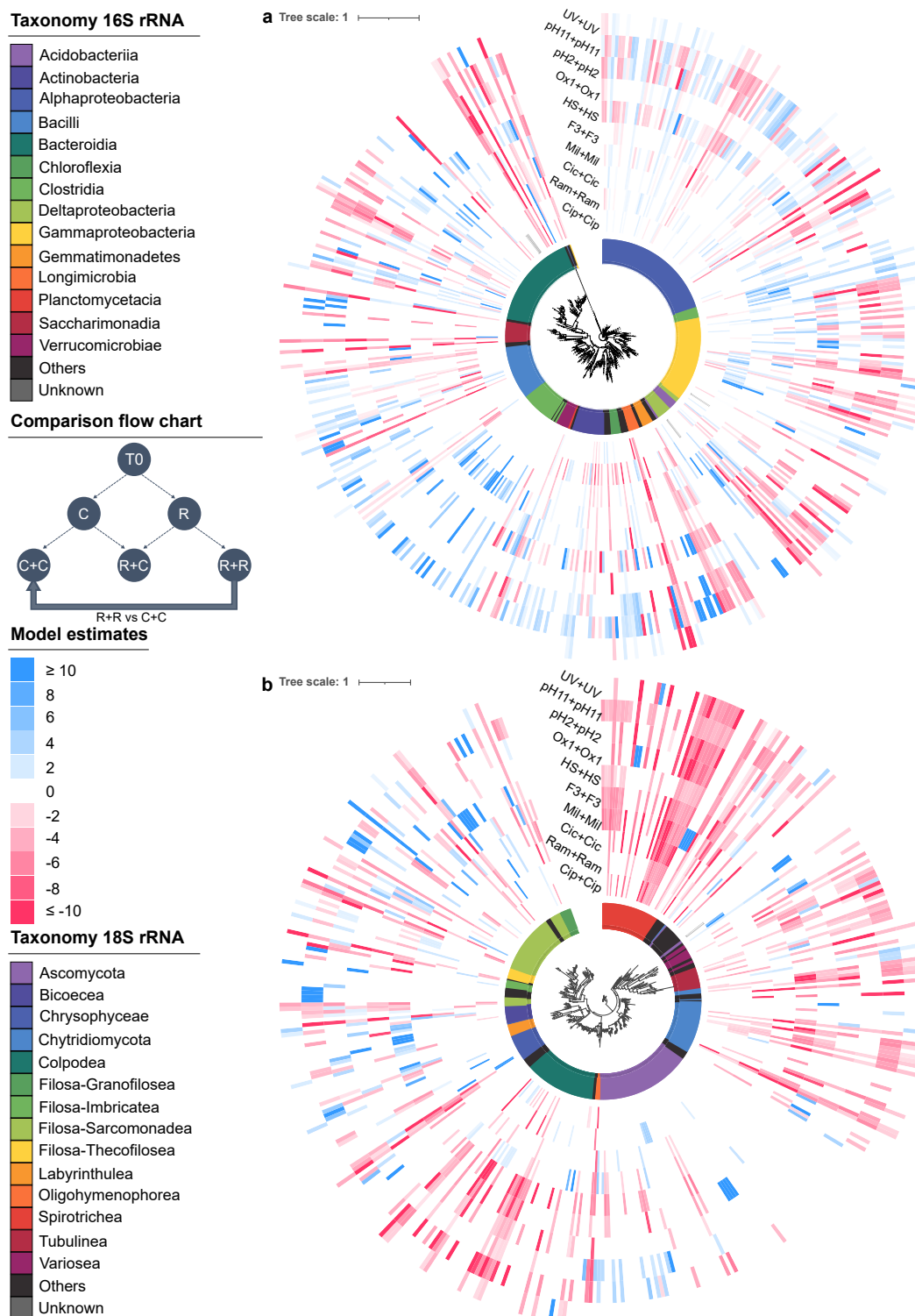


Figure A.9: Taxonomic relationships and distribution of OTUs significantly affected by the Step 2 removal treatments compared to the Step 2 control. The outer rings show the effect of each self-mixed removal treatment on the relative abundance of the 515 most abundant 16S rRNA OTUs (a) and the 439 most abundant 18S rRNA OTUs (b) compared to the self-mixed control (R+R vs C+C), as estimated using a generalized linear mixed model. The blue and red boxes in the outer rings indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment. The OTU class level is indicated by different colors on the innermost ring. (page 28)

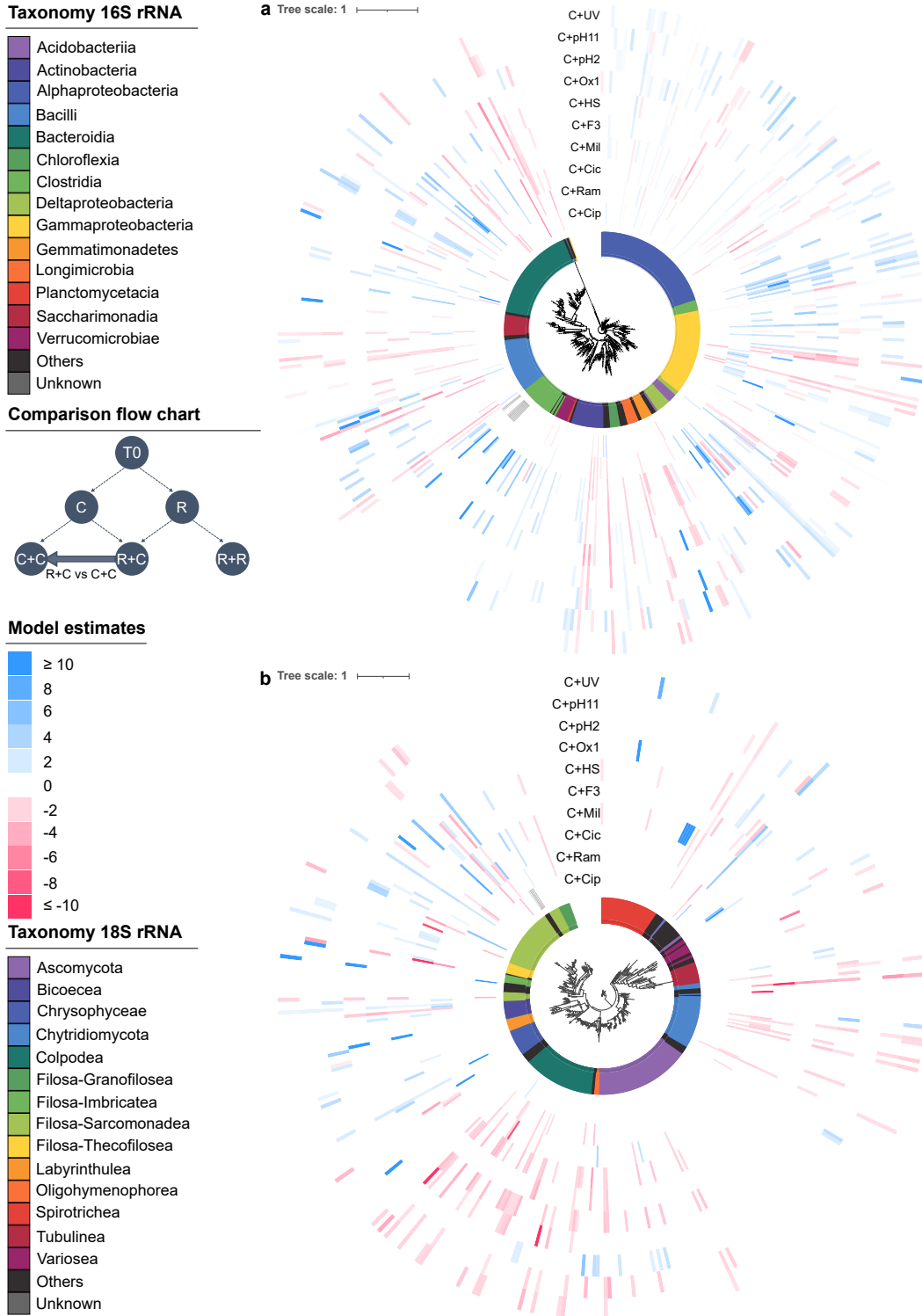


Figure A.10: Taxonomic relationships and distribution of OTUs significantly affected by the Step 2 coalescence treatments compared to the Step 2 control. The outer rings show the effect of each coalescence treatment on the relative abundance of the 515 most abundant 16S rRNA OTUs (a) and the 439 most abundant 18S rRNA OTUs (b) compared to the self-mixed control (R+C vs C+C), as estimated using a generalized linear mixed model. The blue and red boxes in the outer rings indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment. The OTU class level is indicated by different colors on the innermost ring. (page 28)

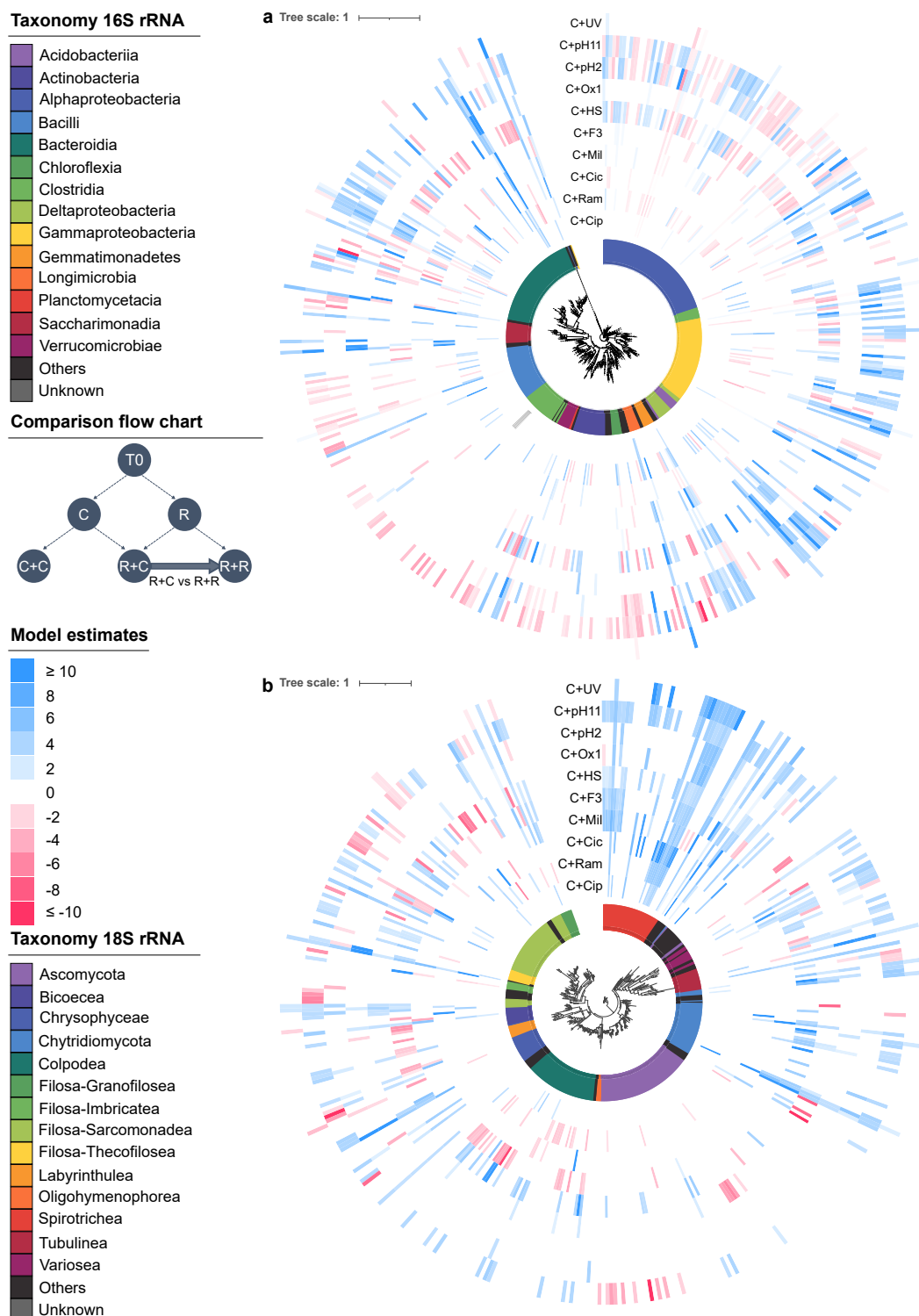


Figure A.11: Taxonomic relationships and distribution of OTUs significantly affected by the Step 2 coalescence treatments compared to the Step 2 removal treatments. The outer rings show the effect of each coalescence treatment on the relative abundance of the 515 most abundant 16S rRNA OTUs (a) and the 439 most abundant 18S rRNA OTUs (b) compared to its corresponding self-mixed removal treatment (R+C vs R+R), as estimated using a generalized linear mixed model. The blue and red boxes in the outer rings indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment. The OTU class level is indicated by different colors on the innermost ring. (page 28)

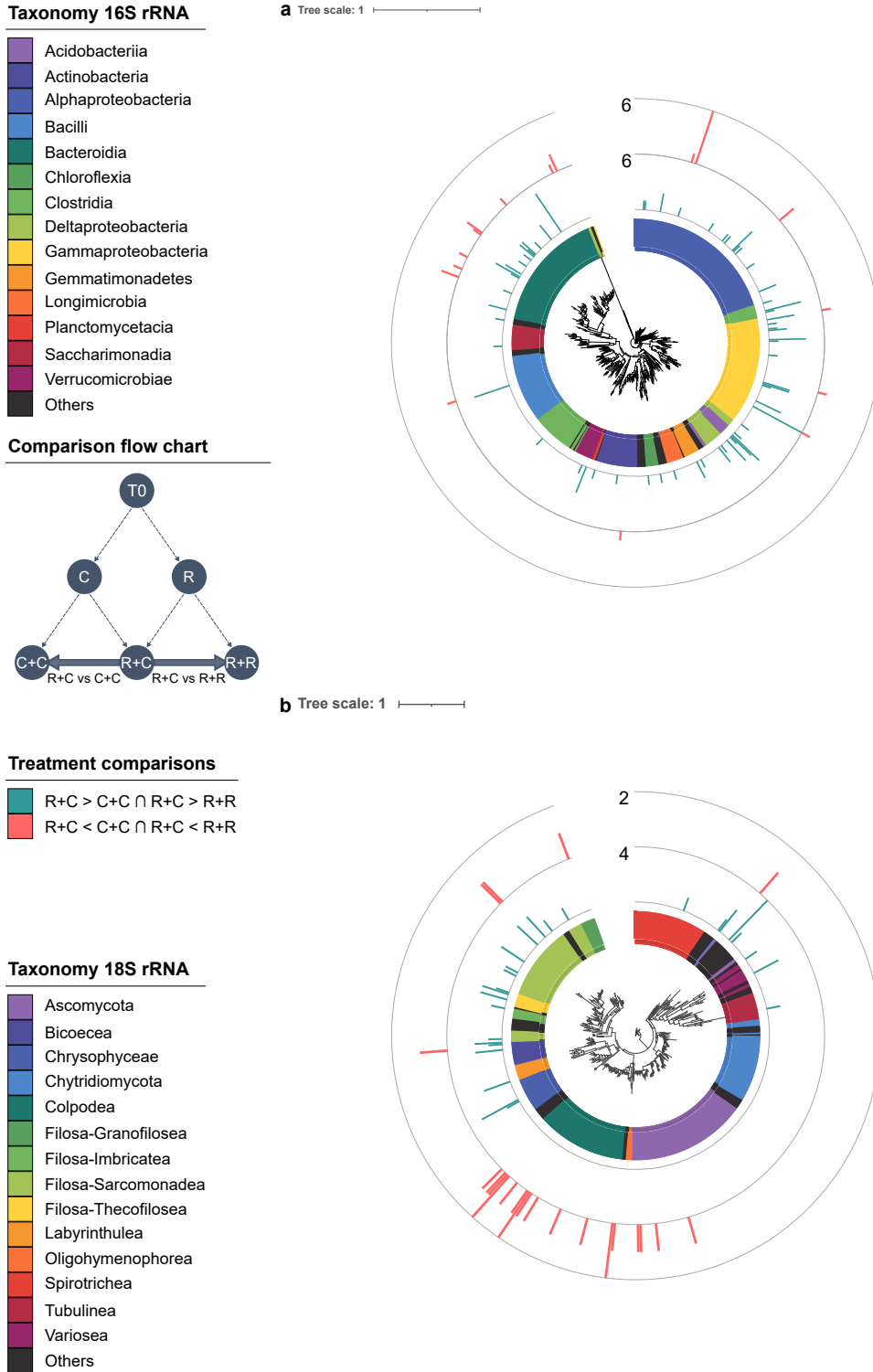


Figure A.12: Taxonomic relationships and distribution of significantly differentially abundant OTUs across treatments. Outer rings show prokaryotic (a) and eukaryotic (b) OTUs exhibiting relative abundances significantly higher or lower in the coalescence treatments than in the self-mixed source community separately ($R+C > C+C \cap R+C > R+R$ and $R+C < C+C \cap R+C < R+R$). Bar scale is proportional to the number of treatment where the OTU is significantly different, with a maximum indicated for each comparison ring. The OTU class is indicated by different colors on the innermost ring. (pages 28 and 33)

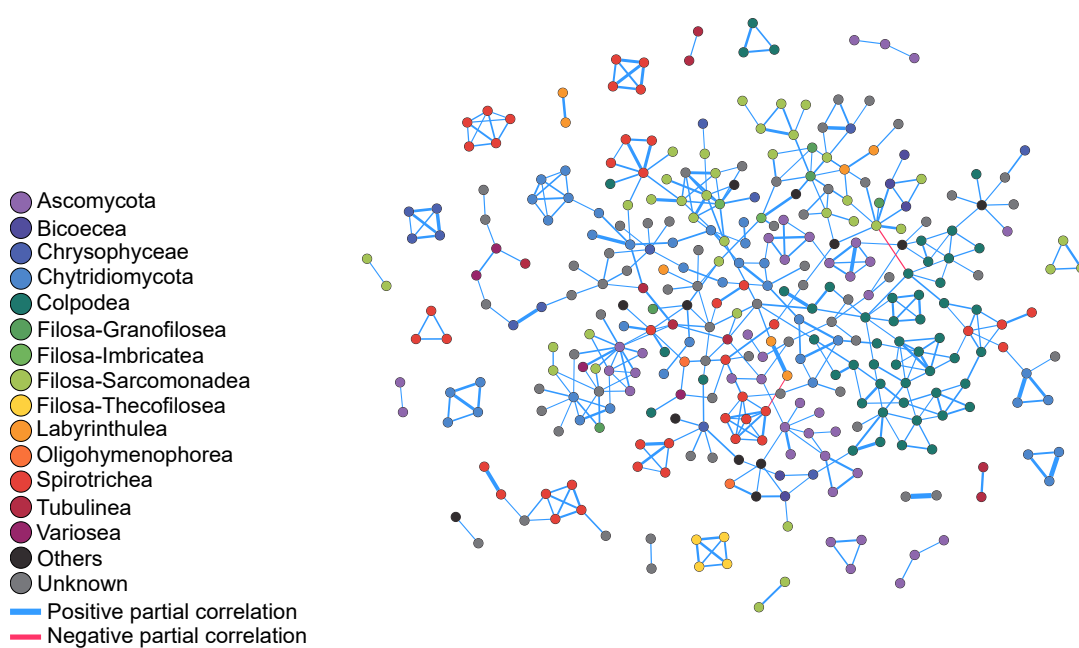


Figure A.13: Global eukaryotic network inferred from all samples across both experimental steps. Nodes represent OTUs and they are colored according to the OTU taxonomic class. Edges represent partial correlations ρ and they are colored blue if $\rho > 0$ and red if $\rho < 0$. Edge width is proportional to $|\rho|$. (page 28)

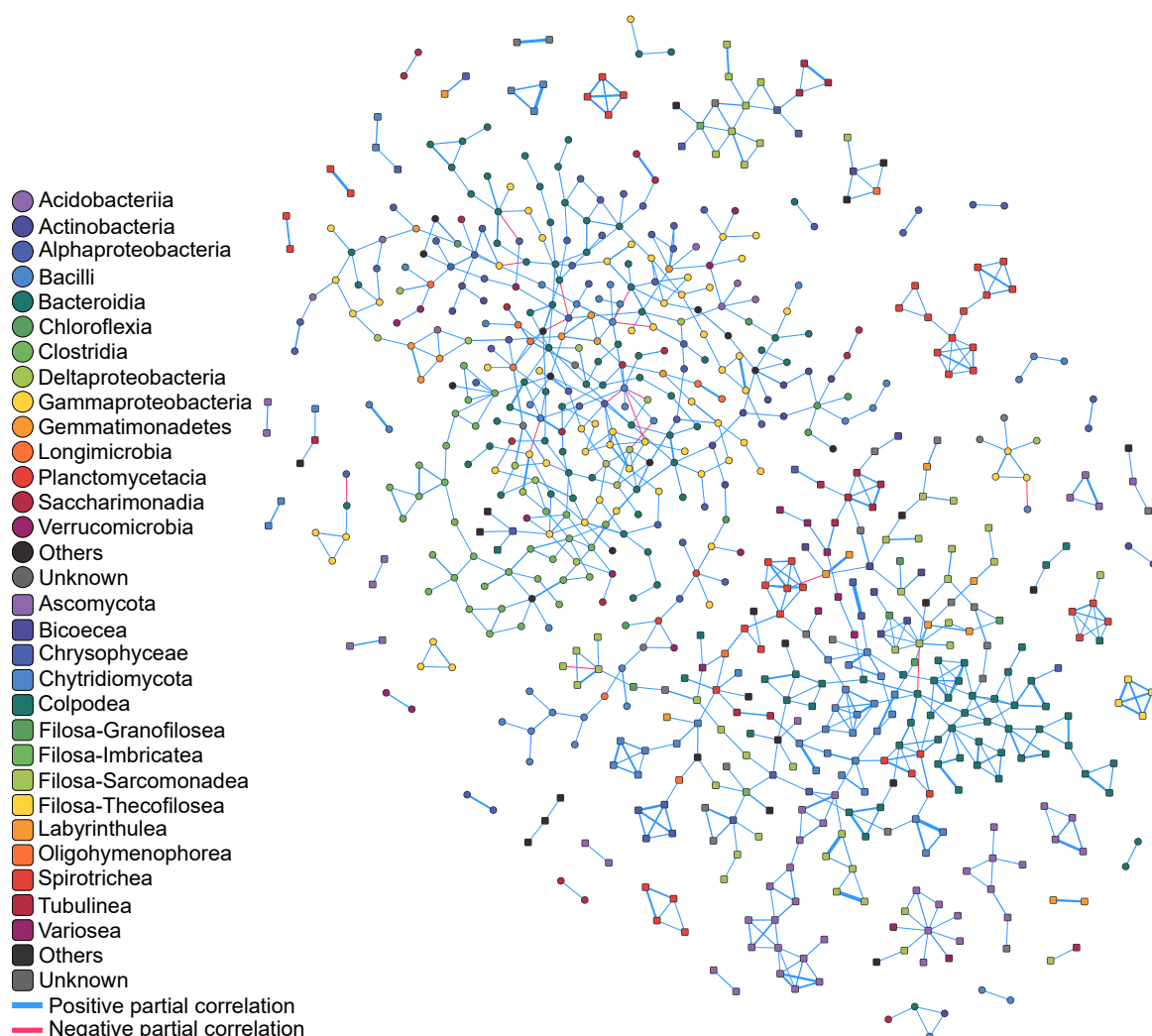


Figure A.14: Global inter-domain network inferred from all samples across both experimental steps. Nodes represent OTUs and they are colored according to the OTU taxonomic class. Edges represent partial correlations ρ and they are colored blue if $\rho > 0$ and red if $\rho < 0$. Edge width is proportional to $|\rho|$. (page 28)

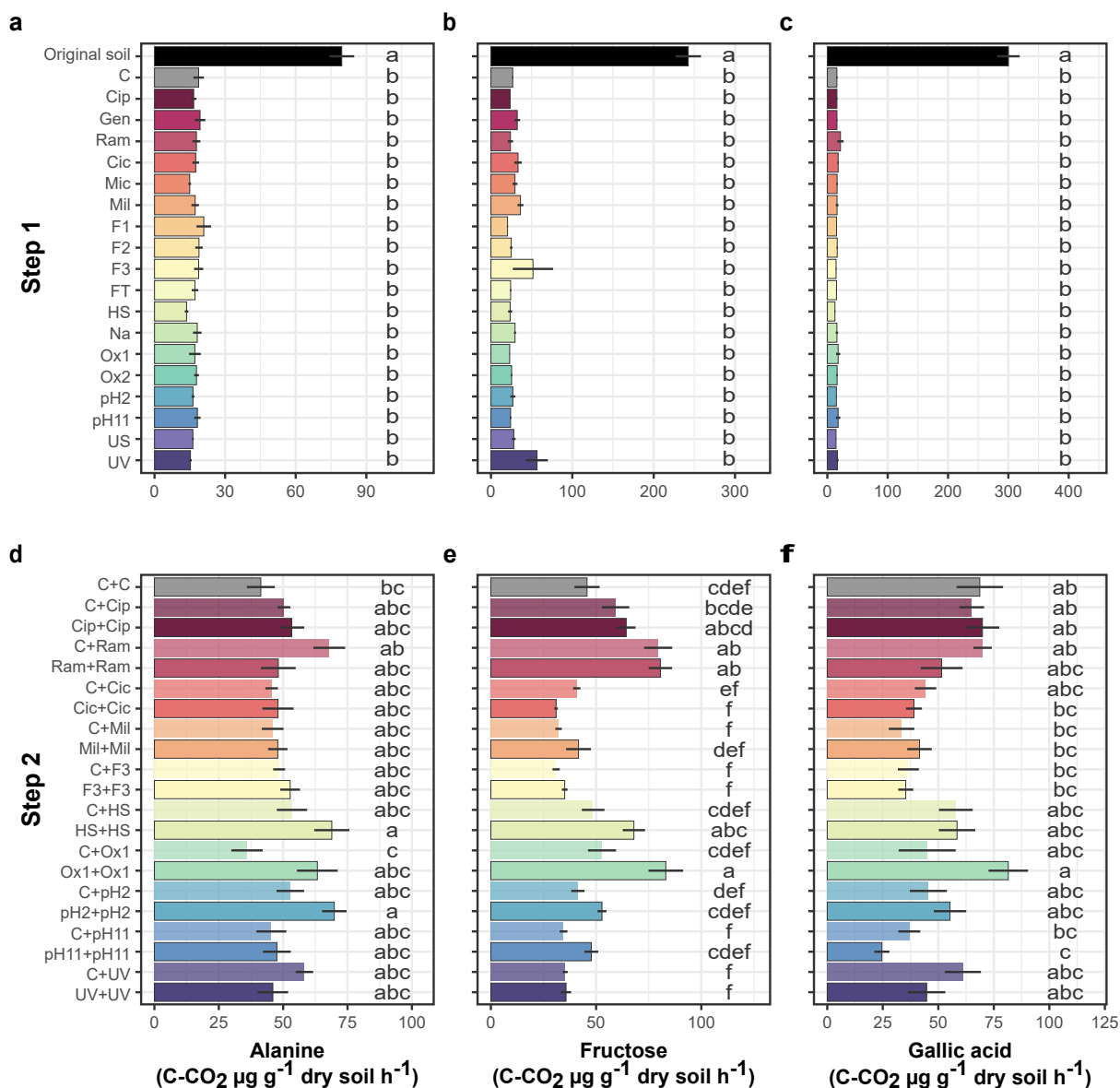


Figure A.15: Treatment-induced changes in soil respiration rate as proxies of C-cycling. Substrate-induced respiration was measured by the MicroResp™ method using the substrates alanine (a and d), fructose (b and e) and gallic acid (c and f) in the original soil, the removal treatments and the control (Step 1; a, b and c) or in the coalescence treatments and the self-mixed treatments (Step 2; d, e and f) (mean ± s.e.). Letters indicate significantly different statistical groups (Tukey's test, p-value ≤ 0.05). (page 30)

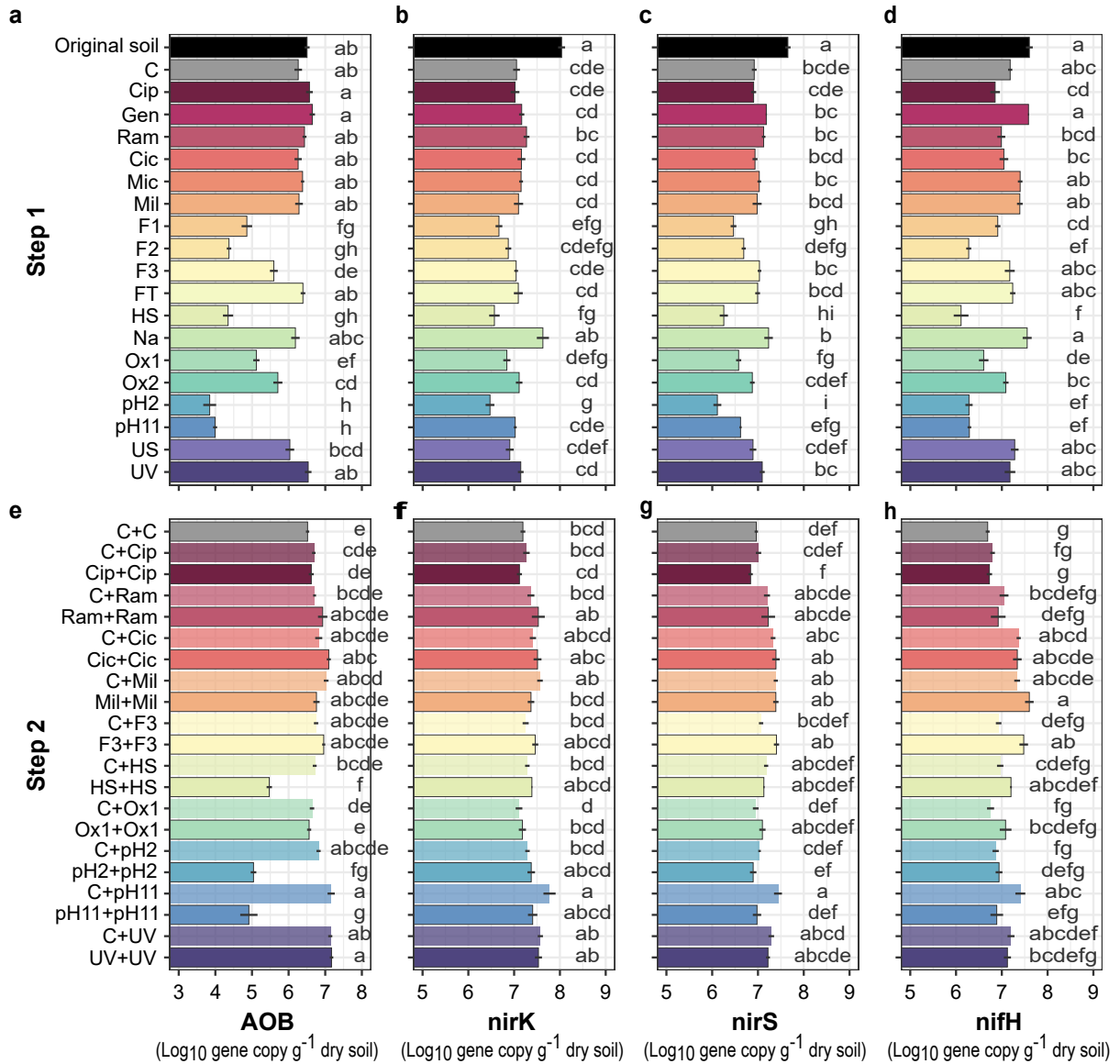


Figure A.16: Treatment-induced changes in abundance of N-cycle microbial guilds as proxies of N-cycling. Abundances of ammonia-oxidizing bacteria (AOB in **a** and **e**), bacterial denitrifiers (nirK in **b** and **f**; nirS in **c** and **g**) and nitrogen-fixing bacteria (nifH in **d** and **h**) in the original soil, the removal treatments and the control (Step 1; **a**, **b**, **c** and **d**) or in the coalescence treatments and the self-mixed treatments (Step 2; **e**, **f**, **g** and **h**) (mean \pm s.e. of log-transformed data expressed as gene copy g⁻¹ dry soil). Letters indicate significantly different statistical groups (Tukey's test, p-value \leq 0.05). (page 30)

B

Chapter 3 Supplementary Materials

Table B.1: Abundances of total bacteria. Quantification of 16S rRNA gene copy numbers in the raw manipulated and inoculated suspensions, the original and microcosm soils (mean \pm s.e. expressed as gene copy.mL⁻¹ suspension or ⁻¹ dry soil, respectively). For suspensions, only DNA from the control and the raw manipulated suspensions were extracted and quantified. The inoculated densities of the manipulated suspensions (in grey) were calculated afterwards from the raw manipulated suspension 16S rRNA gene copy numbers according to the dilution protocol (see Methods). (pages 38, 39 and 42)

Treatment	Suspension		Soil	
	Mean	s.e.	Mean	s.e.
Original Soil	-	-	2.49x10 ⁸	7.32x10 ⁶
C	2.55x10 ⁶	1.53x10 ⁵	1.94x10 ⁸	1.31x10 ⁷
MAC a1	1.23x10 ⁹	2.56x10 ⁷	-	-
MAC a1 d1	6.14x10 ⁶	-	2.27x10 ⁸	5.07x10 ⁶
MAC a1 d2	6.14x10 ⁵	-	2.72x10 ⁸	2.17x10 ⁷
MAC a1 d3	6.14x10 ⁴	-	2.36x10 ⁸	9.00x10 ⁶
MAC a2	7.76x10 ⁸	3.11x10 ⁸	-	-
MAC a2 d1	3.88x10 ⁶	-	2.32x10 ⁸	8.29x10 ⁶
MAC a2 d2	3.88x10 ⁵	-	1.95x10 ⁸	8.51x10 ⁶
MAC a2 d3	3.88x10 ⁴	-	2.13x10 ⁸	4.56x10 ⁶
PEB a1	1.84x10 ⁹	3.16x10 ⁷	-	-
PEB a1 d1	9.21x10 ⁶	-	1.98x10 ⁸	8.36x10 ⁶
PEB a1 d2	9.21x10 ⁵	-	2.29x10 ⁸	1.61x10 ⁷
PEB a1 d3	9.21x10 ⁴	-	2.59x10 ⁸	1.31x10 ⁷
PEB a2	1.57x10 ⁹	1.97x10 ⁸	-	-
PEB a2 d1	7.85x10 ⁶	-	2.30x10 ⁸	1.33x10 ⁷
PEB a2 d2	7.85x10 ⁵	-	2.37x10 ⁸	7.03x10 ⁶
PEB a2 d3	7.85x10 ⁴	-	2.36x10 ⁸	3.95x10 ⁶

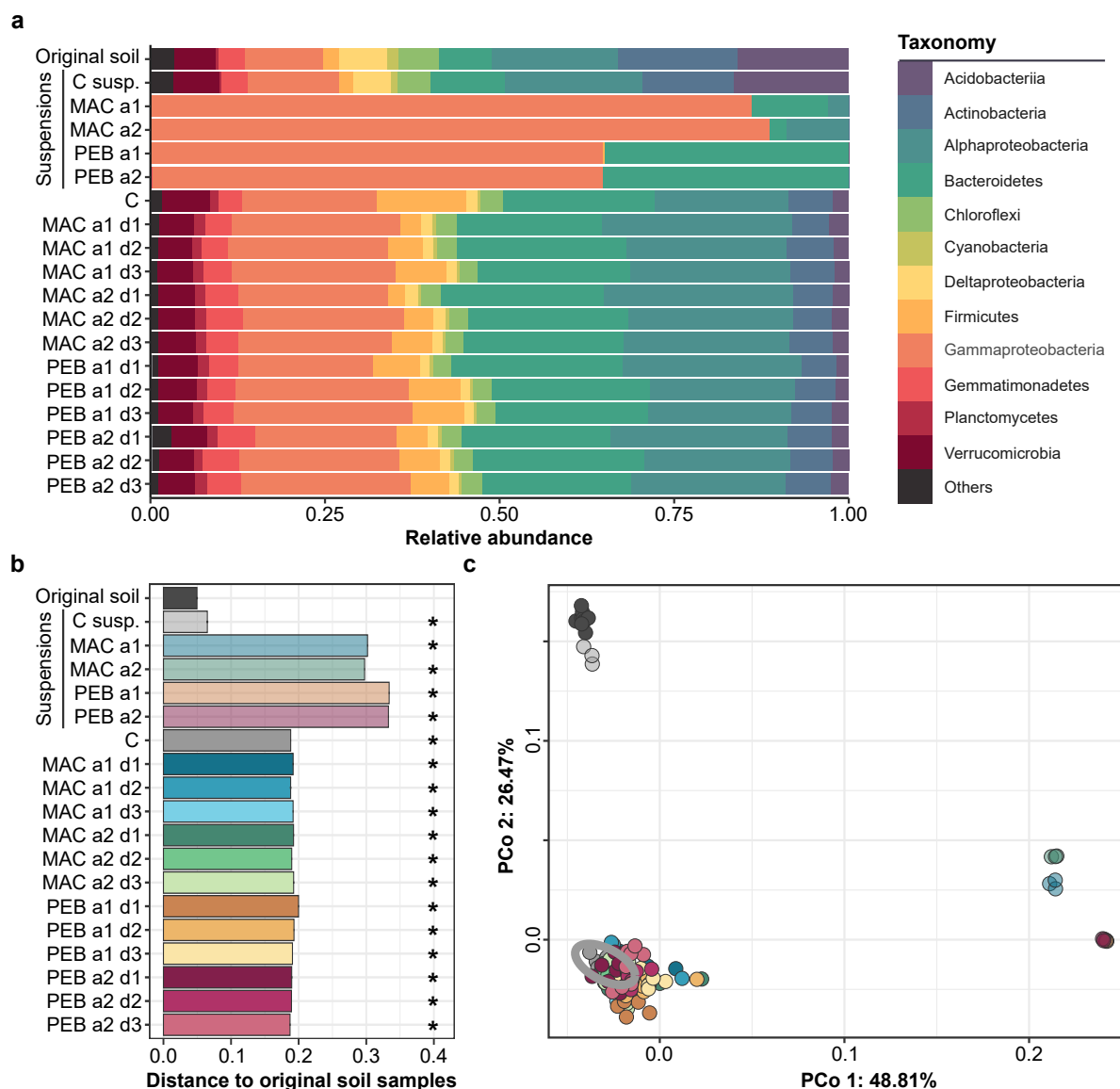


Figure B.1: Composition and Structure of the communities in the original soil, the non-treated control suspension, the manipulated suspensions, the non-coalesced control and the coalescence outcomes. (a) Relative abundances of the fourteen most abundant class of bacteria the original soil, the non-treated control suspension, the manipulated suspensions, the non-coalesced control and the coalescence outcomes. (b) Weighted UniFrac distances between the the non-coalesced control samples and between the non-coalesced control and either the original soil, the non-treated control suspension, the manipulated suspensions and the coalescence outcomes (mean \pm s.e.). Asterisks indicate communities significantly different than the original soil (Welch's t-test p -value ≤ 0.05). (c) Principal coordinate analysis (PCoA) based on the weighted UniFrac distance matrix showing the original soil, the non-treated control suspension, the manipulated suspensions, the non-coalesced control and the coalescence outcomes and the 95% joint confidence ellipse for the non-coalesced control samples. The dot colours correspond to the bar colours in **b**. (page 42)

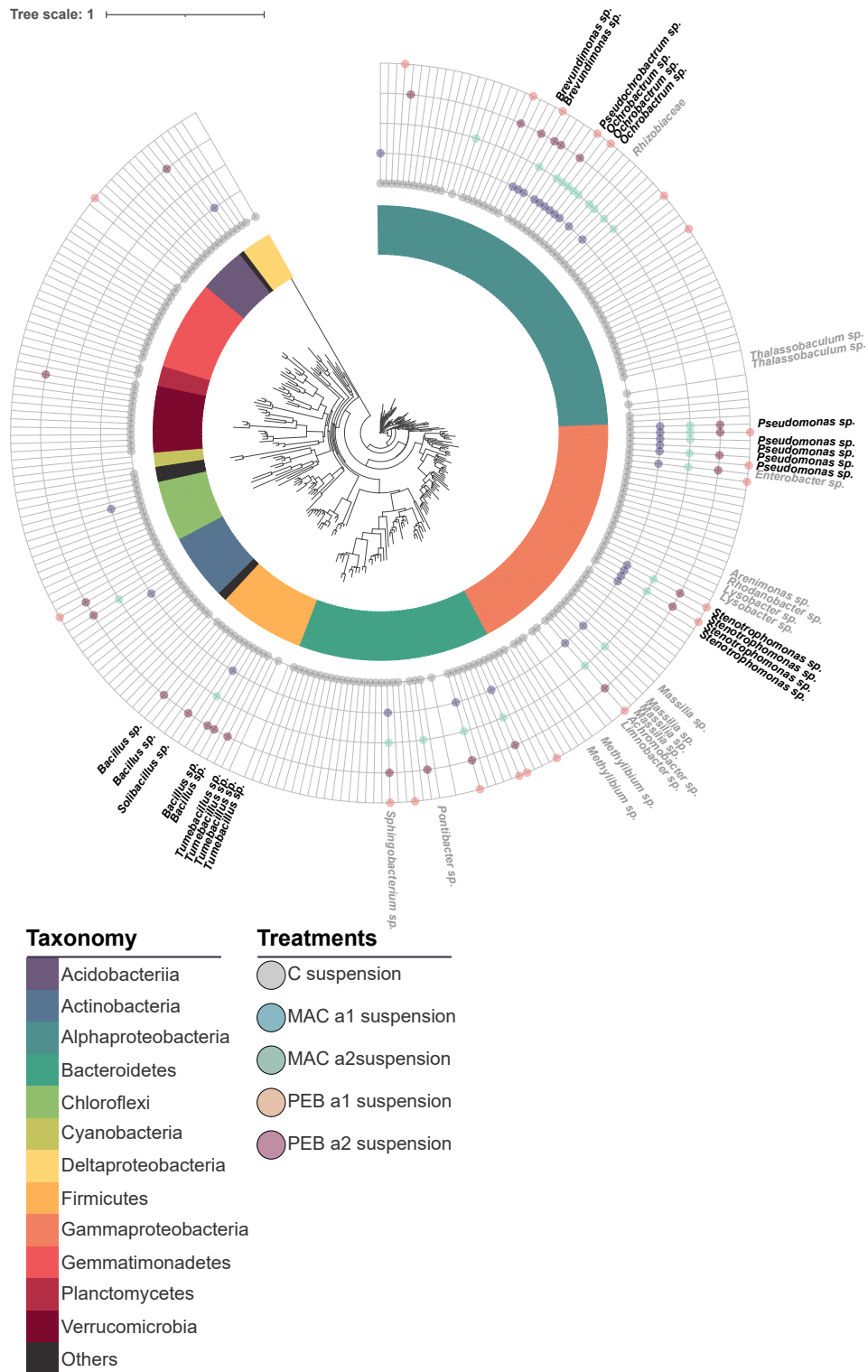


Figure B.2: Taxonomic relationships and distribution of the 258 most abundant 16S rRNA OTUs. The OTU class level is indicated by different colours on the innermost ring. The middle ring shows the detection of an OTU in the non-manipulated control suspension and/or in the manipulated suspensions. The outer ring shows the genera of the OTUs involved in the network. (page 43)

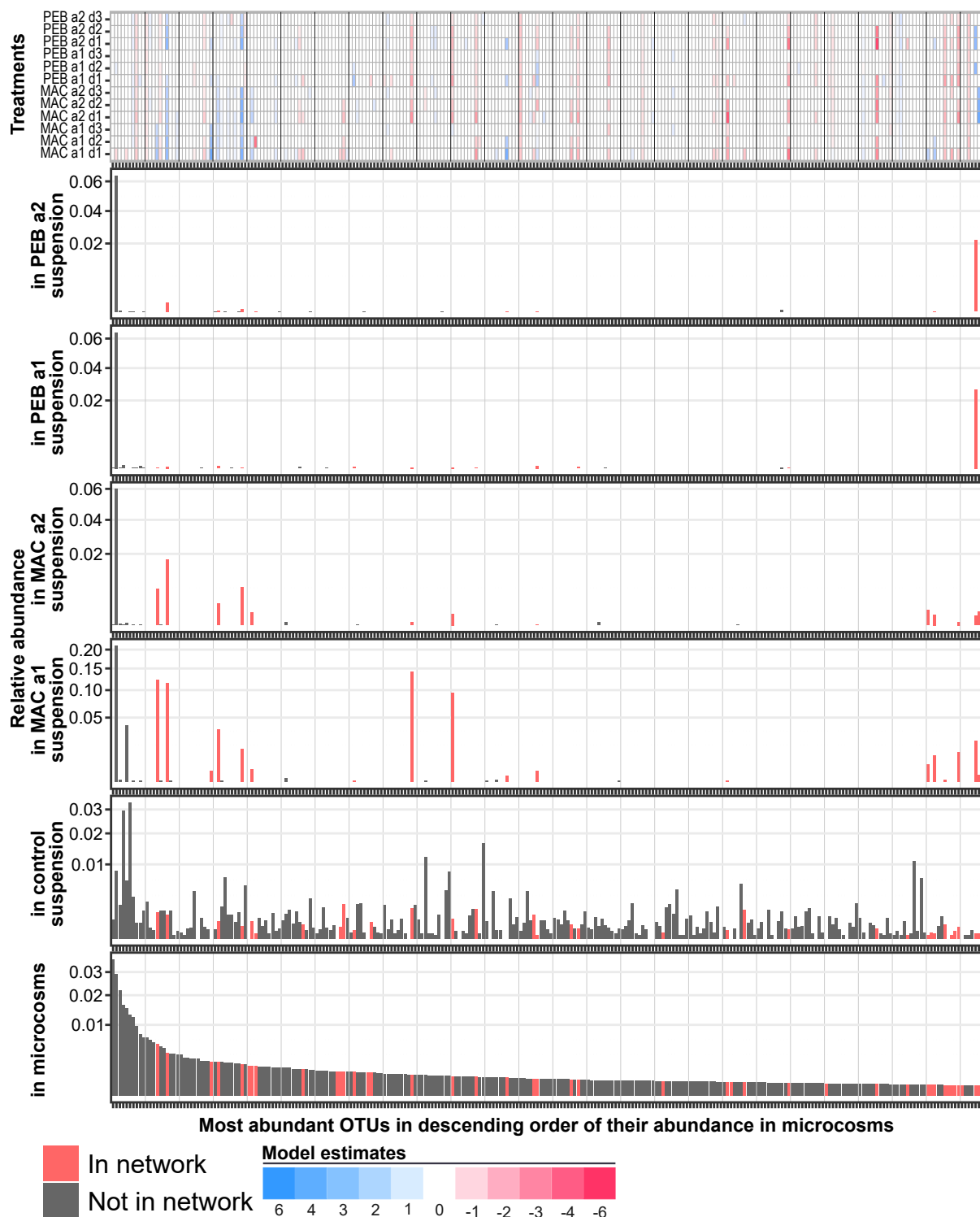


Figure B.3: Relative abundance of the 258 dominant OTUs. OTUs are placed in descending order of their abundance in microcosms and those involved as nodes in the inferred correlation network are displayed in red. The top part shows the coalescence treatment effects on the OTUs' relative abundances compared to the non-coalesced control, as estimated using a generalized linear mixed model. The blue and red boxes indicate OTUs with increasing and decreasing fitness respectively, while white boxes indicate OTUs that are not affected by the treatment (Bonferroni adjusted p -value ≤ 0.05). The following parts shows the OTU relative abundance in manipulated suspensions, in control suspension, and, in microcosms, with a hyperbolic tangent scale. (pages 43 and 45)

C

Chapter 4 Supplementary Materials

Table C.1: PERMANOVA results of Step 1 experiment assessing differences in the bacterial community structure linked to removal treatments, density treatments, and their interactions using weighted UniFrac distance. (page 56)

Comparison	Df	Sums of Sqs	Mean Sqs	F. Model	R ²	P-value
Removal Treatment	2	3.5275	1.76376	243.089	0.6961	< 0.001
Density Treatment	3	0.4824	0.16078	22.160	0.09512	< 0.001
Removal x Density	6	0.2776	0.04627	6.377	0.05474	< 0.001
Residuals	108	0.7836	0.00726	-	0.15452	-
Total	119	5.0711	-	-	1.00000	-

Significant terms are in bold

Table C.2: Pairwise comparisons assessing differences in the bacterial community structure related to the density treatment in the control (C), heat-shock (HS), and ramoplanin (RA) communities using the weighted UniFrac distances with Benjamini–Hochberg corrections for multiple testing. (page 56)

	Sums of Sqs	F. Model	R ²	P adjusted
Control				
d1 vs d2	0.906	111.27871	0.86076594	0.002
d1 vs d3	0.842	94.1304424	0.83947267	0.002
d1 vs d4	0.751	75.9496674	0.80840805	0.002
d2 vs d3	0.007	1.26845068	0.06583044	0.281
d2 vs d4	0.027	3.97500725	0.18088764	0.015
d3 vs d4	0.010	1.3346381	0.06902835	0.281
Heat shock				
d1 vs d2	0.328	80.5692796	0.81738732	0.002
d1 vs d3	0.169	6.80473156	0.274332	0.002
d1 vs d4	0.290	49.0635058	0.73159769	0.002
d2 vs d3	0.082	3.27800894	0.15405619	0.087
d2 vs d4	0.015	2.57913893	0.12532784	0.087
d3 vs d4	0.095	3.57390541	0.16565871	0.087
Ramoplanin				
d1 vs d2	0.492	38.7171571	0.68263572	0.002
d1 vs d3	0.486	32.734357	0.64521084	0.002
d1 vs d4	0.403	29.5890591	0.6217618	0.002
d2 vs d3	0.006	0.94818479	0.05004093	0.493
d2 vs d4	0.012	2.18703831	0.10833874	0.121
d3 vs d4	0.005	0.68869231	0.03685075	0.540

Significant terms are in bold

Table C.3: PERMANOVA results of Step 2 experiment assessing differences in the bacterial community structure linked to the community, density, and their interactions using weighted UniFrac distance. (page 60)

	Df	Sums of Sqs	F. Model	R²	P-value
Control					
Community	2	0.16120507	18.713436	0.40648813	< 0.001
Density Treatment	1	0.06480913	15.0808382	0.16379092	< 0.001
Community x Density	2	0.06625069	7.78300166	0.16906023	< 0.001
Residuals	24	0.10131031	-	0.26066072	-
Total	29	0.4035752	-	1	-
Heat shock					
Community	2	0.09315436	6.00074563	0.24976919	< 0.001
Density Treatment	1	0.05853254	7.54100776	0.15693978	< 0.001
Community x Density	2	0.04275121	2.7539141	0.11462624	0.002
Residuals	23	0.17852367	-	0.47866479	-
Total	28	0.37296178	-	1	-

Significant terms are in bold

Table C.4: Number of OTUs significantly differentially abundant among all treatments as estimated using a generalized linear mixed model, among the 515 most abundant 16S rRNA OTUs and the 439 most abundant 18S rRNA OTUs. (page 60)

	Number of significant OTUs	% of significant OTUs
Control		
$(Cd1+Cd2)_{d1}$ vs $Cd1_{d1}$	153	30.90
$(Cd1+Cd2)_{d1}$ vs $Cd2_{d1}$	69	13.93
$(Cd1+Cd2)_{d2}$ vs $Cd1_{d2}$	116	23.43
$(Cd1+Cd2)_{d2}$ vs $Cd2_{d2}$	64	12.92
Heat-shock		
$(HSd1+HSd2)_{d1}$ vs $HSd1_{d1}$	42	13.00
$(HSd1+HSd2)_{d1}$ vs $HSd2_{d1}$	49	15.17
$(HSd1+HSd2)_{d2}$ vs $HSd1_{d2}$	22	6.81
$(HSd1+HSd2)_{d2}$ vs $HSd2_{d2}$	24	7.43

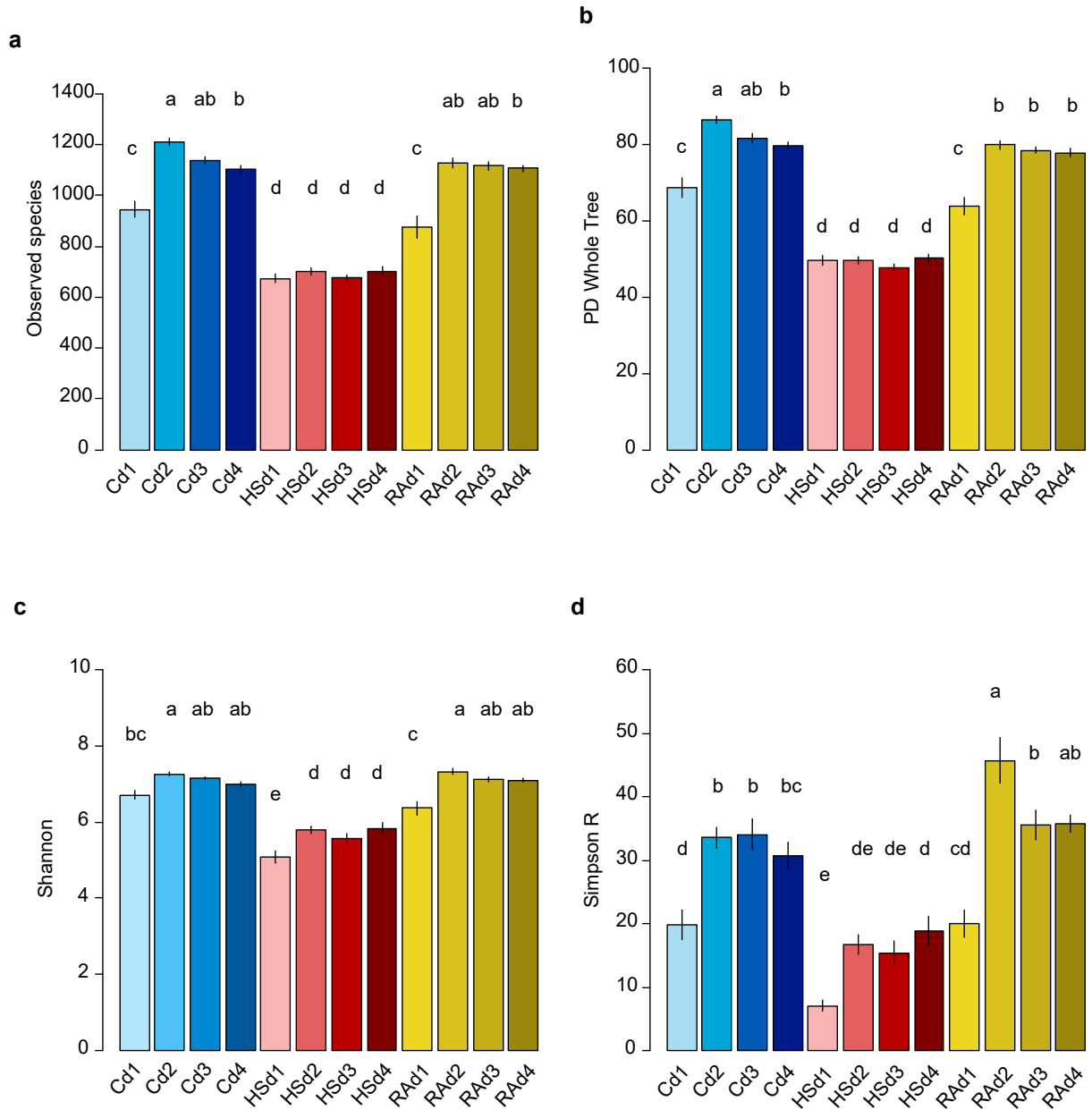


Figure C.1: Diversity levels of the bacterial community after Step 1 experiment. The observed species (a), Faith's phylogenetic diversity (b), Shannon (c), and Simpson's reciprocal (d) indices are shown (mean ± s.e.) in the control (C), heat-shock (HS), and ramoplanin (RA) communities within the density gradient (d1, d2, d3, and d4). Different letters indicate significant differences according to Tukey's test (p-value ≤ 0.05). (page 56)

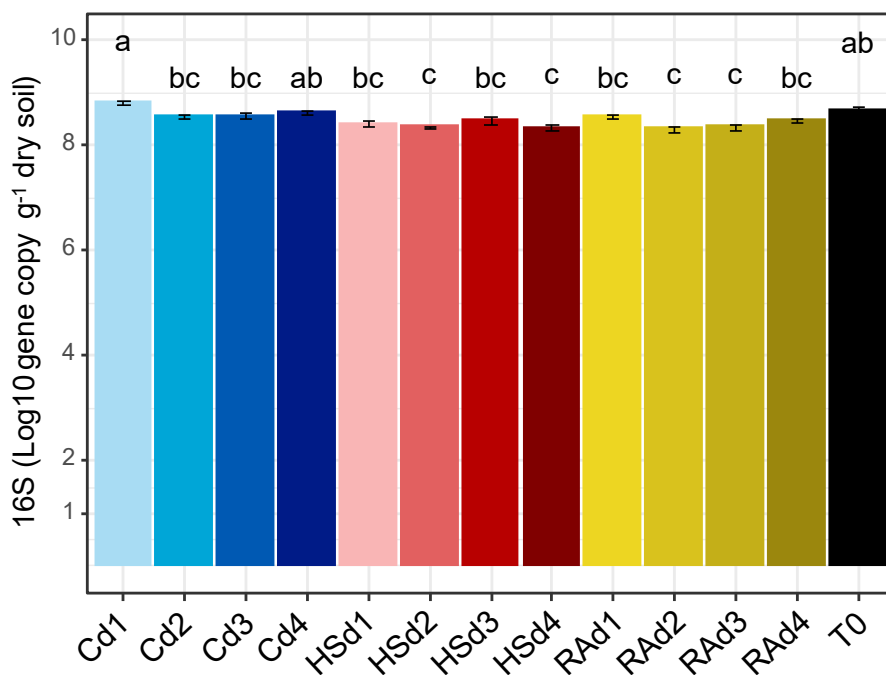


Figure C.2: Abundances of total bacteria (16S rRNA) in the control (C), heat-shock (HS), and ramoplanin (RA) communities within the density gradient (d1, d2, d3, and d4) after Step 1 experiment (mean \pm s.e. of \log_{10} -transformed data expressed as gene copy.g⁻¹ dry soil). Different letters above the bars indicate significant differences according to Tukey's test (p-value \leq 0.05). (page 56)

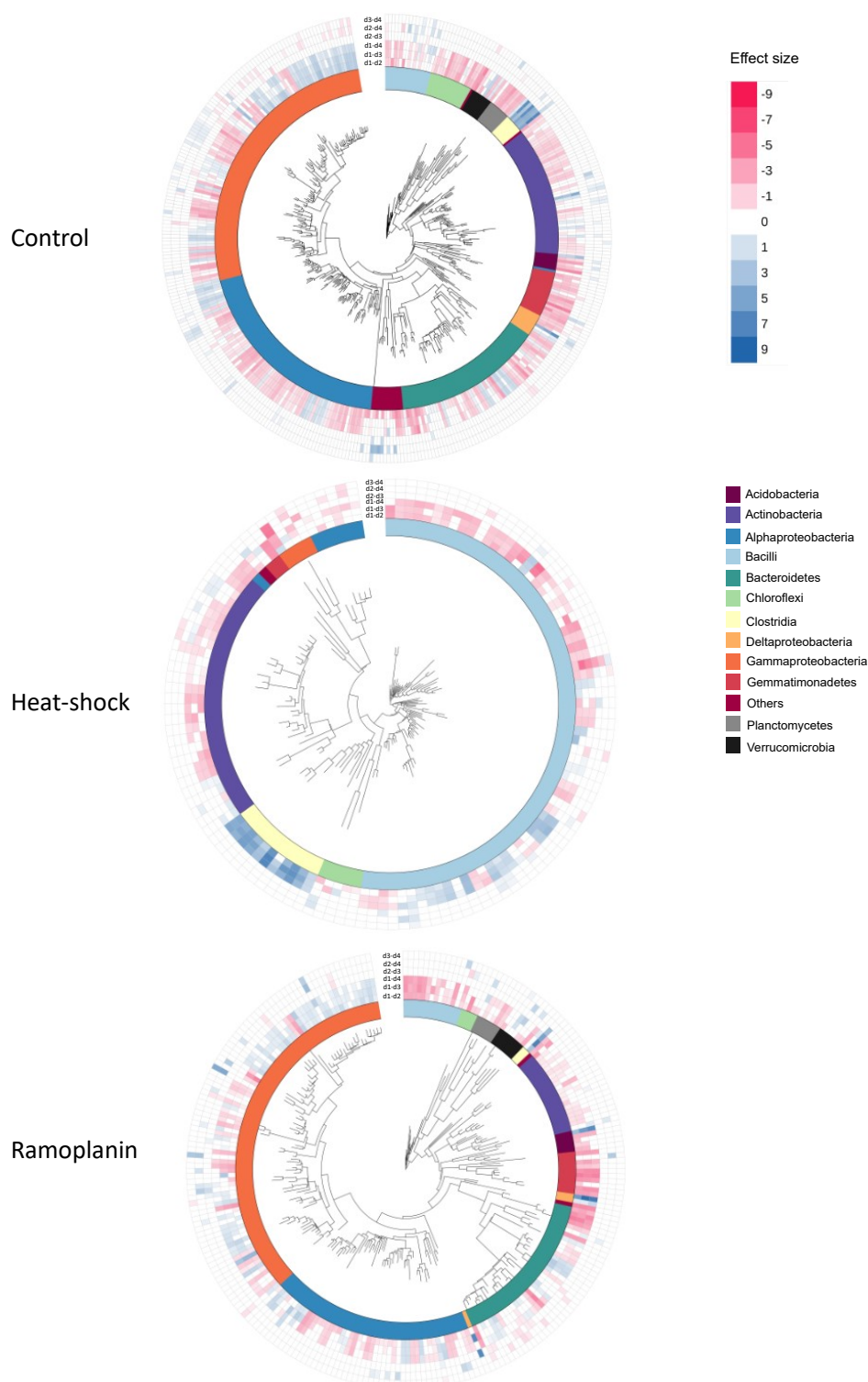


Figure C.3: Phylogenetic relationships and distribution of OTUs with significant increasing/decreasing relative abundances between density treatments according to the generalized linear mixed model for the control (C), heat-shock (HS), and ramoplanin (RA) communities. Changes in the relative abundances as measured by the coefficient estimates (effect size) are represented by the blue-to-red color. The affiliation of OTUs at the phylum or class levels is indicated by different colors on the internal ring. (page 57)

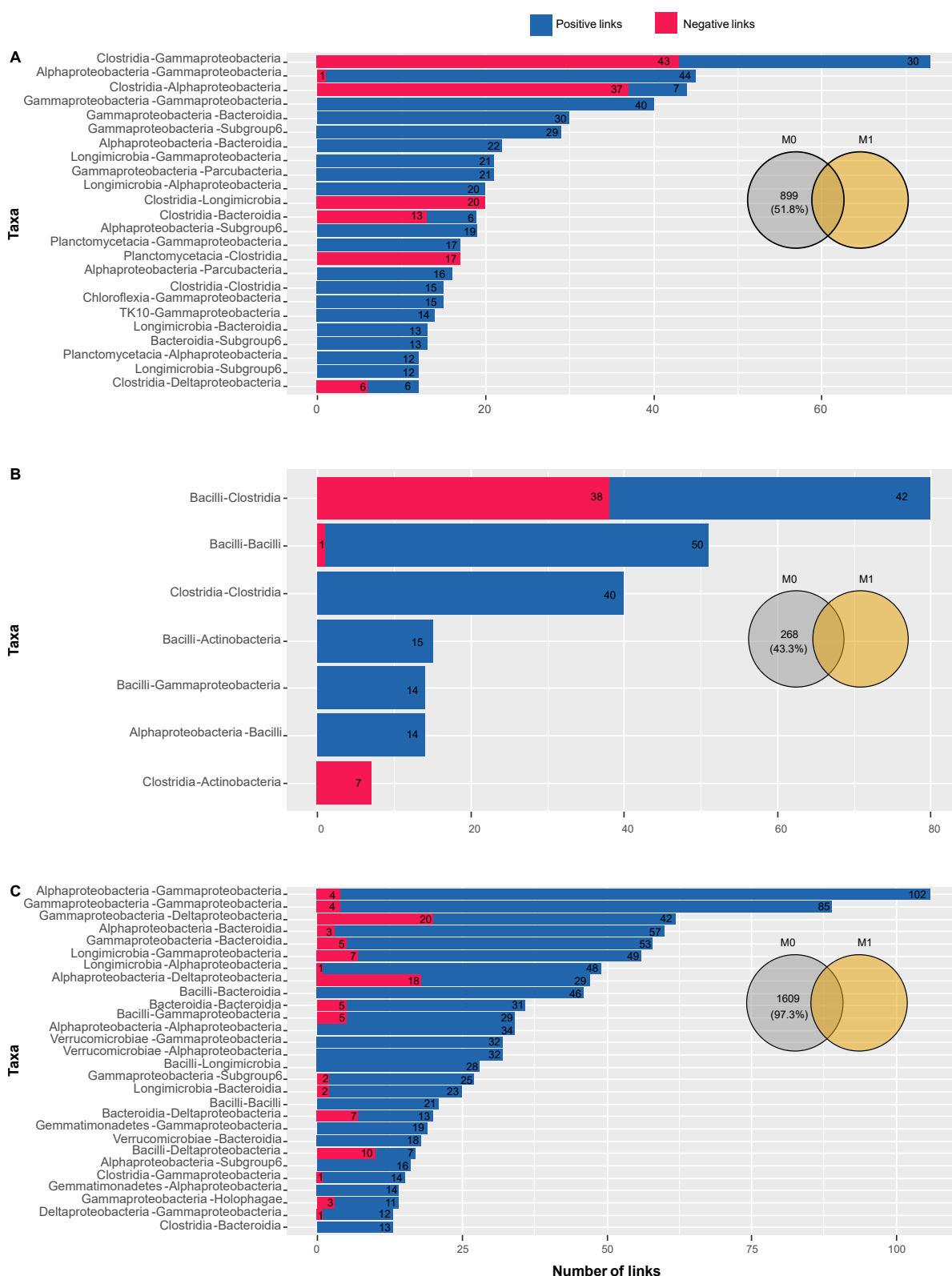


Figure C.4: Number of positive (blue) and negative (red) links that are related to the physical distance (M0-M1) for the control (a), heat-shock (b), and ramoplanin (c) communities. The Venn Diagrams show the number of links that are related to the physical distance (M0-M1). (pages 58 and 59)

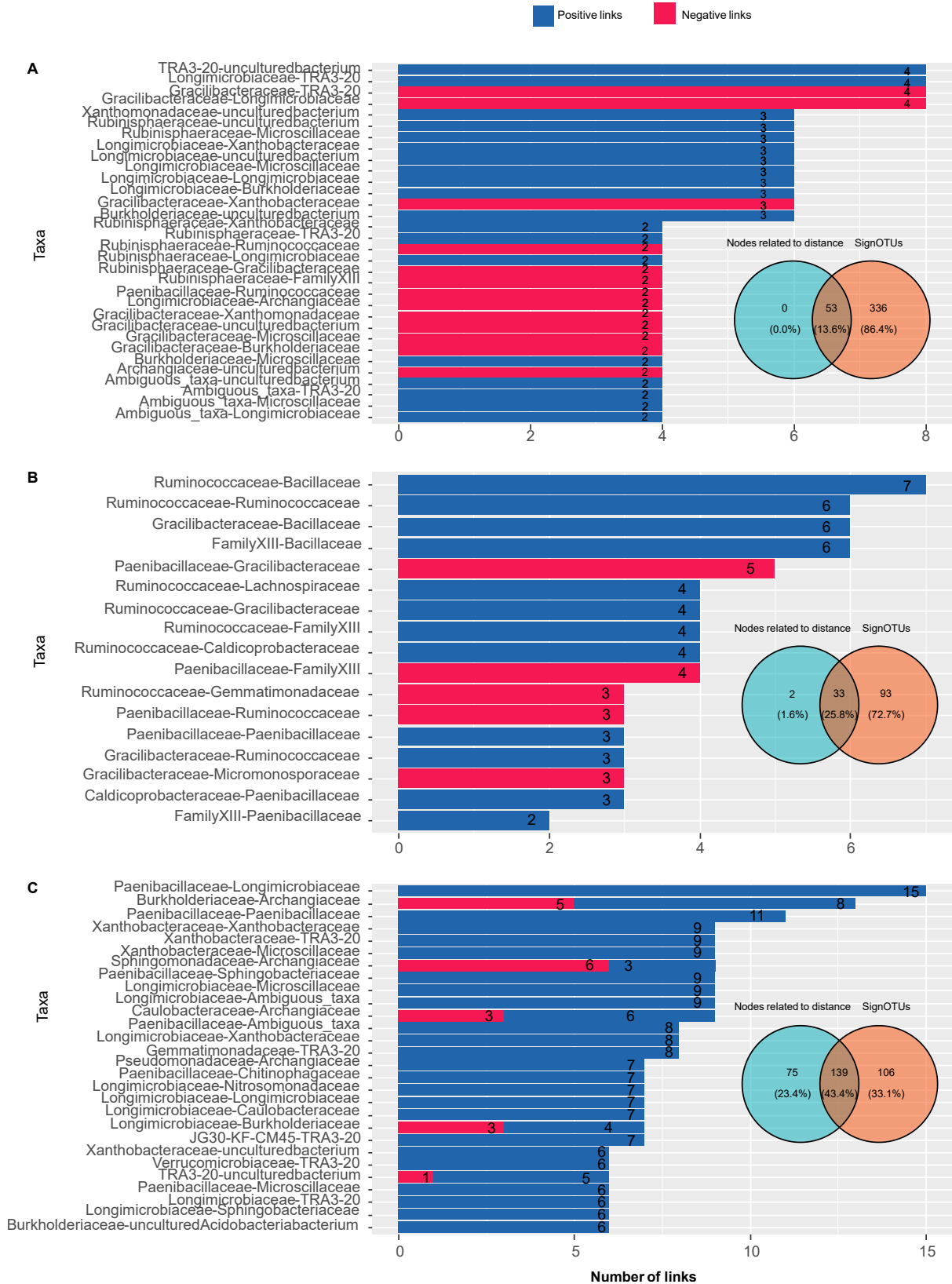


Figure C.5: Number of positive (blue) and negative (red) links between nodes that are related to physical distance in co-occurrence networks (M0-M1) and exhibiting significant changes in relative abundances as determined by the differential abundance analysis, for the control (a), heat-shock (b), and ramoplanin (c) communities. (page 59)

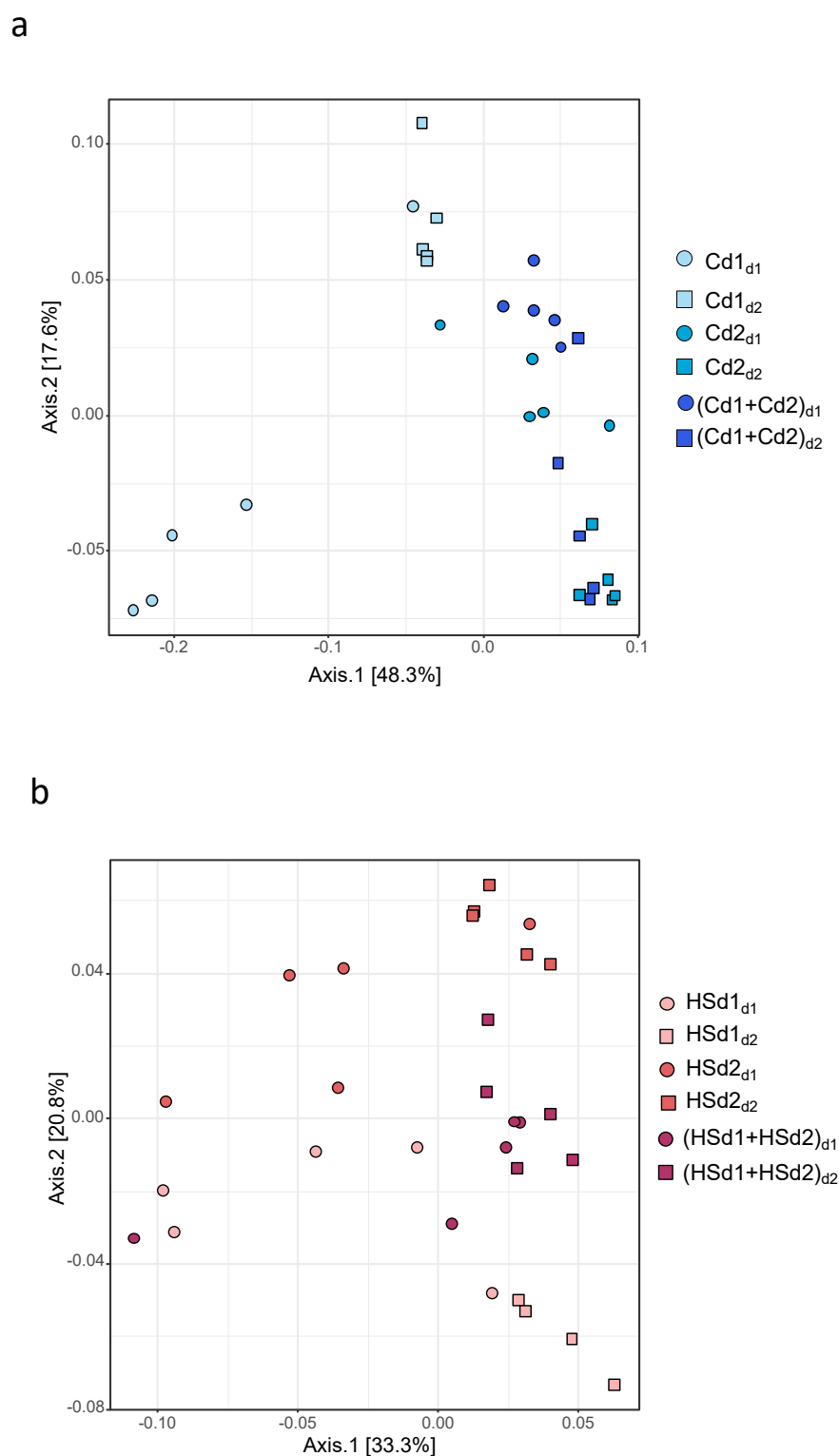


Figure C.6: Differences in bacterial community composition across treatments for the Step 2 experiment. Principal Coordinates Analysis (PCoA) of the weighted UniFrac distance matrix of *16S rRNA* gene amplicons of coalesced and references communities for the control (a) and heat-shock (b) at low (d1) and high densities (d2). The different treatments are represented by different colors and symbols as specified in the legend. (page 60)

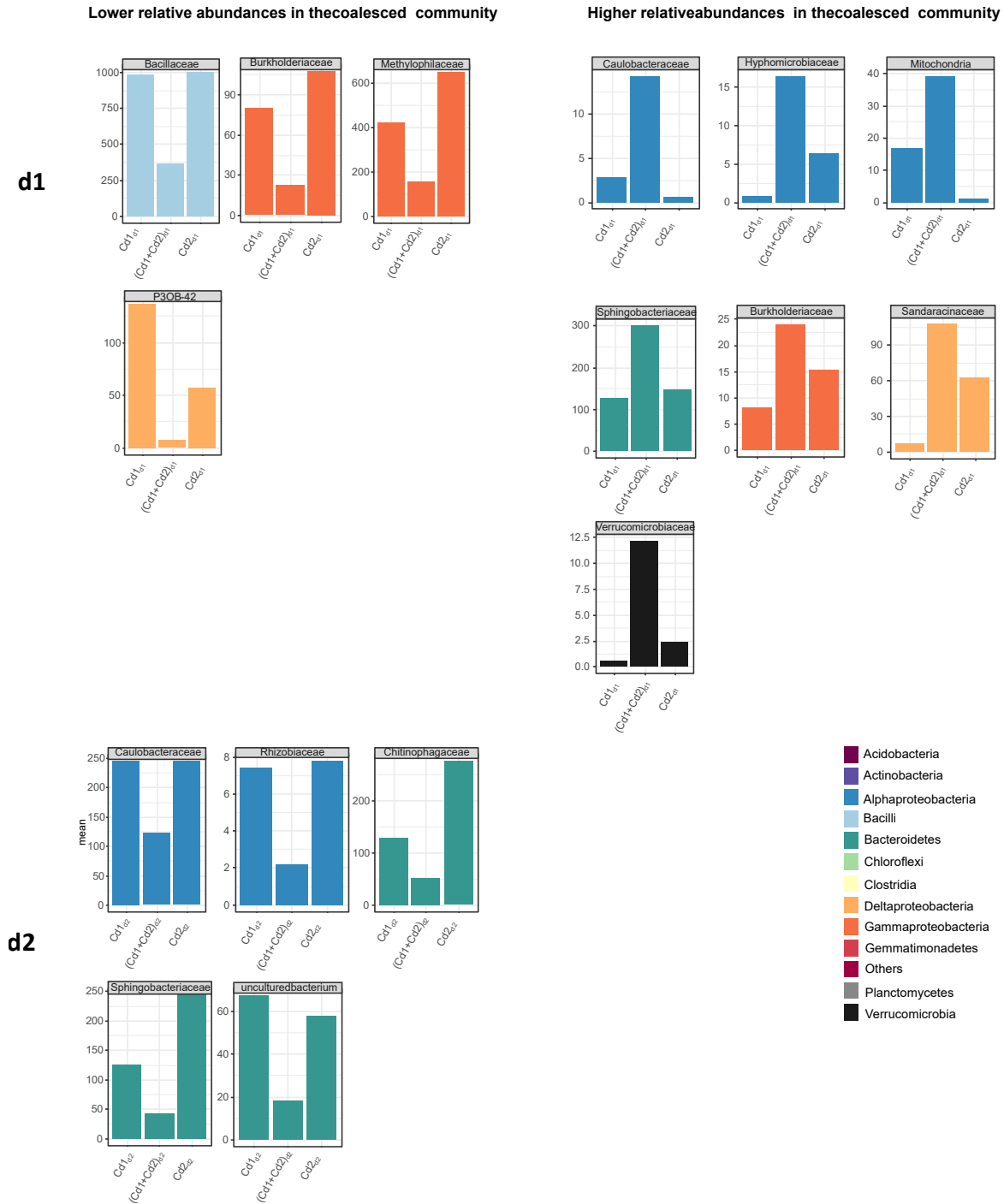


Figure C.7: OTUs exhibiting significantly higher or lower relative abundances in the coalesced communities than reference communities as identified by the generalized linear mixed model at low (d1) and high densities (d2) in the control community. Relative abundances are shown at the family level and the affiliation of OTUs are indicated by different colors at the phylum or class levels. (page 60)

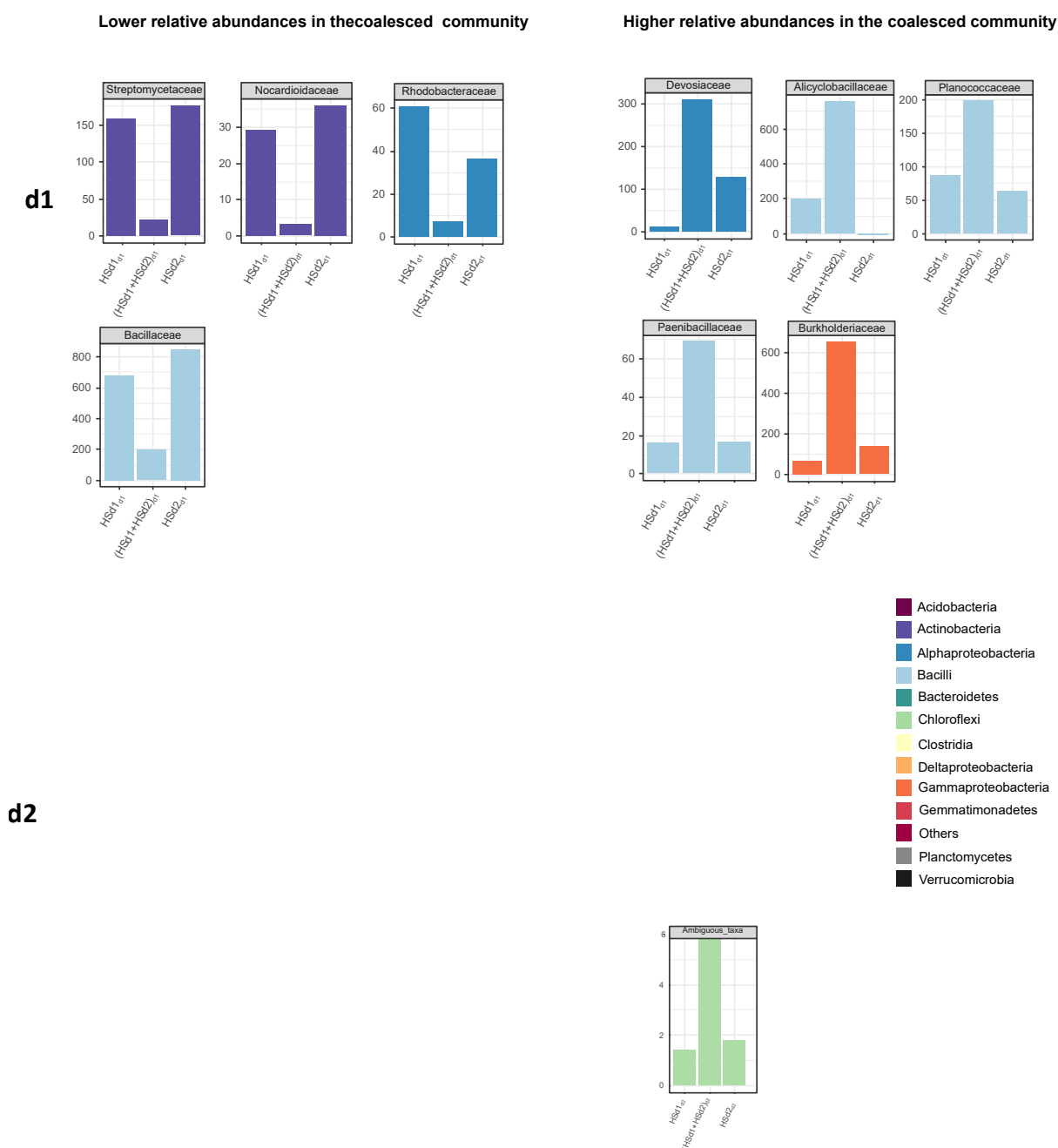


Figure C.8: OTUs exhibiting significantly higher or lower relative abundances in the coalesced communities than reference communities as identified by the generalized linear mixed model at low (d1) and high densities (d2) in the heat-shock community. Relative abundances are shown at the family level and the affiliation of OTUs are indicated by different colors at the phylum or class levels. (page 60)

Abstract Microbial communities play a key role in ecosystem functioning as well as in plant, animal, and human welfare. Their assembly relies on different processes commonly referred to as abiotic and biotic filters. Despite the widespread emphasis on abiotic filters in terrestrial ecosystems, a growing body of evidence suggests that interactions between microorganisms play a critical role in community assembly. However, the contribution of these interactions to microbial community assembly and the factors influencing them have not been clearly established. In most cases, biotic interactions between microorganisms have been investigated based on cocultures of a handful of strains, completely overlooking the high complexity of microbial communities in nature.

This thesis objectives were to decipher biotic interactions occurring between microbial community members and to assess the role of these interactions in the assembly of complex communities. Thereby, we used three top-down experiments based on the manipulation of native soil microbial communities and their reinoculation in sterile soil microcosms allowing them to reassemble during soil recolonisation.

Our results suggest that between 15% and 28% of the bacterial community could be subjected to negative interactions during soil recolonization. Proteobacteria and Firmicutes exhibited a recurrent negative correlation, indicating that competition between them might be a rule governing microbial community assembly. Our approach also uncovered the importance of density-dependent interactions in microbial community assembly, the competitiveness of individuals strongly depending on the density and identity of surrounding neighbours. Furthermore, we showed that coalescence (mixing of manipulated and control communities) could also restore -at least partly- community diversity and soil functions. While the diversity of the coalesced communities was related to that of the source communities, their structure was mostly influenced by the density and the composition of the source communities.

Altogether, we bring here additional evidence that community manipulation could become a standard tool to decipher microbial interactions in complex communities, their contribution to community assembly, and the links between community structure and function. Furthermore, our work highlights the potential of using community manipulation to design new microbial engineering strategies allowing to steer microbial communities in situ, without adding non-indigenous species, by using the interactions occurring between the native community members.

Résumé Les communautés microbiennes sont primordiales dans le fonctionnement des écosystèmes ainsi que pour la santé des plantes, des animaux et des humains. Leur assemblage repose sur différents processus communément appelés filtres abiotiques et biotiques. Malgré l'accent mis sur les filtres abiotiques dans les écosystèmes terrestres, de nouvelles preuves suggèrent que les interactions entre les microorganismes jouent un rôle clé dans l'assemblage des communautés. Cependant, la contribution de ces interactions à l'assemblage des communautés microbiennes et des facteurs qui les influencent demeure floue. Traditionnellement, les interactions biotiques entre microorganismes ont été étudiées par coculture d'une poignée de souches, négligeant complètement la complexité des communautés microbiennes naturelles.

Les objectifs de cette thèse étaient d'identifier les interactions biotiques se produisant entre les membres de communautés microbiennes et d'évaluer le rôle de ces interactions dans l'assemblage de communautés complexes. Pour cela, nous avons utilisé trois expériences basées sur la manipulation de communautés microbiennes natives de sol et sur leur ré-inoculation dans des microcosmes de sol stérile leur permettant de se réassembler lors de la recolonisation du sol.

Nos résultats suggèrent qu'entre 15% et 28% des communautés bactériennes seraient soumises à des interactions négatives pendant la recolonisation du sol. La corrélation négative récurrente entre Proteobacteria et Firmicutes indique que la compétition entre ces deux familles bactériennes pourrait être une règle régissant l'assemblage des communautés microbiennes. Notre approche a également mis en évidence l'importance de la densité dans l'assemblage des communautés microbiennes, la compétitivité des individus dépendant fortement de la densité et de l'identité de leurs voisins. En outre, nous avons montré que la coalescence (correspondant ici au mélange de communautés manipulées et contrôle) pourrait restaurer - au moins partiellement - la diversité et les fonctions des communautés. Alors que la diversité des communautés coalescées dépend de celle des communautés sources, leur structure était principalement influencée par la densité et la composition des communautés sources.

Nos travaux apportent des preuves supplémentaires que la manipulation des communautés peut devenir un outil de référence pour identifier les interactions microbiennes dans des communautés complexes, leur contribution à l'assemblage de ces communautés, et les liens entre structure et fonction des communautés. De plus, notre travail met en évidence le potentiel de la manipulation de communautés pour concevoir de nouvelles stratégies d'ingénierie écologique permettant de guider les communautés microbiennes in situ, sans ajouter d'espèces non indigènes, en utilisant les interactions qui se produisent entre les membres de la communauté native.