Technische Universität München
TUM School of Life Sciences

The effect of increasing plant species richness on soil and seed microbiome and its importance to stress response

Yuri Pinheiro Alves de Souza

Vollständiger Abdruck der von der TUM School of Life Sciences der Technischen Universität München zur Erlangung eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitz: Prof. Dr. Wolfgang Weisser

Prüfende der Dissertation:

1. Prof. Dr. Michael Schloter
2. Prof. Dr. Nico Eisenhauer

Die Dissertation wurde am 24.04.2024 bei der Technischen Universität München eingereicht und durch die TUM School of Life Sciences am 11.07.2024 angenommen.

The tree that never had to fight
For sun and sky and air and light,
But stood out in the open plain
And always got its share of rain,
Never became a forest king
But lived and died a scrubby thing.

The man who never had to toil
To gain and farm his patch of soil,
Who never had to win his share
Of sun and sky and light and air,
Never became a manly man
But lived and died as he began.

Good timber does not grow with ease:
The stronger wind, the stronger trees;
The further sky, the greater length;
The more the storm, the more the strength.
By sun and cold, by rain and snow,
In trees and men good timbers grow (…)

Good Timber by Douglas Malloch

Table of Contents

Summary

Diversity is a key trait to community stability and resilience face to environmental disturbances. This stability is achieved through increasing metabolic complementarity and redundancy, which ensures the smooth functioning of different physiological processes at different trophic levels in a given community. That is true for animal, plant and microbial communities present in soil, water and in the air. Due to its ubiquity and essential role in nutrient cycling, microbial community diversity, mainly in the soil where it is exceptionally diverse compared to water and air, has been demonstrated to be an important indicator of overall ecosystem health, quickly responding to environmental changes. This diversity seems to be tiddly connected with the plant community. As the lowest level of trophic chains and essential supply of food, energy and pharmacological compounds, the interactions between microbial and plant community diversity have been vastly investigated in previous decades by botanists, ecologists, and engineers to disentangle the complex relationships between productivity, sustainability, and conservation of both natural and vegetal communities.

However, despite the extensive research on the biodiversity topic in vegetal and soil microbial communities, the factors driving the interactions between those two communities during stress events and the effects of increasing diversity on those responses are still vaguely explored.

In this thesis, we approach the effects of plant diversity increase over soil and endophytic microbial community composition. We made use of the structure of a long-term biodiversity experiment in Germany (The Jena experiment) to investigate the effects of a plant diversity gradient in the soil microbial response to long term drought and how this increasing plant diversity shapes the composition the endophytic microbiome of *Plantago lanceolata,* a widespread European herb, commonly used as model for greenhouse experiments. In the frame of soil microbial communities, we also investigated the effects of different inoculation loads in the microbial colonization of soils after disturbance (in this case, autoclavation). We used metabarcoding sequencing to access the microbial response to our experimental designs in soil and seed samples. We identified changes in diversity and microbial composition by taxonomical assignment of the resulting reads and calculating the amount amplicon sequencing variants assigned to each of those taxa.

Our results indicates that microbial communities deterministically colonize soil after sterilization, independently of the initial inoculum. Soil communities diversity also surprisingly was positively correlated with long term drought. Lastly, we successfully identified the core microbiome of *Plantago lanceolata* seeds, as being composed by *Paracoccus*, *Alteribacillus*, *Sphingomonas*, *Pseudomonas*, *Massilia* and *Pirellula* genera, being *Sphingomonas faeni* and *Pirellulla spp.* the dominant species. This

result indicates possible transgenerational link between individuals growing in different diversity levels.

Zusammenfassung

Diversität ist ein entscheidendes Merkmal für die Stabilität und Widerstandsfähigkeit einer Gemeinschaft gegenüber Umweltstörungen. Diese Stabilität wird durch eine zunehmende metabolische Vielfalt und Redundanz erreicht, die sicherstellt, dass unterschiedliche physiologische Prozesse auf verschiedenen trophischen Ebenen innerhalb einer Gemeinschaft reibungslos ablaufen. Dies gilt sowohl für Artengemeinschaften im Tier- und Pflanzenreich als auch für mikrobielle, welche in Böden, Gewässern und in der Luft vorkommen können. Aufgrund der Allgegenwärtigkeit von Mikroorganismen, sowie ihrer essenziellen Rolle im Nährstoffkreislauf, hat sich gezeigt, dass die Vielfalt der mikrobiellen Gemeinschaften, welche schnell auf Umweltveränderungen reagieren, einen wichtigen Indikator für die Gesamtgesundheit eines Ökosystems darstellt. Diese außergewöhnliche mikrobielle Vielfalt findet sich, im Vergleich zu Gewässern und der Luft, vor allem im Boden. Diese Diversität scheint eng mit der Pflantzengemeinschaft verbunden zu sein. Da Mikroorganismen die niedrigste Ebene der Nahrungskette darstellen und sowohl als essenzielle Nahrungs- und Energiequellen als auch als Produzenten von pharmakologischen Verbindungen dienen, wurden die Wechselwirkungen zwischen der mikrobiellen Gemeinschaft und der Pflantzengemeinschaft in den vergangenen Jahrzehnten umfassend untersucht. Botaniker, Ökologen und Ingenieure analysierten diese Wechselwirkungen, um die komplexen Beziehungen zwischen Produktivität, Nachhaltigkeit und Erhaltung natürlicher mikrobieller als auch pflanzlicher Gemeinschaften zu erklären, zu bewahren und zu nutzen. Trotz der umfangreichen Forschung zum Thema Biodiversität in pflanzlichen und bodenmikrobiellen Gemeinschaften ist der Stand des Wissens in Forschung und Literatur zu den Faktoren, welche die Wechselwirkungen zwischen diesen beiden Gemeinschaften während Stressereignissen steuern, als auch die Auswirkungen einer zunehmenden Vielfalt auf diese Reaktionen, begrenzt. In dieser Arbeit wurden die Auswirkungen einer erhöhten Pflanzenvielfalt auf die Zusammensetzung der bodenmikrobiellen sowie endophytischen mikrobiellen Gemeinschaft untersucht. Hierbei wurden die Ressourcen und Infrastruktur eines Langzeit-Biodiversitätsexperiments in Deutschland (das Jena-Experiment) verwendet, um die Auswirkungen eines Pflanzenvielfalt-Gradienten auf die Reaktion der Bodenmikroorganismen mit dem zusätzlichen Stressfaktor der andauernden Trockenheit zu untersuchen. Zudem wurde der Einfluss der zunehmenden Pflanzenvielfalt auf die Zusammensetzung des endophytischen Mikrobioms von *Plantago lanceolata* analysiert. *Plantago lanceolata* ist eine weit verbreitete europäischen Pflanze, die häufig als Modellpflanze für Gewächshausexperimente verwendet wird. Im Rahmen des Versuches wurden die Auswirkungen verschiedener Animpfmegen autoklavierten und damit gestörten Bodens, auf die nachfolgende Besiedlung von Böden und der sich entwickelnden mikrobiellen Gemeinschaft, untersucht. Die Reaktion der mikrobiellen Gemeinschaft auf das zugrunde

liegende experimentelle Design wurde sowohl für Boden als auch für Samenproben von *Plantago lanceolata* mittels Metabarcode-Sequenzierung analysiert. Es konnten Änderungen in der mikrobiellen Vielfalt und Zusammensetzung durch taxonomische Zuordnung der erhaltenen Sequenzen identifiziert werden. Zudem wurde die absolute Zahl für jede der erhaltenen und einer spezifischen mikrobiellen taxonomischen Einheit zugeordneten Amplikon-Sequenzvarienten (ASV) pro Probe berechnet. Unsere Ergebnisse zeigen, dass die mikrobielle Gemeinschaft des Bodens nach der Sterilisation deterministisch besiedelt wird, unabhängig von der für die anfängliche Inokulation verwendeten Menge an autoklaviertem Boden.

Die Vielfalt der Bodengemeinschaften ist, entgegen der ursprünglichen Erwartungen, positiv mit dauerhafter Trockenheit korreliert. Weiterhin konnte erfolgreich das Kernmikrobiom der *Plantago lanceolata* Samen identifizieret werden, welches aus den bakteriellen Gattungen *Paracoccus*, *Alteribacillus*, *Sphingomonas*, *Pseudomonas*, *Massilia* und *Pirellula* besteht.

Dabei stellen *Sphingomonas faeni* und *Pirellulla spp*. die dominanten Arten dar. Dieses Ergebnis deutet auf eine mögliche transgenerationale Verbindung zwischen Individuen von *Plantago lancelotata*, welche in Bereichen mit verschiedenen Graden der Pflanzendiversität wachsen, hin.

List of abbreviations

16S rRNA gene - gene coding for a RNA of the small ribosomal subunit of prokaryotes

ASV - Amplicon sequence variant

ANOVA - Analysis of variance

bp - Base pairs

BSA - Bovine serum albumine

°C - Degree Celsius

CO_2 – Carbon dioxide

DEPC-MiliQ - Diethyl pyrocarbonate treated ultrapure water

DNA - Deoxyribonucleic acid

dNTP - Deoxyribonucleotide triphosphate

DOC - Dissolved organic carbon

kb - Kilobases

MB – Megabases

mWHC – Maximum water holding capacity

N_2 – Molecular nitrogen

ng – Nanogram

NH_3 – Ammonia

NPK – Nitrogen phosphorus and potassium

PCR – Polymerase chain reaction

PCoA - Principal Coordinate Analysis

PERMANOVA - Permutational analysis of variance

PMA - Propidium monoazide

qPCR - Quantitative polymerase chain reaction

R2A - Reasoner's 2A medium

R^2 - Coefficient of determination

RubisCO - Ribulose-1,5-bisphosphate carboxylase-oxygenase

SRA - Sequence Read Archive

1. List of publications and contributions

This thesis is based on the following publications:

- **Pinheiro Alves de Souza Y**, Schloter M, Weisser W et al. Deterministic Development of Soil Microbial Communities in Disturbed Soils Depends on Microbial Biomass of the Bioinoculum. Microb Ecol 2023, DOI: 10.1007/s00248-023-02285-9.
  (P1, first author, published)

- **Pinheiro Alves de Souza Y**, Schloter M, Siani R et al. The effect of plant species richness on the recovery of bacterial diversity after successive summer drought periods.
  (**P2, first author, submitted to the FEMS Microbiology Ecology Journal in January 2024**)

- **Pinheiro Alves de Souza Y**, Schloter M, Weisser W et al. The seeds of Plantago lanceolata comprise a stable core microbiome along a plant richness gradient. Environ Microbiome 2024;19:4–11.
  (**P3, first author, published**)

1.1. Publication 1

Deterministic development of soil microbial communities into disturbed soils depends on microbial biomass of the bioinoculum.

Yuri Pinheiro Alves de Souza, Michael Schloter, Wolfgang Weisser, Stefanie Schulz

Short description:

Despite its enormous importance for ecosystem services, factors driving microbial recolonization of soils after disturbance are still poorly understood. In this publication, we compared the microbial recolonization patterns of a soil disturbed by autoclavation using different amounts of the original non-disturbed soil as inoculum. By using this approach, we manipulated microbial biomass, but did not change microbial diversity of the inoculum. We followed the development of a new soil microbiome after reinoculation over a period of 4 weeks using a molecular barcoding approach as well as qPCR. Focus was given on the assessment of bacteria and archaea. We could show that one week after inoculation in all inoculated treatments bacterial biomass exceeded the values from the original soil as a consequence of high dissolved organic carbon (DOC) concentrations in the disturbed soil resulting from the disturbance. This high biomass was persistent over the complete experimental period. In line with the high DOC concentrations, in the first two weeks of incubation, copiothrophic bacteria dominated the community, which derived from the inoculum used. Only in the disturbed control soils which did not receive a microbial inoculum, recolonization pattern differed. In contrast, archaeal biomass did not recover over the experimental period and recolonization was strongly triggered by amount of inoculated original soil added. Interestingly, the variability between replicates of the same inoculation density decreased with increasing biomass in the inoculum, indicating a deterministic development of soil microbiomes if higher numbers of cells are used for reinoculation.

Contributions:

- Experimental design
- Soil collection
- Conducted the microcosm experiment
- Conducted the sample processing (DNA extraction and sequencing)
- Conducted the bioinformatical analyses and statistics
- Wrote the manuscript

1.2. Publication 2

The effect of plant species richness on microbial diversity maintenance during extended drought periods

Yuri Pinheiro Alves de Souza, Roberto Siani, Cynthia Albracht, Yuanyuan Huang, Nico Eisenhauer, Anja Vogel, Cameron Wagg, Michael Schloter, Stefanie Schulz

Short description:

Drought is a major stressor factor to soil microbial communities and the intensification of climate changes is predicted to intensify hydric stress all over the globe in the next decades. The absence of water leads to osmotic stress, decrease in nutrient viability and in the connectivity of ecological processes, especially in the soil, leading to decrease in diversity and ecosystem functioning. As possible mitigating factor to the consequences of prolonged drought periods, diversity manipulation can increase ecosystem resistance and resilience by improving metabolic redundancy and complementarity as biodiversity increases. In this sense, here we investigated the interaction between increasing plant diversity and the response of soil microbial communities to prolonged drought stress. For that, we made use of a well-established biodiversity experiment (The Jena Experiment) to investigate the oscillations in diversity and composition of soil bacterial community exposed to long drought periods alongside a plant diversity gradient. Plots were covered from natural precipitation during summer in a period of 8 years, in a gradient of plant diversity ranging from monoculture to 60 species. Our data indicates that bacterial diversity increased after the exposition to drought, being the increase stable along the plant diversity gradient. This data indicates the long-term drought actually promotes soil diversity, by increasing niche differentiation.

Contributions:

- Conducted the sample processing and sequencing
- Conducted the bioinformatical analyses and statistics
- Wrote the manuscript

1.3. Publication 3

The seeds of Plantago lanceolata comprise a stable core microbiome along a plant richness gradient.

Yuri Pinheiro Alves de Souza; Michael Schloter; Wolfgang Weisser; Yuanyuan Huang; Stefanie Schulz

Short description:

Seed endophytic bacteria are plant-beneficial bacteria that thrive inside seeds. They improve seedling growth by enhancing plant nutrient uptake, modulating stress-related phytohormone production, and targeting pests and pathogens with antibiotics. Seed endophytes can be influenced by pollination, plant cultivar, and soil physicochemical conditions. However, the effects of plant community diversity on seed endophytes are unknown. To investigate the effects of increasing plant diversity on the diversity and composition of the seed microbiome, we made use of a well-established long-term biodiversity experiment in Germany (The Jena Experiment). We sampled seeds from different *Plantago lanceolata* blossoms in a plant diversity gradient ranging from monocultures to 16 species mixtures. Seeds were surface sterilized to remove seed surface-associated bacteria and subjected to a metabarcoding approach to assess bacterial community structure. Our data indicates a very stable core microbiome, which accounted for more than 90 % of the reads and was present in all seeds independent of the plant diversity, from which the seeds originated. It consisted mainly of reads linked to *Pseudomonas rhizosphaerae*, *Sphingomonas faeni* and *Pirellulla spp*. The number of unique ASVs in each diversity level was positively correlated with the plant diversity. Our data indicates that the seed microbiome can be influenced by the surrounding plant diversity. Thus, impacting the next plant generation.

Contributions:

- Experimental design
- Seed sampling and processing
- Conducted the sample processing (DNA extraction and sequencing)
- Conducted the bioinformatical analyses and statistics
- Wrote the manuscript

2. Introduction

2.1. Biodiversity – Definitions and measures

According to the United Nations Environment Programme (https://www.unep.org/unep-and-biodiversity), Biodiversity is the variety of life on Earth, including all organisms, species, and populations; the genetic variation among these; and their complex assemblages of communities and ecosystems. It also refers to the interrelatedness of genes, species, and ecosystems and in turn, their interactions with the environment. Three levels of biodiversity are commonly discussed: genetical, species and ecosystem diversity. The genetic diversity comprehends the different genes contained in all the living species, including individual plants, animals, fungi, and microorganisms. Species diversity refers to the different species, as well as the differences within and between different species while ecosystem diversity accounts for the diversity of all different habitats, biological communities, and ecological processes, as well as variation within individual ecosystems.

This diversification of life is considered to be the cornerstone of the successful colonization of earth. It came from the need to occupy different niches and therefore the opportunity to exploit different energy resources, which led to intricate networks of relationships and dependencies we can observe today. The molecular bases of life itself, shared by all domains of life, actively incentivize and support the diversification of life forms through mutations, recombination and genetic drifts, which ultimately leads to different metabolism and strategies (Crawford and Whitney 2010; Tilman, Isbell, and Cowles 2014).

Local changes in the biodiversity of a specific environment can be accessed by the estimation of several different metrics which account for changes in both the number of different species on that environment and changes in the abundance of those species. *Species richness* accounts for the simple number of different species present in each environment. Therefore, a *species rich* environment would harbour a higher number of different species then a *species poor* environment. If species abundance is taken in consideration, the species *evenness* can be estimated. Evenness measures how evenly individuals are distributed among different species in a community. A community with high evenness has a more balanced distribution of species abundances. Shifts in species evenness can indicate changes in the pattern of dominant species, resulting from internal competition or differences in ecosystem pressure (X. Wang et al. 2021). Diversity indices combine both richness and evenness information in the same metric, estimating how those two factors are connected (Hubálek 2000). For that, different diversity indices were proposed during the years, each taking in consideration different aspects of the relationship between richness and evenness. The Shannon Diversity Index, also known

as Shannon-Wiener Index, was proposed by Claude Shannon and Warren Weaver in their book "The Mathematical Theory of Communication," which was originally published in 1949 and is still to this date one of the most used diversity metrics in ecology to estimate biodiversity. The index calculation takes in consideration both the number of different species and their relative abundance and reach higher numbers as more species are added and as the species present become more evenly distributed. In this case, a rich environment with very low evenness (high dominance) will have lower Shannon diversity estimation then a less rich environment with evenly distributed species. Another widely used diversity index, The Simpson Diversity Index, sometimes referred to only as the Simpson Index, was proposed by Edward H. Simpson, a British statistician, in 1949. Like the Shannon Diversity Index, Simpson's Index is a measure of biodiversity in a community, but it focuses more on the dominance or concentration of species rather than considering both species richness and evenness as in the Shannon Index. Simpson's Diversity Index ranges from 0 to 1, where 0 indicates infinite diversity (maximum evenness), and 1 indicates no diversity (all individuals belong to a single species). In this case, the index calculates the probability that two individuals randomly selected from the community belong to the same species. Therefore, while high diverse environments have higher Shannon diversity due to increase in species richness and evenness, they´ll consequently decrease in Simpson Index estimation, due to the smaller chance to repeatedly detect the same species. Those metrics are useful tools to access changes in biodiversity caused by both natural and anthropogenic sources and are commonly used in ecology (Tucker et al. 2017). Those metrics, which describe the diversity *withing* a given habitat, are termed Alpha diversity metrics, in contrast to Beta diversity metrics, which describe the variation in species composition between different habitats or ecosystems, quantifying the change in species diversity between two distinct communities. The nomenclature of alpha and beta diversity in the context of ecology and biodiversity was first introduced by R.H. Whittaker, an American ecologist, in a paper published in 1960 (Whittaker 1960) and its still widely utilized in ecology research in current literature.

Beta diversity metrics in ecology are based on the comparison of species composition between different sites or habitats. These metrics aim to quantify the variation in species composition or turnover among distinct communities. Therefore, beta diversity is a value derived from the differences between the composition and/or abundances of species present in a given habitat. Commonly used beta diversity metrics are the Jaccard's Index, which measures the proportion of shared species between two communities (A and B) relative to the total number of species in both communities (Jaccard 1912) and the Bray-Curtis Dissimilarity Index, which quantifies the dissimilarity between two communities based on the abundance or presence-absence of species (Bray and Curtis 1957). It considers both the shared and unique species in each community. Since the result of beta diversity

analysis are exponentially complex as the number of observed habitats increase (each habitat or sample is compared against all the other samples in the dataset) visualization techniques are often implemented to assist the data interpretation. An example of technique utilized for the visualization and interpretation of beta diversity results is the Non-Metric Multidimensional Scaling (NMDS), which consists of a dimensionality reduction technique that transforms the dissimilarity matrix into a lower-dimensional space while preserving the original pairwise distances as much as possible and the result can be displayed as an scatterplot represents a community, and the distance between points reflects the dissimilarity between communities (Kruskal and Shepard 1974). Points that are closer together are more similar in species composition. The same is true for Principal Coordinates Analysis (PCoA), which similar to the NMDS can be used to spatially represent the dissimilarity between the habitats, however utilizing and creates a set of axes (principal coordinates) that capture the maximum variation in the dissimilarity matrix (Mead 1992). Other visualization strategies can also be implemented, as clustering analyses utilizing dendrograms, heatmaps and networks, being however less commonly used. Both NMDS and PCoA have become important tools in ecological and environmental sciences for visualizing and interpreting patterns of beta diversity and dissimilarity in community data. They are widely used in the analysis of species composition and ecological community structure. Altogether, alpha and beta diversity metrics help to distinguish complex ecological patterns and are useful tools in biodiversity experiments.

2.2. Biodiversity and ecosystem functioning

Ecosystem biodiversity and the increase in the number of different species has beneficial effects on the whole ecosystem. More diversity leads to an efficient occupation of different niches, promoting complementarity between the species in that specific environment and protecting the ecosystem as a whole from external disturbances (Hector et al. 1999). Those beneficial effects of biodiversity include the enhancement of ecosystem productivity, caused by the presence of a diverse array of species contributing to increased primary productivity and biomass production within ecosystems (Balvanera et al. 2014), the promotion of the stability and resistance to stress (Isbell et al. 2015) and ultimately the promotion of biodiversity itself, once more diverse environments favour the accumulation of genetic diversity over time (Hughes et al. 2008). Those effects can be attributed to the diversification of niches, metabolisms, and morphologies presented in a species-diverse environment which, in contrast to a species-poor environment, can more efficiently use the physicochemical resources available, optimizing the energy flow in the process (Barnes et al. 2018).

Ecosystem biodiversity also significantly contributes to the resilience of communities and populations against pathogen outbreaks. Both genetical and species diversity play a role in protecting the widespread infection agents (Alizon et al. 2009), since pathogenic and opportunistic bacteria, fungi, and especially viruses, rely on specific infection mechanisms, often species-specific ones, to successfully proliferate on their hosts. The same is true for invertebrate predators, such as caterpillars, locusts and aphids, which can easily unbalance food chains and destroy complete habitats, if left unchecked (Baker 2015). Monoculture plantations, for example, heavily rely on pesticides to reduce the herbivores population, and are extremely susceptible to viral and bacterial infections, due to the low genetic diversity of plant hosts (Letourneau and Bothwell 2008), which are usually monoclonal offerings. In this scenario, increasing the number of species can contribute to better resilience by recruiting competing and predating species against the invertebrate predators, increasing the genetic diversity of members in the same species, or simply diluting the number of susceptible hosts, which will further decrease and regulate the spreading rate of that given pathogen (Ostfeld and Keesing 2000). Altogether, those beneficial effects contribute to less severe outbreaks and give more time to the community to recover from those events.

Species biodiversity promotes higher biomass production (Balvanera et al. 2014) and ecosystem productivity. The increase in species number leads to higher complementarity between the species in the given environment, increasing overall biological activity. This increase in biological activity results in an improved nutrient turnover, leading to faster detoxification from contaminants (Dell'Anno et al. 2012) and faster recovery from disturbance events, like wildfires (Pugh et al. 2022), floods (Wright et al. 2015) as well as anthropogenic disturbance (Moreno-Mateos et al. 2017). The matter of increasing biomass production is especially relevant in the context of increasing atmosphere pollution. The annual report from NOAA's Global Monitoring Lab (2022) indicates that carbon dioxide concentration in the atmosphere in 2022 was, on average, 417 parts per million (ppm), in contrast to the 360-ppm concentration in 1960. The Global Carbon Project estimates that 43 billion tons of CO_2 are released into the atmosphere every year (2019), including agricultural, land use, fertilization and burning of fossil fuels. The accumulation of CO_2 in the atmosphere (among other greenhouse gases), as well as the catastrophic environmental consequences of global warming, can be mitigated by carbon sequestration and imprisonment in organic molecules, such as cellulose, which is potentialized by increasing biodiversity (Farrelly et al. 2013; Nanda et al. 2016).

2.3. The effect of biodiversity loss on ecosystem functioning

In the same way that biodiversity promotes ecosystem functioning and resilience against disturbance, diversity loss has serious consequences for ecosystem quality. Diversity loss can happen as result of natural disturbances, as for example geological and tectonic events, as well as species migration, invasion, and predation. However, the diversity loss in the Anthropocene (geological period of important human impact in the biosphere - Waters et al. 2016) reached astonishingly high levels on the global scale. Despite the low accuracy of current knowledge on the impact of human activity on species extinction and environmental impact, current estimations indicated that, due to human activity, around 600 species of plants have been extinct in the previous 250 years (Humphreys et al. 2019), while for vertebrates this number is 363 in the past 500 years (Johnson et al. 2017). Those numbers exemplify the direct and irreversible impact increasing human activity exerts on natural ecosystems. The extinction events are the result of extensive hunt (Price and Gittleman 2007), habitat destruction and fragmentation (Fahrig 2003), species relocation (McDonald-Madden et al. 2011), land use (Haines-young 2009; Jha and Bawa 2006), deforestation and/or overall consequences of climate change (Habibullah et al. 2022).

Regardless of whether extinct or not, the local effect of species loss has a direct impact on ecosystem quality and functioning. The lack of pre-existing species might result in empty niches and therefore disrupt the intricate web of interactions within ecosystems, making them more vulnerable to disturbances. This can lead to reduced ecosystem stability and resilience, increasing the likelihood of ecological shifts and even ecosystem collapse (Moreno-Mateos et al. 2017; Tilman, Isbell, and Cowles 2014; Wagg et al. 2017). Those effects can be observed in the drop of ecosystem services, like pollination (Hoiss et al. 2013; Isbell et al. 2017), climate regulation (Isbell et al. 2015), and nutrient cycling (Weisser et al. 2017). In terms of nutrient cycling, biodiversity loss is especially detrimental to ecosystem stability. While highly diverse environments harbour a quick turnover and acquisition of organic forms of carbon (Lange et al. 2015), nitrogen and phosphorus (Isbell et al. 2017), increasing the productivity in those ecosystems (Y. Yang et al. 2019), low diversity environments see a decrease in nutrient uptake and, therefore, in the overall ecosystem fertility. This phenomenon can be easily observed in intensely exploited agricultural fields, where the prevalence of monoculture cultivations, coupled with rapidly growing crops, drastically reduce soil carbon and nitrogen stocks (Cong et al. 2014; Gregorich, Drury, and Baldock 2001). The lack of nutrients impairs other ecosystem services like reducing decomposition rates (Handa et al. 2014), unbalancing soil chemistry stoichiometry (Aanderud et al. 2018) and increasing nutrient leaching (Grant et al. 2019). Since monoculture crops cannot sustains their own nutritional needs and also struggle to attend commercial demands for productivity,

growth rates in those artificial envrioments highly rely on intense fertilization, which ultimately leads to water (Trimble 2020), soil and (Kopittke et al. 2019) air pollution (Fluegge and Fluegge 2017), alongside direct consequences to human health (Dhankhar and Kumar 2023). The relationship between pesticide exposure and several types of cancers (Hu et al. 2015) and other chronical diseases as diabetes (Jaacks and Staimez 2015) have already being documented.

As previously established, although the addition of fertilizers supplements the lack of soil nutrients caused by the drop in crop diversity, monoculture crops lack other important ecosystem functions, like the protection against herbivory and pathogen infections. Low-diversity environments are more susceptive to weeds and pests, as well as infections by viruses, bacteria and fungi (King and Lively 2012). In this situation, the lack of genetic diversity intensifies the infection rates across the crops, leaving a very short time for individual recovery, while high diversity environments tend to have milder outbreaks and recover faster (King and Lively 2012; Ostfeld and Keesing 2000). Experimental evidence (Jr, Origin, and Haven 2000) from rice monoculture fields indicates the active manipulation of genetic diversity increased production yields in 89% and reduced the severity of rice blast (an infectious disease caused by the *Magnaporthe grisea* fungi) in 94%. Spatial distribution of different plant species has also being demonstrated to significantly reduce the spread of pathogens (Sapoukhina et al. 2010), posing a natural barrier for the pathogen dispersion.

Interestingly, nutrient-poor and disturbed environments tend to host very specialized species (especially in the case of plant communities), making the development of biodiversity itself more challenging than in healthy environments (Wardle 2006). That said, the absence of diversity creates a hostile environment where less competitive species struggles to thrive, creating a positive feedback loop where diversity loss promotes itself. In this loop, the ecosystem's capacity to recover from stress events and buffer extreme fluctuations is compromised (Berendse et al. 2015; Geisen, Wall, and van der Putten 2019), lowering the recovery process. A classic example of positive feedback loop, where loss of diversity potentialize itself, it´s the case of desertification of arears with damaged vegetation. As vegetations covering is removed, increasing soil erosion and soil moisture evaporation lead to a decrease in soil fertility, consequently reducing the successful establishment of new a new plant community and so on (Higginbottom and Symeonakis 2014). Figure 1 summarises the effects of increasing biodiversity (consequently the impaired ecosystem functions during diversity loss) over several ecosystem processes.

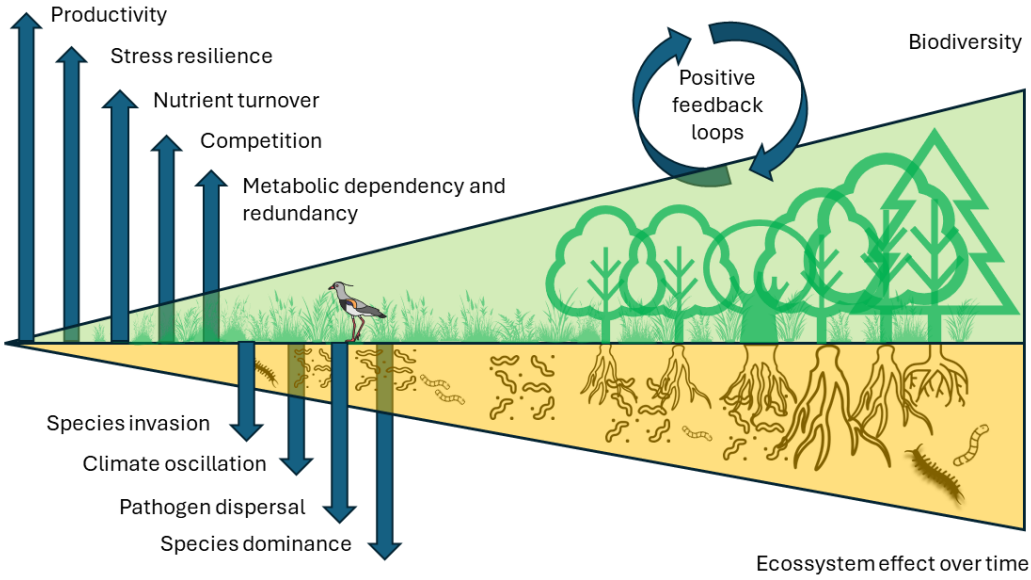


Figure 1: Schematic representation of increasing and decreasing ecosystem processes alongside biodiversity increase. Upwards arrows indicate increasing processes, while downward arrows represented decreasing processes (the size of arrows do not represent the increase/decrease dimension). As biodiversity increases, positive feedback loops (like increasing species interaction and ecosystem resilience) also intensify, potentializing beneficial effects. The effects of increasing biodiversity also increase over time, often taking seasons or years to be noticed. Those effects linger over time even though the causes are still not present, characterizing legacy effect.

2.4. Plant biodiversity services to ecosystem

Plants are the most abundant kingdom on earth. They correspond to 80% of the total life biomass on the planet, with an approximated amount of 450 Gt (Bar-On, Phillips, and Milo 2018), while bacteria are on the second place with approximately 10% (70 Gt), followed by fungi, archaea, protists, animals, and viruses, which together account for the remaining <10%. The Royal Botanic Gardens, Kew, launched the *Plant List* initiative to compile a comprehensive database of accepted plant names, and their latest update, published in 2013, listed around 1.04 million plant names representing roughly 350,699 accepted species.

The impressive diversity and overwhelming prevalence of plant species around the globe is reflected on the fundamental character plant communities perform on the maintenance of ecosystems. Despite it´s obvious role as primary producer in terrestrial ecosystems (Woodwell and Whittaker 1968), plant communities provide a multitude of essential services to ecosystem functioning, contributing to the overall health and balance of natural environments. One of the primary services is carbon sequestration through photosynthesis (Elbasiouny et al. 2022), where plants absorb carbon dioxide and release oxygen, helping regulate the Earth's atmospheric composition and providing oxygen for

autotrophic organisms. In the context of actual climatic changes (Huntington 2006; Nanda et al. 2016) the storage of carbon in the form of biomolecules, as cellulose, is a crucial factor for the stabilization of environmental changes, as has been used as strategy to mitigate those changes (Cong et al. 2014). Plants also play a crucial role in nutrient cycling and turnover, improving soil fertility. Due to the association with soil microbes and the recruitment of important microbial taxa (Park, Seo, and Mannaa 2023; Vives-Peris et al. 2020), as the ones responsible for nitrogen fixation and phosphorus solubilization, plant communities improve nutrient availability and nutrient storage in soil. This trait is especially interesting in the context of biodiversity studies, since different plant species contribute differently to the soil nutrient availability. In this sense, an environment with higher plant richness has access to a vaster diversity of beneficial traits, provided by its individuals. Current literature associate higher plant diversity to a higher environment productivity and stability in face of stressing conditions (Isbell et al. 2017; Tilman 1996; Wagg et al. 2022). This observation can be attributed to the complementary use of resources and the potentiation of synergistic interactions between the different species in a given environment. Diverse root traits (as length, dept and width), for example, lead to an optimal occupation of the soil´s tri dimensional space, increasing overall community water and nutrient uptake (Freschet et al. 2021). Long roots reach deeper depts in the soil, while short and thinner roots occupy smaller spaces among the bulk soil. The different roots in the soil also present different degrees of association with mycorrhiza and Rhizobia, also producing a more complex array of secondary metabolites, which are the main providers of carbon sources to soil microbial community (X. Wan et al. 2021).

A common example of the practical use of biodiversity manipulation is the rotation of crops and co-plantation, a farming practice that involves systematically growing different crops in the same field over a sequence of years or seasons. It is a traditional and sustainable agricultural technique aiming at the improvement of soil health, reducing pest and disease pressure, and optimizing crop yields. Crop rotation has been practiced for centuries and remains a fundamental strategy in modern farming systems (Dias, Dukes, and Antunes 2014). This approach increases productivity by matching farming systems management and genotypes and can reduce the use of fertilizers (Kirkegaard and Hunt 2010). Plant root systems help prevent soil erosion and stabilize landscapes, maintaining the integrity soil ecosystems by physically binding soil particles together. This process, known as soil aggregation (Lehmann, Zheng, and Rillig 2017), creates pore spaces in the soil, allowing water and air to infiltrate, therefore, promoting better drainage and aeration. Improved soil structure also reduces the risk of erosion by preventing topsoil from being easily washed away (Kopittke et al. 2019; Lal and Moldenhauer 1987). The root exudates, compounds including sugars, amino acids, organic acids, and enzymes (Park, Seo, and Mannaa 2023; Vives-Peris et al. 2020) are released by plant roots as secondary

metabolites, working as energy sources and recruitment baits to beneficial microbes present in the soil, forming a symbiotic relationship. Mycorrhizal fungi, for example, can form mutualistic associations with plant roots, enhancing nutrient uptake, particularly phosphorus.

2.5. Microbial biodiversity and its ecological role in terrestrial ecosystems

In complement to the importance of plant communities to ecosystem quality, Bacteria, the second most abundant group on earth (Bar-On, Phillips, and Milo 2018), are remarkably more diverse then plants and animals all together. This immense diversity can be observed phylogenetically, morphologically, and metabolically which leads to a complete ubiquity of the microscopic organisms as saprophytic, parasitic, and symbiotic organisms. Bacteria and archaea are especially diverse, when compared to their eukaryotic counterparts. While animals, fungi and non-photosynthetic protists display a strictly chemoautotrophic metabolism and plants and photosynthetic protists (also commonly known as eukaryotic algae, as the Chlorophyta and Bacillariophyta phyla) present a photoautotrophic metabolism, bacteria and archaea go further (DeLong and Pace 2001; Downs 2006). If classified according to their nutritional needs and energy acquisition strategy, bacteria and archaea can use both mineral (autotrophic) and organic carbon sources (heterotrophic), as well as acquiring energy from either light (phototrophic) or chemical reactions (chemotroph). The electron donors can be either organic (organotroph) or inorganic (lithotroph). The genus *Rhodospirillum,* for example, belong to the purple non-sulphur bacteria group, found in various aquatic and terrestrial environments where light and organic carbon sources are available and it´s rarely observed in oxygenated environments. The bacteria of this genus are capable of performing anoxygenic photosynthesis, using light as energy source but not fixing its own carbon, so a photoheterotrophic organism (Schultz and Weaver 1982). In the other hand, *Nitrosomonas* is a genus of bacteria that belongs to the group of ammonia oxidizing bacteria. These bacteria are chemoautotrophs, which means they derive their energy from the oxidation of inorganic chemicals (in this case ammonia - NH_3) and use carbon dioxide (CO_2) as their sole carbon source for growth. Specifically, Nitrosomonas bacteria are known for their role in the nitrogen cycle, where they participate in the process of nitrification, a two-step process that converts NH3 into nitrite (NO_2-) and then into nitrate (NO_3-). Nitrosomonas performs the first step of nitrification, which is the oxidation of ammonia to nitrite (Koops et al. 1991). Chemoautotrophs specifically can utilize a wide variety of subtracted for energy generation. *Hydrogenovibrio marinus,* abundant in hydrothermal vents, can use molecular hydrogen (H_2) as primary energy source (Nishihara, Igarashi, and Kodama 1991), while *Acidithiobacillus ferrooxidans,* commonly found in acidic

environments, including acid mine drainage sites, oxidizes ferrous iron (Fe_2+) for energy generation (Valdés et al. 2008).

The metabolic diversity of prokaryotes is reflected on the assembling and development of microbial diversity. This diversity leads to the ubiquity of bacteria and archaea in every environment on earth, from the ocean tranches to mountains, performing different wholes as free-living, parasites and commensals (Zeigler 2014). In soil, bacterial communities display a diverse array of metabolic capabilities, being the heterotrophic and aerobic metabolism the most abundant in the top layers in the soil. As soil dept increases, oxygen availability diminishes, opening space to alternative, less efficient lifestyles. This special and metabolic complementarity between soil bacterial communities' results in an intricate net of dependencies and highly complementary metabolic networks, where bacteria utilizes both available nutrients and secondary metabolites for growth. The assembly of microbial communities is, therefore, shaped by the environmental conditions, the metabolic needs of its components and the ecologic relationships between the species in that given environment, similar to the assembly of plant and animals communities (Nemergut et al. 2013). The microbial communities, however, display unique characteristic which differentiate them from the assembling of macroscopic communities. Microbes can also evolve faster to adapt to new environments. While a generation time for plants and animals can be months, or even years, microbes can multiply in a matter of minutes. This trait seems to be specially important during the colonization of extreme environments, where the stressful and demanding conditions seems to speed up the evolutionary rates (S. J. Li et al. 2014). This demonstrates how environmental conditions are important for microbial colonization, determining how the ecological succession will develop over time.

The biosphere greatly benefits for the ubiquity and diversity of microbes. According to the circumstances, specific microbial taxa can be selected according to its metabolically needs and physicochemical capabilities. Environments with high concentration of ammonia, for example, will naturally selects ammonia-oxidizing bacteria like the ones in the *Nitrosomonas* genus (Nakagawa and Takahashi 2015), capable of converting NH_3 into NO_2^-. In the other hand, environments with lack of nitrogen will represent an empty niche for nitrogen fixation bacteria, as the *Rhizobium* genus, and so on. Microbes are catalysers for unique chemical reactions on the element cycling, which are essential for the supply of those nutrients on the further trophic levels. Besides the above cites nitrification and nitrogen fixation, other examples of nutrient cycling reactions catalysed only by bacteria and archaea are denitrification (*Paracoccus* - Carlson and Ingraham 1983), methanogenesis (*Methanobacterium* and *Methanococcus* - Goyal, Zhou and Karimi 2016) and sulphate reduction (*Desulfovibrio* - Goldstein *et al.* 2003).

Microbial species diversity is specially interested when put the in the context of soil microbiology, where microbes directly interact with plants and animals. Soil is one of the most diverse environments on the planet (Kennedy and Smith 1995). Its heterogeneity leads to complex three-dimensional spaces where microbes interact and compete for nutrients and niche formation, promoting species diversification and interaction (Morris, Lenski, and Zinser 2012). A single gram of soil can host up to 10^{10} bacterial cells and an estimated species diversity of between $4{\cdot}10^{3}$ to $5{\cdot}10^{4}$ species (Torsvik, Goksoyr, and Daae 1990). Soil environments are often dominated by metabolically diverse phyla, as Proteobacteria, Actinobacteria and Firmicutes (Janssen 2006), related to nutrient turnover, decomposition of recalcitrant compounds and plant growth modulation (van Bergeijk et al. 2020; Spain, Krumholz, and Elshahed 2009; Wrighton et al. 2008). The importance of soil for agriculture (Kopittke et al. 2019) and species preservation (Decaëns et al. 2006) led to extensive research in the past decades. Responsible for most of the human food production, soil is one of the most extensively used natural resources. According to the Food and Agriculture Organization of the United Nations (FA0) the fertilization demand in 2019 was four times higher than it was in 1961, a necessary expansion to keep up with the increasing population, which on 15 November 2022, was projected to reach 8 billion people, a huge milestone in human development.

In this scenario, the study of soil sciences and especially soil microbiology becomes extremely relevant. The majority of the fertilization consumption aims to replace macronutrients used for plants during their growth, as nitrogen (in the form of ammonium nitrate, urea and ammonium sulphate), phosphorus (superphosphate, triple superphosphate, and diammonium phosphate), and potassium (as potassium chloride, potassium sulphate, and potassium nitrate.), often applied as NPK mineral fertilizer (Ludwig et al. 2011).

Despite the necessity to sustain humanity growth, current fertilization strategies are extremely deleterious for the environment, contaminating water bodies (Trimble 2020), altering the soil's pH, nutrient balance, and microbial composition (Geisseler and Scow 2014), polluting the air (Fluegge and Fluegge 2017) and heaving lingering effects in human health (Ahmed et al. 2017). The Haber–Bosch process, for example, is the industrial reaction utilized to convert the extremely stable molecular nitrogen (N_2), abundant in the atmosphere, into ammonia (NH_3), which can later be assimilated by plants. This process alone accounts for 1.4% of global carbon dioxide emissions and consumes 1% of the world’s total energy production (Capdevila-Cortada 2019).

Therefore, the study and investigation on how to manipulate and optimize the use of soil microbial communities, often coupled with the study of plant communities (Lange et al. 2015; G. Yang et al. 2021) appears as an alternative to the conservation and sustainability in soil usage. Bacteria and

archaea are the only known organisms capable of performing natural nitrogen fixation, which is enhanced by a symbiotic relationship with plants (Powlson 1993; Schulz et al. 2013). On the other hand fungi (specially mycorrhiza), can tightly associate with plant roots, greatly improving plant nutrient and water uptake, promoting plant growth and stress tolerance. (Branco et al. 2022). The manipulation of plant community composition (Abalos et al. 2021; J. Wang et al. 2021) as well as the use of microbial inoculants (Santos, Nogueira, and Hungria 2019) has already been demonstrated to improve soil quality, nutrient cycling and plant nutrient uptake, collaborating for a healthier agricultural system

2.6. Interactions between plant and microbial communities

Due to its ubiquity, microbes interact with basically all other living beings on the planet. Plants and animals co-evolved alongside microbes, profiting from this relationship as much as possible (Groussin, Mazel, and Alm 2020). Plants develop specific mechanisms to exploit microbes, optimizing nutrient acquisition, promoting plant growth and improving protection against pathogen infection (Miliute et al. 2015). Besides the already mentioned association with nitrogen-fixing bacteria, which facilitate nutrient acquisition by the plant and the accumulation of more label forms of nitrogen in the soil, plants also utilize the association with bacteria and fungi to, for example, improve phosphorus acquisition by association with phosphate-solubilizing bacteria (Zhonghua Wang et al. 2022). Those bacteria can solubilize mineral phosphorus by releasing organic acids as a result of their secondary metabolism, releasing this nutrient to the surrounding plants (Saeid, Prochownik, and Dobrowolska-Iwanek 2018). Fungi can also create intricate connections with plant tissues, especially roots, where mycorrhizae fungi, as the *Rhizophagus* genus, can improve water acquisition by increasing the surface contact area for the roots and also has been shown to produce important phytohormones as cytokinin (isopentenyl adenosine) and an auxin (indole-acetic acid) (Pons et al. 2020).

More interestingly, besides the so-called *epiphytic* microbes, which are deposited on the plant surface, and loosely associated with the plant individual, some bacterial interactions happen at the cellular level. The *endophytic* microbes, usually bacteria and fungi, resides inside the plant tissue, between the cellular space, stablishing a symbiotic relationship with their plant hosts (Khare, Mishra, and Arora 2018; Wu et al. 2021). In this case, as plant provides shelter and nutrients filtered from the environment to their microbial guests, microbes directly modulate plant metabolisms and protect their hosts against stress and infections. Taking in consideration the metabolic dependencies between plant hosts and its microbial commensals, currently literatures refer to the plant-microbe system as a *holobiont* (Baedke, Fábregas-Tejeda, and Nieves Delgado 2020), making a reference to an eukaryotic

host (in this case a plant, but the term has also being use to describe animal-microbe associations - Thompson *et al.* 2014; van de Guchte, Blottière and Doré 2018) and its associated microbiome. The term acknowledges the fact that multicellular eukaryotic organisms evolve in the context of its surrounded microbiome, relying on microbes for certain metabolic task, creating a dependency relationship from both sides.

The already mentioned association between leguminous roots such as soybeans, peas and clover with nitrogen-fixing bacteria as the ones in the *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium* genera (Oldroyd 2013) are a clear example of holobiont interaction. Roots can also associate with taxa as *Pseudomonas*, *Bacillus*, *Enterobacter,* which, as previously stated, can solubilize phosphate (Oteino et al. 2015). Regarding phytohormone production, some of those compounds can be produced by the plant or by its endophytic microbiome. Auxins, for example, are a class of plant hormones that play a crucial role in regulating various aspects of plant growth and development, and can be produced by *Azospirillum* (Somers et al. 2005), *Bacillus* (Wagi and Ahmed 2019) and *Pseudomonas* (Ouzari et al. 2008) bacterial genera, as well as *Trichoderma* (Contreras-Cornejo et al. 2009) and *Penicillium* fungi (Babu et al. 2015). The auxins are involved in processes such as cell elongation, root and shoot development, apical dominance, phototropism, gravitropism, and tissue differentiation (Ludwig-Müller 2011; Spaepen and Vanderleyden 2011). Auxins are primarily produced in the tips of growing plant parts, such as shoot apices and young leaves, and are then transported downward to other parts of the plant (Spaepen and Vanderleyden 2011).

Endophytic bacteria can influence the production and modulation of various plant defence compounds, enhancing the plant's ability to defend against pathogens and herbivores. Phytoalexins, as resveratrol produced in grapes (Lekli, Ray, and Das 2010) and stilbenes in various plant species (Valletta, Iozia, and Leonelli 2021), for example, are antimicrobial compounds produced by plants in response to pathogen attack. Endophytic bacteria can induce the production of phytoalexins, enhancing the plant's resistance to pathogens (Khare, Mishra, and Arora 2018). Terpenoids, another diverse group of compounds that include essential oils and resinous substances, can have their production stimulated by bacterial and fungal endophytes, improving the antimicrobial and repellent action against herbivores and pathogens (Galindo-Solís and Fernández 2022; Helfrich et al. 2019). The same is true for the production of phenolic compounds (Marhuenda-muñoz et al. 2019), glycosides (Z. Liu et al. 2023; Mraja et al. 2011), alkaloids (Y. Liu, Liu, and Liang 2015) and hydrolytic enzymes (Dogan and Taskin 2021) which can either have their production stimulated by endophytic bacteria or can be produced by the microbes themselves. Those substances are compounds that can deter herbivores by causing toxicity or unpleasant taste, increasing the plant's defence against pests and also improving their resistance against microbial infections (Afzal et al. 2019).

The modulation of plant growth and stress response is essential during challenging environmental conditions and is key for plant survival and productivity in low-diversity environments. When diversity and ecosystem services are compromised, plant microbiome can complement plant defences (Tyc et al. 2020; Zhu et al. 2018), help with nutrient, and water acquisition and protect the plant individual against opportunistic pathogens (Miliute et al. 2015; Tyc et al. 2020). Recent experimental evidence indicates that the modulation of plant response to stress can actively influence plant evolution. Hawkes, Bull, and Lau (2020) used infectious disease and quantitative genetics models to demonstrate that plant-associated microbiome can alter the plant perception of the environment, potentially reducing the strength of selection acting on plant stress tolerance or defense traits and/or altering the traits that are the target of selection.

This microbial colonization changes according to the plant species, seasons and also reacts to environmental changes. A study from Ding and Melcher (2016) using leaf samples from five plant species collected at four sampling times from four different locations identified that both plant species and collection point had a significant impact of leave endophytic microbiome composition. Despite the changes over time and according to the plant species, the study identified a concise core microbiome, composed by Proteobacteria, Bacteroidetes and Actinobacteria. A different study investigating different Mulberry cultivars identified significantly different microbial compositions amongst different cultivars of the same plant species and also a significant effect of different season samplings. This data indicates that microbial colonization can easily adapt according to individual traits between individuals of the same species. The microbial colonization patterns can also change according to external influences, like the use of pesticides (Salam and Kataoka 2023) and changes in environmental conditions, like temperature, light exposure and rain patterns (Trivedi et al. 2022). Those changes in the microbial colonization are a result from plant physiological adaptations to environmental changes, which can include reduced respiration rates, increase in total leaf area and sometimes increased assimilation rates at a warmer growth temperature, increasing carbon uptake and growth (Crous 2019; Trivedi et al. 2022).

Plant endophytic microbiome is also compartment dependent, being different organs and structures colonized by different microbial species. A less study compartment, seeds also present an intimate relationship with their endophytic microbiome, heavily relying on them for their development (Abdelfattah et al. 2022). Seeds are a crucial development stage in the plants' life, being essential for the successful dispersion of species along the landscape (Ungar Irwin A. 1987) and the resilience of plant communities through the formation of seed banks that can withstand challenging environmental conditions (V., Warrier, and Kunhikannan 2022). Germinating seeds and seedlings are, however, especially vulnerable to mortality from drought, herbivore predation, and fungal seed-borne and soil

pathogens (Bever, Mangan, and Alexander 2015). In this case, the seed microbial endophytic community can contribute to seed survival rates and later on its productivity and fitness. As observed for other compartments, the seed microbiome, comprises a diverse community of microorganisms residing both on (epiphyte) and within (endophytes) the seeds, offering a range of critical functions that profoundly influence plant health and development. These functions encompass nutrient acquisition and cycling, disease suppression, stress tolerance, and plant growth promotion (Berg and Raaijmakers 2018; Johnston-Monje, Gutiérrez, and Lopez-Lavalle 2021; Nelson 2018). Microbes constituting the seed microbiome contribute to the breakdown of organic matter (Fadiji and Babalola 2020), releasing essential nutrients for seed germination and subsequent growth, improving it (Tyc et al. 2020). Additionally, they engage in intricate interactions that help shield plants from pathogenic invaders by outcompeting or inhibiting harmful microbes (Lata et al. 2018). The microbiome's capacity to enhance stress tolerance equips plants with the ability to withstand adverse conditions like drought, salinity, and extreme temperatures, by actively inducing the activation of stress tolerance genes and producing molecules that reduce reactive oxygen species, for example (Lata et al. 2018).

More interestingly, seeds represent a transgenerational link between the parental plants and their offspring. In this sense, beneficial traits and environmental effects could possibly be vertically transmitted between generations, representing an advantage to seeds coming from healthy environments (less fertilization, pesticides and higher diversity, for example) to the detriment of seeds coming from unbalanced environments (Abdelfattah et al. 2022). Those effects, also called *legacy* effects, represent the long-lasting impacts that past land use, management practices, and environmental conditions can have on the characteristics and functioning of soil ecosystems. These effects can persist for years, decades, or even centuries after the initial disturbance or intervention (Cuddington 2011; Kostenko and Bezemer 2020). The legacy effects can be the result of management of nutrient cycling (e.i. crop rotation), lingering effects of Pesticides, herbicides, and other chemicals applied to the soil, changes in soil structure caused by activities like construction, mining, or compaction can influence water infiltration and, naturally, adaptation and selection of microbial communities (Cuddington 2011). The transmissions routs of seed microbiome and its interaction with environmental microbes are summarized in Figure 2.

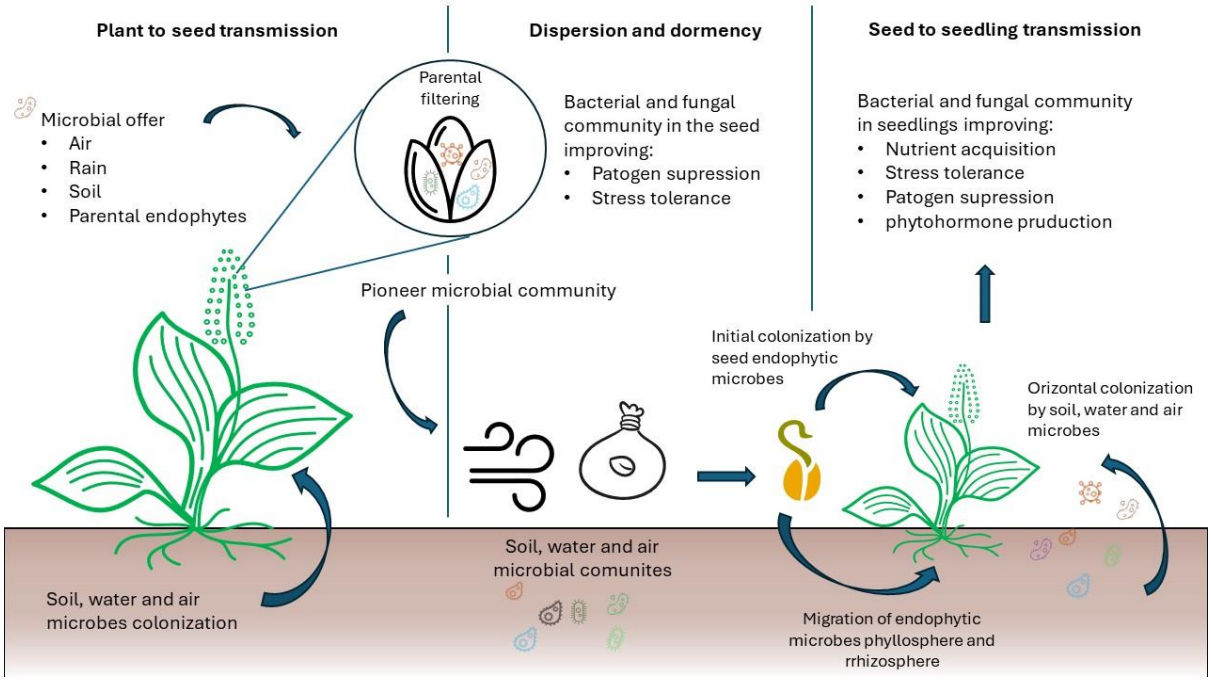


Figure 2: Schematic representation of the process of microbial transmission between parental plant and their offspring. The initial seed microbial community resembles environmental offering (microbes coming from air, water and soil) growing through parental filtering, which selects beneficial microbes among the ones in the environmental pool. During and after seed dispersion, the seed microbiome interacts and might be modified by microbes present in the seed bank and in the soil, constituting an important step on determining the microbes which will compose the plant individually during germination. While in the seed bank, exophytic microbes protect the seed from fungal and bacterial infections by competition and antibiotic production. During germination, seed microbiome is transmitted to the seedling, being specific strains migrated to either roots or leafs. The adult plant microbiome is initially composed exclusively by seed microbes, which are further complemented by soil, water and airborne microbiome.

2.7. Long-term biodiversity experiments as experimental platform

The beneficial effects of biodiversity, as well as the negative effects of biodiversity loss on ecosystem function and preservation can be experimentally investigated. Biodiversity experiments manipulate diversity composition and compare diversity features related to biodiversity, as the relationship between plant diversity and ecosystem functioning (M. Loreau et al. 2001), ecosystem stability in face of environmental disturbances (Tilman and Downing 1994), nutrient cycling and turnover (Cornwell et al. 2008), community dynamics (Michel Loreau and de Mazancourt 2013), invasion resistance (Civitello et al. 2015) and genetic diversity and individual adaptation to increasing biodiversity (Reed and Frankham 2003).

Those biodiversity experiments also benefit from long term runs, where legacy effects can be investigated through the seasons and year, dissentingly long-lasting and also delayed benefits of biodiversity (Youhua Chen and Shen 2017). The Cedar Creek Biodiversity Experiment (https://cedarcreek.umn.edu/), for example, was initiated in 1994 by a team of researchers from the

University of Minesota led by Dr. David Tilman. The experiment was designed to investigate the relationship between plant diversity and ecosystem functioning and consists of plots with varying levels of plant species richness. Different plant species were selected to create a gradient of diversity within the experimental plots. Some plots contain a single species (monocultures), while others have a mix of several species (Spohn et al. 2023).

Figure 3: Aerial view of the Jena Experiment field site. The experiment has been running since 2002 and aims to uncover the mechanisms that determine biodiversity-ecosystem functioning (BEF) relationships in the short and in the long term. During the last years, the Jena Experiment provided novel empirical evidence that ecological and evolutionary processes are intertwined in determining BEF relationships, and long-term experiments are key not only to gain a basic understanding of the relative importance as well as interactions of these processes but also to apply these concepts to better provisioning ecosystem functions and stability. In the next few years, we will focus on the biodiversity drivers of ecosystem stability, including temporal stability using unique time series and stability in response to extreme climate events, such as drought, flooding, hot spells, and exceptional frost periods.

In Europe, similar initiatives were also established to investigate similar questions. The Biodiversity Exploratories (https://www.biodiversity-exploratories.de/en), for example, project was initiated in 2006 and it's a large initiative involving researchers from various institutions in Germany and is designed to investigate the relationships between biodiversity, land-use practices, and ecosystem functioning across different types of landscapes. The project involves multiple study sites across different regions in Germany and encompasses a variety of ecosystems, including grasslands and forests (Bramble et al. 2024).

The research on this thesis was conducted in the frame of one of those biodiversity experiments. The Jena Experiment (https://the-jena-experiment.de/), a long-term (Roscher et al. 2004; Weisser et al. 2017) biodiversity experiment established in 2002 focusing on understanding the mechanisms and dynamics of plant communities and their interactions in grassland ecosystems, which focus on element cycling and trophic interactions. The primary goal of the Jena Experiment is to understand how changes in plant diversity influence various ecosystem processes, such as productivity, nutrient cycling, and stability (Weisser et al. 2017). The experiment was established on the floodplain of the river Saale in the city of Jena in Germany and consists of large outdoor plots that vary in plant species richness (Figure 1). Different combinations of plant species are sown to create a gradient of plant diversity, ranging from monocultures to mixtures with up to 60 different species (B. Schmid et al. 2004). Similar to other long-term biodiversity experiments, the experiment involves continuous and extensive monitoring. Data on plant growth, community dynamics, and ecosystem processes are collected over multiple years to capture the long-term effects of plant diversity on the ecosystem. Over the years, the experiment has demonstrated positive influence of plant richness on ecosystem productivity and resilience, showing the influence of species and functional groups on those responses, and highlighted the important of long-term experiments to distinguish actual ecologic trends from transitory effects (Weisser et al. 2017).

Plots with higher species richness generally exhibited greater biomass production compared to monocultures (Roscher et al. 2011; Scherber et al. 2010) and shown increased stability and resistance to invasion by non-native species (Roscher, Schmid, and Schulze 2009), suggesting that ecosystems with higher plant diversity may be more resilient to disturbances and less susceptible to colonization by invasive species. In terms of plant-microbiome interactions, previous experiments on the Jena Experiment field site investigated the relationship of increasing plant richness with soil, ophitic and endophytic microbial community. This data indicates that the diversity of plant species richness influences the diversity and composition of belowground microbial communities diversity (Lange et al. 2014; Steinauer et al. 2016) and abundance (Guenay et al. 2013), being different plant species supporting distinct microbial communities, and higher plant species richness associated with increased microbial diversity.

Those changes on soil and host-associated microbial communities were shown to have important consequences to nutrient cycling and biomass decomposition (Mellado-Vázquez et al. 2016), plant fitness and stress tolerance (Long, Schmidt, and Baldwin 2008). In this case, the increasing microbial diversity promoted by increasing plant species richness leads to improved plant-microbial interactions,

as increasing water and nutrient acquisition promoted by soil mycorrhiza (Baslam, Garmendia, and Goicoechea 2011; Kakouridis et al. 2022; Oldroyd 2013), increasing nutrient availability (as phosphorus and nitrogen) promoted by root associated bacteria and improved plant resilience against stress (Porter et al. 2020). Those potentializing interactions generate a feedback loop where plant diversity promotes microbial diversity which improve plant health and consequently overall ecosystem quality (Strecker et al. 2016).

2.8. Aims, research gaps and hypothesis of the thesis.

In the context of the beneficial effects of increasing biodiversity and its role on ecosystem resilience against stress, the worked developed on this thesis aimed to investigate the effects of artificially manipulated biodiversity on the diversity and composition of soil and plant associated bacterial community. As demonstrated previously, extensive research already indicated that increasing diversity has positive effects on ecosystem productivity and resilience to stress (Isbell et al. 2015; B. Schmid et al. 2004; M. W. Schmid et al. 2021; Weisser et al. 2017), however the interaction between increasing species diversity and the associated microbial diversity in both soil and plant is still poorly investigated.

Although plant community has already being show to directly affect and modulate soil microbial community (Chaparro et al. 2012; Lange et al. 2014; Park, Seo, and Mannaa 2023), the dimension of this effect hasn't being demonstrated yet, being the direct relationship between plant species richness and soil microbial diversity still to be investigated. The same can be said about the effect of increasing surrounding richness on the recruitment of beneficial microbes from the environmental pool to compose the seed endophytic microbiome. This research gap is intrinsically connected to the change caused by increasing biodiversity on soil, since the content of seed microbiome is a subset of the overall microbial offer from air, water and soil. The content of seed endophytic microbiome, as already established, can be influenced by sternal factors such as pollination and changes on physicochemical soil conditions (Bergna et al. 2018; Nelson 2018), which leads to the speculation that changes on soil caused by increasing plant richness can also influence the content of seed endophytic microbiome.

Moreover, we also investigated the effects of biodiversity on stress response and recovery of soil bacterial communities. Current literature already indicates that increasing biodiversity might mitigate negative effects over ecosystem functioning during stressing events (Isbell et al. 2017; Maron et al. 2018; Wright et al. 2015), but the dimension of this buffering effects, as well as whether it linearly increases alongside ecosystem diversity, is still unclear. Besides that, the importance of biodiversity for

the recovery of heavily disturbed environments, and until which extend the recovery process is consistent or not according to the provided biodiversity is still not know.

Therefore, we focus our research on the correlation between plant species richness and bacterial diversity, as a proxy of a direct effect of increasing plant biodiversity on soil and endophytic microbial communities. We also investigated the behaviour of stress exposed soil bacterial community (drought stress) and the role of biodiversity inoculum on the recovery of heavily disturbed soil environments (autoclavation). The research them broken down to three research experiment (or work packages), approaching those open topics. The specific research question on each experiment is described below.

2.8.1. Effect of initial soil diversity on microbial assembly

The first experiment part or our research (referred to in this document as **P1**) was design to investigate the assembly of soil bacterial communities over time, parting from different initial diversity loads. For that, we designed a microcosm experiment where a sterilized soil was reinoculated with its non-sterilized counterpart in different proportions, so we could investigate how deterministic is the development of a soil bacterial community in the same soil starting from different inoculation points and how this microbial community develops over time, inferring whether or not the initial diversity loads determine the community development. The experiment was conducted in a 4-week incubation period, where soil was incubated in different inoculation loads, alongside non-inoculated and original soil controls. DNA was extracted from the soil samples and the abundance of bacteria and archaea was estimated via quantitative PCR (qPCR) and changes in microbial composition were accessed via metabarcoding sequencing.

2.8.2. Effect of plant diversity on microbial stress recovery

The research followed by work package 2 (**P2**), where we directly investigated the effects of prolonged drought on the soil microbial communities in the Jena experiment, investigating how the plant diversity gradient in the field site affects these responses. We aimed to clarify whether plant community diversity could possibly buffer the loss of soil microbial diversity due to drought (Isbell et al. 2015; Wagg et al. 2017). For this, 80 plots with plant diversity of monoculture, 2, 4, 8, 16 and 60 species were split in half. The plots were sheltered from environmental precipitation during the summer season from the year of 2008 to 2016. Control plots had equivalent precipitation manually added, while drought-exposed plots were derived of water. Soil samples were collected in 2017 and DNA extraction

and metabarcoding sequencing were performed to assess changes in microbial composition along the diversity gradient. The microbial community diversity and composition were then compared between drought and control samples alongside the plant diversity gradient.

2.8.3. Effect of plant diversity and soil and seed microbial diversity.

Finaly, work package 3 (**P3**) consisted of the investigation of the effects of increasing biodiversity in the seed microbiome composition of a model plant, the herb *Plantago lanceolata*. As previously stabilised, seed endophytic microbiome can greatly improve plant health and resilience in the face of stress (Nelson 2018) and we speculate this effect can be potentialized by the increase in surrounding diversity. For this, blossoms from *Plantago lanceolata* individuals coming from plots with different diversity levels were sampled. Seeds were surface sterilized to remove epiphytic microbes and DNA extraction was performed in the seeds. The resulting DNA was amplified with chloroplast blocking primers to reduce the amplification of chloroplast sequences, abundant in plant samples. The amplified DNA was then submitted to metabarcoding sequencing. The diversity and composition of the seed endophytic community were then compared alongside the plant diversity gradient.

Therefore, in this work we approach the following hypothesis: 1) - Soil microbial community deterministically responds to environmental pressure during community assembly, being the community development over time and the final community composition significantly impacted by the initial diversity on the inoculum (**P1**); 2) - Plant species richness significantly impacts soil (**P2**) and seed endophytic microbiome (**P3**) composition and diversity, being the bacterial diversity in both compartments positively correlated with the increase in plant species richness due to increasing recruitment of microbial species; 3) – increasing plant species richness buffers diversity loss during extensive stress periods, due to increasing ecosystem resilience provided by increasing biodiversity (**P2**).

3. Materials and methods

3.1. Experiment design and sampling

This thesis is composed of three independent experiments. **P1** is a microcosms experiment testing the microbial colonization of sterilized soil by different microbial inoculum loads. **P2** and **P3** are field experiments where we investigated the effects of increasing plant diversity in the response of microbial soil communities to extensive drought periods and in the composition of seed endophytic microbiome, respectively. **P1** was executed in laboratory conditions in the facility of the Research Centre of Comparative Microbiome Analysis (COMI) in the Helmholtz Zentrum Muenchen, while **P2** and **P3** were executed in The Jena Experiment field site, in Jena.

3.1.1. Microcosm experiment (P1)

For **P1** soil samples were taken in June 2020 from" The Jena Experiment" field side (Roscher et al. 2004; Weisser et al. 2017 - http://the-jena-experiment.de/), which is located in Jena (Thuringia, Germany, 50°55'N, 11°35'E, 130 m a.s.l.) on the floodplain of the Saale River (altitude 130 m a.s.l.). The mean annual air temperature is 9.9 °C (1980–2010), and the mean annual precipitation is 610 mm (Hoffmann et al. 2014). The Jena Experiment is composed of 82 plots with dimensions of 20 X 20 m where plant diversity has been manipulated for more than 20 years. The soil for **P1** experiment was taken outside the treatment plots, reflecting the original soil where the experiment was built. The soil is classified as a Eutric Fluvisol (World Reference Base for Soil Resources 2015) with a pH value range from 7.1 to 8.4 and Corg 5–33 g C kg-1 (B. Schmid et al. 2004). A total of 50 kg of fresh soil was sampled from the top 20 cm of a 1 m2 area, using a shovel, and transported to the lab. For homogenization, the soil was sieved to 5 mm. The soil was split into two parts: one part to be disturbed and the other part to be used as control for the natural soil as well as to generate the inoculum. Soils were kept at 4 °C until further processing.

Disturbed soil was obtained by autoclaving. Autoclaving was done at 130 °C and 1.5 ATM for 1 h. After autoclaving the soil was incubated at 4 °C for one week to allow for potential spore germination and tested for successful sterilization. Therefore, 0.5 g soil and 100 µl of 0.8% NaCl sterile saline buffer were mixed. The obtained soil slurry was diluted 1:10, 1:100 and 1:1000, and 100µl of each dilution was plated on R2A agar plates. Plates were incubated for a week at room temperature, and growth was evaluated at the end of this period. This cycle of autoclaving, incubation and testing for sterility

was repeated four times until no microbial growth on R2A medium was observed. After four rounds of autoclaving no microbial growth on R2A medium was observed anymore.

To evaluate the soil microbial recolonization after autoclaving, disturbed soil was reinoculated with original soil in three different proportions: 1:10 (10% inoculation), 1:50 (2%) and 1:100 (1%) by mixing. The amount of inoculum was calculated based on soil dry weight (w/w). In addition, both non-autoclaved soil (termed Original) and the autoclaved non-inoculated soil (termed No inoculum) served as controls alongside the experiment. Per treatment 15 g of the soil mixtures were incubated in open, 50 ml Falcon Tubes (Universal Medical - Germany) at 20 °C in the dark for four weeks. During the incubation, soil moisture was kept constant at 50 % of maximum water holding capacity (mWHC) by watering every second day. Samples were taken at the beginning (T0) and after one (T1), two (T2) and four weeks (T4) of incubation from three independent replicates per treatment. Samples were immediately frozen at -20 °C for DNA analysis. Overall, 60 samples (5 treatments x 3 replicates x 4 samplings) were analysed. Figure 4 displays the experimental design.

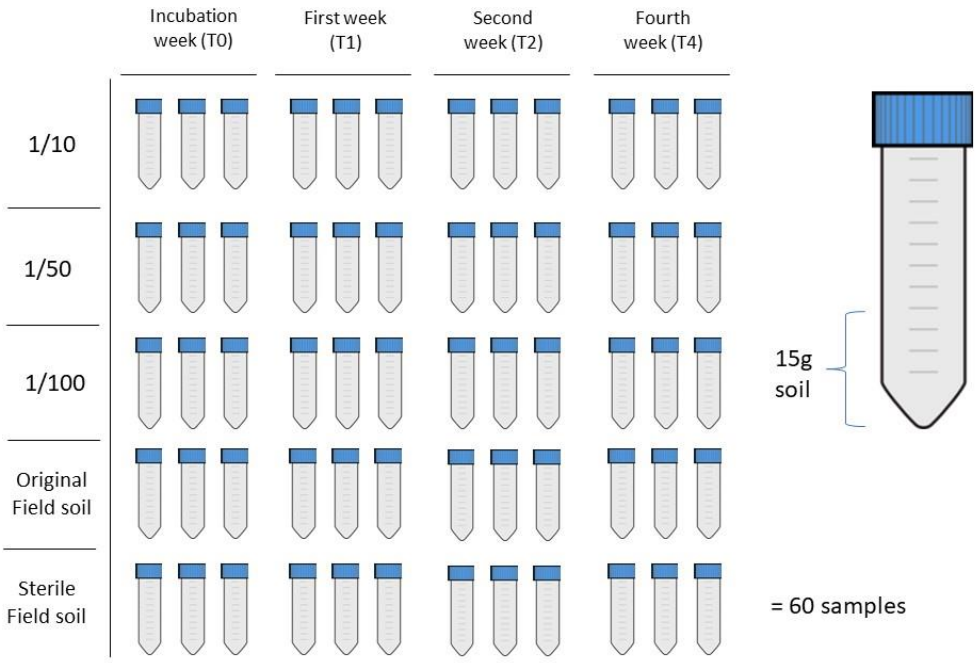


Figure 4: Experimental design from the soil inoculation experiment. Each tube contained 15g of the respective soil dilution (1:10, 1:50, 1:100, non-inoculated soil or original soil). Tubes were incubated at room temperature and water holding capacity was kept at 50% during the experiments' duration. Destructive samples were taken weekly and soil was frozen until DNA extraction.

3.1.2. Drought experiment (P2)

The experiment for **P2** was established in the field site of the Jena Experiment (Weisser et al. 2017). To investigate the effects of long-term drought over the soil microbial communities and how the increase on plant diversity can interact with those effects, a simulated drought experiment was established. In 2008 subplots were established inside the 80 experiment plots of the main experiment in the Jena experiment field site, nested in the preexisting plots across 6 different diversity levels (monocultures, 2, 4, 8, 16 and 60 species). Prior to the second annual mowing in September, transparent rain shelters (wood and PVC sheets, 2.6 x 3 m – Figure 6) were installed for 6 weeks every year to induce a prolonged summer drought period over a span of 9 years (2008-2016) (Vogel, Eisenhauer, et al. 2013). Of the two sheltered subplots, one received no water after installation (the 'drought' treatment), and one received collected rain water as equivalent precipitation after rain events ('control'), thereby controlling for non-drought roofing effects such as altered light and temperature (Vogel, Fester, et al. 2013). The roof shelters excluded 39.5 mm precipitation in 2009 and reduced summer precipitation by an average of 42% in 2008-2014 (Vogel, Scherer-Lorenzen, and Weigelt 2012).

Figure 5: PVC rain shelters utilized to simulate drought during summer months for our field experiment. In the top left corner the litter decomposition united utilized during the study where soil samples were extracted from are displayed (adapted from Vogel et. al 2013).

In July 2013, the field experiment was completely flooded during a natural flood event of the Saale River, disrupting the drought treatment during the flood period. The experiment and the treatments were continued after the flood recovered.

To access changes in microbial composition after the experiment, soil was sampled in each of the 160 subplots of the drought experiment in August 2017, one year after the last drought period. The subplots of the drought experiment were not covered by a roof construction during 2017. Table 1 displays the distribution of replicates across the field site, with its respective blocks.

Table 1: Replicate distribution across the different diversity levels and its respective blocks during the drought experiment. Plots inside each block are randomly distributed across the different diversity levels.

	Block 1		Block 2		Block 3		Block 4		
Diversity level	control	drought	control	drought	control	drought	control	drought	Total per level
1	3	3	4	4	4	4	3	3	28
2	4	4	4	4	4	4	4	4	32
4	4	4	4	4	4	4	4	4	32
8	3	4	4	4	4	4	4	4	31
16	4	4	3	3	4	3	3	3	27
60	1	1	1	1	1	1	1	1	8
								total samples	158

Soil sampling was conducted by the collection and pooling of 3 soil cores from the topsoil (0-15cm dept) of each subplot. Soil was then homogenized, sifted and store in -80°C until DNA extraction.

3.1.3. *Plantago lanceolata* seed experiment (P3)

For this experiment, we chose *Plantago lanceolata,* an abundant herb widespread in European flora as study model. We sampled the plots containing *Plantago lanceolata* in their mixture. Thus, seeds from all available monoculture plots (3) and from 4-, 8- and 16- species mixtures were sampled, which were 4, 2 and 3 plots, respectively. The *P. lanceolata* coverage at 60-species plots was too low for additional sampling. That sums up to 12 plots, which were sampled in September 2021. The sampling was done in a nested design, which is depicted in Figure 6. Per plot three plant individuals were sampled and per individual three blossoms. Blossoms were collected using gloves and surface disinfected scissors. Intact blossoms were stored in sterile 15 ml falcon tubes and transported to the laboratory at room temperature where the seeds were removed from the blossoms. Resulting seeds were surface sterilized as following: 1 min incubation in 1% tween; 2 min incubation in 70% ethanol;

3-times washing in sterile water; 5 min incubation in 5% NaClO solution; 3-times washing in sterile water (Estendorfer et al. 2017). The surface sterilized seeds were stored at -20°C until further processing.

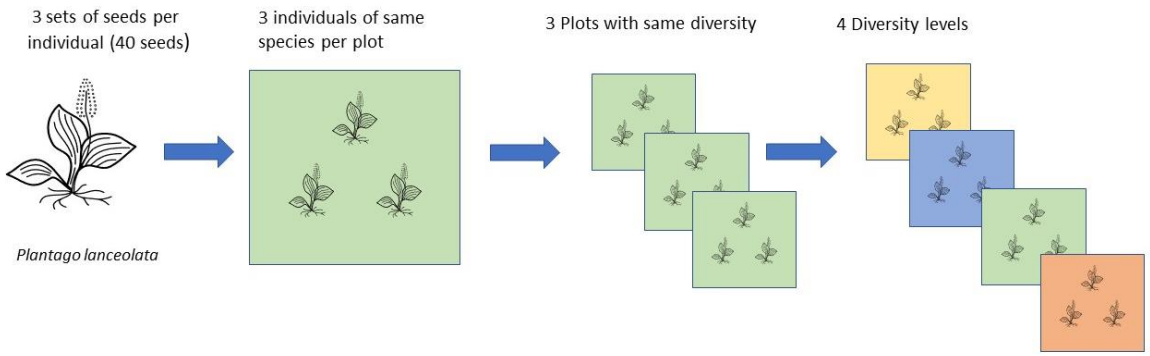


Figure 6: Schematic representation design for the seed sampling of P3. Samples were taken at blossom level, having each individual 3 blossom samples. Per plot, we collected 3 individuals, covering all the samples with that given diversity containing Plantago lanceolata individuals at monoculture, 4, 8 and 16 species levels.

3.2. DNA extraction

DNA extraction was performed for all experiments in this thesis. For **P1** and **P2** soil samples were extracted while, for **P3,** samples consisted of seed. Different DNA extraction techniques were used for each experiment.

For **P1**, DNA was extracted from 0.5 g of fresh soil following a Phenol/Chloroform/Isoamyl alcohol method (modified from Pommerenke and Friedrich, 2007)**.** The sample lysis was done using Lysing Matrix E tubes (MP Biomedicals™ - Germany). The bead beating was done using the TissueLyser II bead beater (QIAGEN®- Germany) at a frequency of 15 Hz during 2 min.

For **P2** DNA extraction was done from 0.5 g bulk soil samples, using the DNeasy powersoil kit (QIAGEN®- Germany) according to the manufacturer's instructions.

For **P3,** seeds were ground using sterilized mortars and liquid nitrogen. Per DNA extraction, 50 seeds were used, equalling 97,5 mg of seeds. The number of seeds was estimated based on the weight of 1000 *P. lanceolata* seeds, which is 1.95 g for plants from "The Jena Experiment" field site (B. Schmid et al. 2004). For blossoms with a lower number of seeds, all material was used. Seed DNA was extracted following a phenol/chloroform/isoamyl alcohol-based method (Lueders, Manefield, and Friedrich

2004) as described above. Sample lysis was performed using Lysing Matrix E tubes (MP Biomedicals™, Germany). The beat beating was done using a TissueLyser II bead beater (QIAGEN®, Germany) at a frequency of 15 Hz for 2 min. In all cases, the DNA quality of resulting DNA was quantified by Qubit fluorometric system (Thermo), and the quality was checked using the Nanodrop photometric system (Thermo) and by gel electrophoresis. The extracted DNA was stored at -20 C° until usage. To exclude contaminations during DNA extraction a blank control without samples was alongside negative PCR reactions were included.

In addition to the normal DNA extraction, for **P1** a smaller test was performed to estimate how much DNA in the original soil derived from intact cells. We used Propidium monoazide (PMA), a photo-reactive DNA-binding dye which, when exposed to light, degrades extracellular DNA (Carini et al. 2016). Therefore, a set of parallel DNA extractions was performed. Those comprise the original soil (-PMA), original soil treated with PMA (+PMA), a "dead" soil control (dead-PMA) and a "dead" soil treated with PMA (dead+PMA). The "dead" soil control was included to determine the capability of PMA to remove DNA from the samples, being used to calculate the efficiency of extracellular DNA removal. The "dead" soil samples were heated at 90 °C for 1 h prior to DNA extraction, to kill vegetative cells on the samples. The test was conducted in triplicate for each treatment. Soil samples with PMA (0.5 g) were incubated in the dark with 25 µM of PMA (Biotium - Germany) for 10 min, then exposed to white light for 25 min. DNA was extracted as described above. The percentage of DNA coming from dead and/or damaged cells was calculated by follows:

$$Extracelular DNA\% = \left[\left(1 - \left(\frac{dead + PMA}{dead - PMA}\right)\right) x \left(1 - \left(\frac{+ PMA}{- PMA}\right)\right)\right] x100$$

3.3. Quantification of bacteria and archaea

For **P1** Bacterial and archaeal abundance was determined by a SybrGreen based absolute quantification using a 7300 Real-Time PCR System (Applied Biosystems, Germany). To quantify bacteria, the primer pair FP 16S and RP 16S (Bach et al. 2002) was used; for archaea the primer pair rSAf(i) (Nicol, Glover, and Prosser 2003) and 958r (Bano et al 2004). Each PCR reactions contained 12.5 µL Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific - Germany), 1 µL of each primer with 10 pmol μl^{-1}, 0.5 µl of 3% BSA, 2 µl of extracted DNA, and 8.0 µL of DEPC treated water. Thermal profiles are summarized in Table 2. As standard curve serial dilutions (10^3 to 10^9 copies per μl^{-1}) of plasmids containing the 16S rRNA gene fragment of *Pseudomonas putida* for bacteria and *Methanobacterium sp*. for archaea were used in three technical replications. Besides three replicates per standard, three

no template controls were included in each run. The obtained copy number were subtracted from samples and controls.

To exclude any inhibitory effects of co-extracted substances a dilution test was performed, which identified a 1:32 dilution as sufficient. The specificity of the PCR product was ensured by doing a melting curve analysis as well as an agarose gel at 1.5%, running for 40 min at 120 V and 400 mA. Final copy numbers were calculated by normalizing the number of copies per µl to g of dry soil. The respective qPCR efficiency (calculated with the formula Eff = $[10^{(1/slope)}-1]$) and R^2 for bacteria and archaea ranged between R^2 0.997 and 0.982 and efficiency 76.47% and 71.12% for bacteria and; R^2 0.998 and 0.997 and efficiency 84% and 85.4% for archaea.

Table 2: Thermal profiles for qPCR of the 16S rRNA gene of bacteria and archaea.

Target Gene	Thermal Profile	N of cycles
16S rRNA Bacteria	95°C - 10 min	1
	95°C - 45 sec/58°C – 45 sec/ 72°C – 45 sec	35
16S rRNA Archaea	95°C-10 min	1
	95°C - 20 sec/55°C – 60 sec/ 72°C – 60 sec	5 (Touchdown: -1°C per cycle)
	95°C - 20 sec/58°C – 60 sec/ 72°C – 60 sec	40

3.4. Metabarcoding of bacterial and archaeal communities

To analyse bacterial and archaeal diversity in **P1, P2** and **P3**, MiSeq 16S rRNA gene metabarcoding was performed. For that, we followed the "16S Metagenomic Sequencing Library Preparation" protocol (Illumina, San Diego, CA, USA) and quality guidelines recommended by Schöler et al (2017). For **P1** and **P2,** bacteria and archaea were targeted together by using the universal primer pair 515FB and 806RB (Apprill et al. 2015; Parada, Needham, and Fuhrman 2016) with Illumina adapters. For **P3**, we amplified regions V3 and V4 region of the 16S rRNA gene using chloroplast exclusion primers S-D-Bact-0335-a-S-17 (338f) and S-D-Bact-0769-a-A-19 (789r) (Dorn-In et al. 2015) with an overhang sequences at the 5' end compatible with the Nextera® XT Index Kit. The used primers reduce over amplification of chloroplast sequences. For all experiments, PCR reactions were performed similarly: each PCR reaction contained 12.5 µL NEB Next High-Fidelity Master Mix (Thermo - Germany), 0.5 µL of each primer at 10 pmol μl^{-1}, 2.5 µL of 3% BSA, 1 µl of 10 ng μL^{-1} diluted DNA (10ng for **P1** and **P2** and 20ng for **P3**), and 8 µL of DEPC treated water. Thermal profile, DNA concentration, primers sequence and references are displayed in Table 3. After PCR reaction, samples were purified with the MagSi-NGSprep Plus Beads (ratio 0,8 beads: 1 sample) according to the manufacturers protocol and quantity of the PCR product was measured using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Germany).

Purified samples were indexed using Nextera® XT Index Kit v2 (Illumina, San Diego, CA, USA) and again purified with MagSi-NGSprep Plus Beads (ratio 0.8 beads: 1 sample). Quality assessment and final quantification of the indexed fragments was done via Fragment Analyser (Agilent - Germany). High-quality DNA was diluted to 4 nM and sequenced on Illumina MiSeq using MiSeq Reagent v3 (600 Cycle) kit. 5 pM 20% PhiX was loaded alongside the samples.

Raw sequences were demultiplexed based on the associated barcodes and adapters were removed using AdapterRemoval version 2.3.1 (Schubert, Lindgreen, and Orlando 2016).

Table 3: Details of PCR amplification for amplicon sequencing utilized on this thesis. P1 and P2 utilized primers optimized for soil samples, targeting both bacteria and archaea while P3 utilized chloroplast blocking primers, optimized for plant material as roots and leaves and, in this case, seeds.

Experiment	PCR cycling profile	DNA amount	Primer set	Sequence	Reference
P1	98°C - 1min (23x) 98 °C - 10 s, 55 °C - 30 72 °C - 30 s final extension 72 °C for 5 min	10ng	515FB / 806RB	515F - GTGYCAGCMGCCGCGGTAA / 806R - GGACTACNVGGGTWTCTAAT	Parada et al., 2016 /Apprill et al., 2015
P2		10ng	515FB / 806RB	515F - GTGYCAGCMGCCGCGGTAA / 806R - GGACTACNVGGGTWTCTAAT	
P3	98°C - 1min (30x) 98 °C - 10 s, 55 °C - 30 72 °C - 30 s final extension 72 °C for 5 min	20ng	S-D-Bact-0335-a-S-17 (338f) / S-D-Bact-0769-a-A-19 (789r)	338f -TCGTCGGCAGCGTCAGATGTGTATAAG AGACAGCADACTCCTACGGGAGGC / 789r-GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAGATCCTGTTTGMTMCCCVCRC	Dorn-In et al. 2015

3.5. Bioinformatic processing

Demultiplexed sequences were processed in different ways for each project: For **P1**, samples were processed using Qiime2 version 2021.2 (Quast et al. 2013). The samples were processed using denoise-paired option with the following parameters: --p-trim-left-f 20; --p-trim-left-r 20; --p-trunc-len-f 240; --p-trunc-len-r 200; --p-max-ee-f 4 ; --p-max-ee-r 4. Taxonomic assignments were done against SILVA database (version 138) using *classify-consensus-blast* option in default parameters. The raw sequencing reads were uploaded to NCBI sequencing read archive under the BioProject number

PRJNA937438 and BioSample SAMN33408602 (**P1**), BioProject number PRJNA937585 and BioSample SAMN37746197 (**P2**), BioProject number PRJNA937585 and BioSample SAMN33409010 (**P3**).

For **P2** and **P3,** after sequencing, demultiplexed samples were uploaded to the European Galaxy server (https://usegalaxy.eu). The Cutadpat tool was used to remove adapters, and read quality was accessed via FastQC and with the dada2 version 1.16 (Callahan, , Paul J McMurdie, Michael J Rosen, Andrew W Han, and Johnson 2016) plotQualityProfile option. Trimming parameters were set to 200 bp for **P2** and 280 bp for the forward reads and 220 bp for the reverse reads for **P3**. dada2 was used to trim the sequences without adapters. We also used dada2 to apply error rates, merge the read pairs and make a sequence table according to the default dada2 pipeline (https://benjjneb.github.io/dada2/tutorial.html). Taxonomy was assigned using assingTaxonomy and addSpecies function, aligning the ASVs against the Silva database (Quast et al. 2013) version 132.

For all experiments, the resulting table of amplified sequence variants (ASVs) was exported to R, where Phyloseq version 1.42.0 (McMurdie and Holmes 2013) and Vegan version 2.6-2 packages were used to construct rarefaction curves to observe sequencing coverage, normalize the number of reads in each sample using median sequencing depth, plot relative abundance and ordination plots (PCoA with Bray–Curtis dissimilarity distance). For **P3** the three blossoms per individual were treated as pseudoreplicates per plant individual level. Thus, only consent sequences detected on all three blossoms per individual were further considered. The normalized dataset was used to plot the taxonomical composition of the most abundant taxa in a form of a heatmap using the pheatmap package (version 1.0.12). ASVs with abundance equal o higher them 5% in at least one of the samples were filtered using the *filter taxa* function on phyloseq package. Dendrograms were drawn using Ward's Hierarchical Clustering.

3.6. Statistical analysis

Further the packages were used to calculate alpha diversity metrics (Observed ASVS, Shannon diversity and Evenness). Those metrics were used as response variable for experimental design and statistical significances across the treatment was calculated via two-way ANOVA (**P1**) and linear mixed effect models (**P2** and **P3**). Data was checked for normality via the Shapiro-Wilk tests and Q-Q plots. Pairwise comparison was done using Tukey Post Hoc test for multiple comparisons of means (**P1**) and estimated marginal means for the linear models (**P2** and **P3)** using emmeans package. For **P2** plant species richness (SR) was log-transformed to improve distribution and reach linearity. We fit the transformed data to a linear mixed-effects model using the lme function in the package *nlme* to investigate the

effects of block (BL), plant species richness (SR), drought (DR) and plot (Pl) on the measured variables (V – Shannon, Inverse Simpson and Observed Richness metrics for alpha diversity). The drought treatment was represented by subplots. The fitting order was m1<-lme(V~log2(SR)*DR,random=~1|BL/PL), moving the BL to a fixed term to investigate the changes in the outcome and changing SR per FG to investigate the effects of functional groups instead of species diversity. For **P3**, we also used Shannon and Observed Richness metrics as response variables, fitting those variables in a model alongside with the plot (PL) and individual (IN) information as random factor. The fitting order was m1<-lme(V~log2(SR), random=~1|PL/IN). We used PERMANOVA to calculate the effect size and significance over beta diversity, using as input a Bray Curtis dissimilarity matrix.

For **P1** we investigated the proportion of ASVs coming from the soil inoculation using Qiime2 plugin SourceTracker version 1.0.1 (Knights et al. 2011), used with default parameters to track the origin of ASVs in different treatments. The original soil at T0 was treated as source. The sink were the re-inoculated soils. Besides that, for **P1** we calculated phylogenetic signal for each sample, determining the mean-nearest-taxon-distance (MNTD) and the nearest-taxon-index (NTI) (Stegen et al. 2012) using 'mntd' and 'ses.mntd' implemented in the package 'picante' version 1.8.2 (Kembel et al. 2010). This procedure allows to determine whether a given community (input is a phylogenetic tree generated by Qiime2) is more phylogenetic related than expected by chance, when compared to a random version of this same community (*null model*) (Stegen et al. 2012). We use this metric to estimate whether microbial communities' assembly stochastically (NTI less than -2) or deterministically (NTI greater than +2). To generate the underlying phylogenetic tree, Qiime2 was used by applying the *alignment mafft* function for alignment, *alignment mask* to mask ambiguously aligned regions, *fasttree* for tree generation and *phylogeny midpoint-root* for tree rooting.

3.7. Dissolved organic carbon

For **P1** Dissolved organic carbon (DOC) was measured from soil samples. DOC was extracted by incubating four grams (g) of soil in 20 ml of 0.01 M $CaCl_2$ solution for 40 min on an overhead shaker (Schulz et al. 2013). Afterwards, samples were filtered using cellulose filter papers (595,5 filter papers, Whatman – Germany). The DOC concentration of the extracts was measured with a DIMA-TOC 100 (Dima Tec, Germany).

4. Discussion

During the work developed in this thesis, we investigated the effects of biodiversity on various aspects of microbial communities and the implications of those changes to ecosystem functioning. Based on the literature indicating that increasing biodiversity promotes higher ecosystem functioning (Civitello et al. 2015; Isbell et al. 2017; M. W. Schmid et al. 2021) and increasing resilience to environmental stress (Kowalchuk et al. 2002; Renard, Mahaut, and Noack 2023; Vogel, Scherer-Lorenzen, and Weigelt 2012), we approached the following questions: 1) does increasing plant richness affects soil and seed endophytic microbial community; 2) is the interaction between increasing plant richness and soil microbial communities capable of mitigating environmental stress and diversity loss for soil bacterial community; and 3) what are the main factors determining microbial community assembly after environmental disturbances. Ahead we´ll disentangle the results of our research and the implications of those findings to biodiversity research.

Those research questions come from the well know beneficial effects on increasing biodiversity for ecosystem functioning and resilience (Kennedy and Smith 1995; Isbell et al. 2017; Habibullah et al. 2022). Investigating those effects on field experiments, although harder to control and more expensive, can give us a more comprehensive understanding of distinct factors responsible for the ecological effects. Ecological systems are highly intricate and involve numerous interactions between organisms and their environment. Field experiments enable researchers to capture this complexity, including factors that may be challenging to replicate in a laboratory setting (Körschens 2006). The ecological validity of the findings on this kind of experiments are generally higher since they take in consideration spatial heterogeneity and ecosystem-level interactions which would be impossible to replicate in vitro. Altogether the robustness of field experiments makes the ecological findings more applicable to real-world situations, this being particularly important when trying to understand how organisms respond to their natural surroundings.

Another important aspect of field studies in long-lasting ecology experiments, is the assessment of long term and delayed biodiversity responses. Some ecological effects can be only perceived in a time scale, taking in consideration the life cycle of plants, animals and microbes present in a given habitat (Chen and Shen 2017). Seasonal changes on temperature and sun exposure, for example, are known to have strong effect on nutrient flux and nutrient stocks in soil (Wu et al. 2021; Uzel, Stanton and Scott 2023), which of course leads to changes on species behaviour over the seasons and can only be accessed and validated in a timeframe of years or decades. Some effects, related to changes in biodiversity composition, diversity loss and stress, are delayed and can be only perceived through the years. Those effects are the result of population dynamics, successional processes, and the slow

accumulation of disturbances effects over the time, which therefore can be only investigated in long term experiments (Essl et al. 2015). The study of phenology, i.e., the study of periodic biological phenomena that are correlated with climatic conditions, is only possible in long-term experiments. The persistent result of long-term ecological interactions is often referred as legacy effects (Cuddington 2011), and in the context of ecology refer to the persistent and lingering impacts of past events or conditions on current ecological systems. These effects can shape the structure, function, and dynamics of ecosystems for an extended period, even after the original trigger has been removed. Legacy effects are important considerations in ecological research since they highlight the interconnectedness of past, present, and future ecological conditions.

With this in mind, we consider that the results on this thesis represent consistent biodiversity effects in both soil and plant microbial community, reflecting two decades of legacy effects on the Jena Experiment, being therefore a robust assessment of biodiversity effects on microbial communities.

4.1. The effect of plant richness over bacterial diversity

The first research question approached in this thesis refers to the interaction between increasing plant richness and the diversity and composition of soil and seed endophytic microbes. The opportunity to investigate both soil and endophytic microbiome can give us a more complete overview of the effects of increasing biodiversity over microbial communities. Soils, harbouring immense microbial biodiversity, are considered to be the main repository of endophytic bacteria colonizing plant tissues (Bulgarelli et al. 2013; Wu et al. 2021). Therefore, while increasing plant richness have direct effects on soil microbial composition due to changes on nutrient turnover, exudate production and microbial recruitment (Roscher et al. 2004), effects on endophytic microbiome are indirect, passing through the physiological filtering of the plant tissue and can represent changes not only on the microbial community pool, but also adaptations on the plant organisms itself (Ding and Melcher 2016; Fadiji and Babalola 2020).

We made use of the Jena Experiment´s structure to disentangle this relationship. Although the covered diversity gradient was not exactly the same in both studies (the drought experiment, **P2,** had a more complete coverage of plant richness, with monocultures, 2-, 4-, 8-, 16- and 60-species plots, while the seed experiment, **P3,** did not covered the 2- and 60- species plots), we observed similar results. In both cases, we did not observe a direct correlation between plant richness and overall microbial diversity. However, despite the lack of significant effects on the overall bacterial diversity, we did observe shifts in the composition of the bacterial communities in both cases. For the soil samples, we could observe a significant effect of plant richness gradient on the beta diversity analyses among the soil sample from

different plots, while for seeds we could observe changes on the core microbiome of seed from plants growing in different diversity levels. Interestingly, we observed that the seed microbiome of Plantago lanceolata is rather consistent along the plant richness gradient, being mostly composed by the genera *Paracoccus*, *Alteribacillus*, *Sphingomonas*, *Pseudomonas*, *Massilia* and *Pirellula,* being *Pseudomonas rhizosphaerae* followed by Sphingomonas faeni and Pirellulla spp., the most abundant species present in the core microbiome. When analysing the complementary core microbiome, e.i., the additional bacteria accumulated on the seed microbiome along the diversity gradient, we observed a positively correlated increase in the number of unique ASVs present in the seed microbiome. In this case, the seed endophytic microbiome of *Plantago lanceolata* seems to display additional microbes as the plant diversity increase. Those ASVs were assigned to the same genus present on the core microbiome (with exemption of the *Pedobacter* genus which was present only in the levels 04 and 08 of plant richness), which indicates that the trait selection in this case might happen on the species, or even strain level, suggesting that bacterial selection by parental plant (Bergna et al. 2018; Johnston-Monje, Gutiérrez, and Lopez-Lavalle 2021) respond to subtle and highly specific processes.

Overall, our data aligns interestingly with current literature. Albracht *et al*. (2023), also using the same richness gradient on the Jena Experiment field site, observed a different trend for fungi community, which significantly responded to increase in plant richness. Previous data on the Jena Experiment indicates that plant species richness significantly affect overall fungal composition, with the association of specific fungal groups with determined plant richness levels (M. W. Schmid et al. 2021). In our case, although we did not associate specific taxa with a specific diversity level, we did identify different taxa responding to drought stress across the plant richness gradient (see next session in detail) and as already shown, different taxa being associated with the core microbiome on seeds. A similar study also observed that biodiversity effects on belowground environments might not be significant even though aboveground effects could be observed (Bessler et al. 2009), which can indicate that diversity effects on soil bacterial diversity can be confounded by other environmental factors, or that responses on soil microbial composition might respond to changes in smaller scales, as for example physiological conditions of plant individuals or the species identity on each plot. A study from Prober *et. al* (2015) counting with grasslands samples from four continents indicates no direct correlation between soil alpha diversity for bacteria, archaea and fungi with the increase in plant community diversity. The study did, however, indicate a strong relationship between plant and soil microbial beta diversity. This data aligns with our findings, indicating that although the increase in plant richness do not linearly increases microbial diversity, plant community does affect and modulate soil microbial composition, with the association with specific microbial taxa to certain plant species.

A possible explanation for this pattern is the recruitment of specific microbial taxa according to specific interactions with plant community. In this case, a further research question could be the assessment of the specific recruitment of microbial taxa according to plant species identity or functional group. The Jena Experiment also counts with a gradient of plant functional groups, running alongside the plant richness gradient which can be used to investigate such question, which unfortunately is not in the scope of this thesis.

Besides, we observed that the effects presented in the soil samples are not necessarily linked to the ones observed in the seed endophytic microbiome. Although we did not see direct increase in soil bacterial diversity alongside the plant richness gradient, we did observe a slight increase in the number of ASVs composing the core microbiome on *Plantago lanceolate* seed. This trend suggested that this complexification is not dependent on the soil diversity increased, which was not observed. The effect might be caused by changes in the filtering processes by parental plants, which have already been demonstrated experimentally (Bergna et al. 2018; Johnston-Monje, Gutiérrez, and Lopez-Lavalle 2021), but the mechanism behind this change is still unknow. This correlation, however, is merely suggestive since soil and seed samples were not collected in the same year and the soil-plant relationships might change over the years or seasons. More investigation in this topic seems to be necessary.

Overall, we conclude that plant species richness does affect soil and seed microbiome, but the effect if not linear or cumulative, being the changes qualitative instead of quantitative. Different plant traits as roots length, exudate production and the association with soil microbial community are reportedly responsible by modulating soil microbial community (Pascale et al. 2020; Santoyo 2022; Vives-Peris et al. 2020) which might consequently influence the endophytic microbiome associated to the plant host, affecting the seed colonization by beneficial taxa (Abdelfattah et al. 2021). Moreover, the interaction between different plant functional groups and/or species might lead to complex scenarios where the simple increase in diversity is not the determining factor.

In the context biodiversity studies, we can observe that the possible beneficial effects of increasing plant biodiversity over soil microbial diversity are not represented by overall increase in microbial diversity, which ultimately makes sense. Soil bacterial diversity in this case would represent an important repository of metabolic potential, which would be “activated” according to induvial needs on each plant community. In this case, the overall bacterial diversity would respond with changes in abundance and activity, with shifts not in the number of different species, but in the dominance of the most suitable taxa for each plant species combination. Plots without legumes, for example, might see an increase in abundance and activity bacteria related with nitrogen fixation, while plots with legumes

might see an increase in denitrifying bacterial groups. Additionally, the abundance versus activity discussion, cutting edge research topic in current microbial ecology, can help us to understand those responses. While microbial responses to environmental changes are always resulting of changes on microbial activity, those changes do not necessarily leads to changes in abundance of responding taxa (Hanson et al. 2012; J. P. Schimel and Schaeffer 2012), consequently not affecting community diversity metrics. Therefore, although soil bacterial diversity is a useful metrics to access changes in soil microbial community in response to environmental changes in, the lack of changes on diversity do not deny the microbial responses in our case. Increasing plant diversity has already been demonstrated to significantly affect soil microbial activity (Lange et al. 2015), corroborating this line of though.

4.2. The role of plant and soil diversity for stress mitigation and community assembly

Still in relation to the biodiversity study, we investigated how stress can shape the adaptation and development of soil microbial communities. In **P1,** we tested how initial diversity influences the colonization patters on soil microbial communities after severe stress (autoclavation), while on **P2** we studied how soil microbial community adapts to long-term, intermittent drought.

The resilience to stress and the ability to recover after disturbances is undoubtedly one of the biggest ecological benefits of ecosystem diversity. Both biological (parasites, invading species, and predators) and environmental (extremes of temperature, pH, precipitation, and chemical contamination) stresses can be attenuated when ecosystem processes are well complemented. Species diversity, for example, can dilute pathogen dispersion, reducing the infection efficiency of pathogens (Civitello et al. 2015; Ostfeld and Keesing 2000) and increase the degradation speed of contaminant compounds (Dell'Anno et al. 2012). In the same way, genetic diversity also plays a role on reducing the spreading efficiency of pathogens, greatly increasing species survivability during outbreaks (King and Lively 2012; Lively 2010). Ecosystem diversity improves stress resistance by providing functional redundancy, granting that even if a few species get eliminated during the process, essential ecosystem services as nutrient cycling and biomass decomposition will continue existing (Mitchell et al. 2000; Renard, Mahaut, and Noack 2023; Wagg et al. 2017). This functional redundancy, also reference as Insurance Hypothesis (Yachi and Loreau 2011), prevents ecosystem breakdown during disturbing events. The buffering effect of increasing biodiversity has been largely observed in ecology. Cesarz *et. al.* (2017) demonstrated that increasing plant sustained higher nematode diversity after a summer flood on the Jena Experiment field site, in comparison to monoculture plots. The same can be said regarding microbial communities, whose stability against stress has already been shown to be directly dependent on the community diversity itself (Tardy et al. 2014).

4.2.1. Plant diversity and drought stress.

During the drought experiment on the Jena Experiment field site (**P2**), we investigated the interaction between increasing plant richness and the effect of intermittent summer drought on soil bacterial communities. Drought events have a drastic impact on plant and animal community abundance and composition, as well as soil microbial communities, limiting nutrient availability (Carson et al. 2010; Lawrence, Neff, and Schimel 2009), reducing community connectivity through dissolved molecules (Carson et al. 2010; Manzoni et al. 2016; Manzoni and Katul 2014) and, obviously, diminishing the availability of water as a resource itself, which is essential to the basic functioning and maintenance of cellular processes (Potts 2001). On the one hand, drought events can quickly change soil properties and nutrient availability, a situation where copiotroph microbial taxa do not have enough time to react to drought exposure or simply do not possess the necessary machinery to cope with the changes imposed by drought. In our experiment, however, we tested long-term, intermittent drought, a difference that might have important implications for the soil microbial community response to drought. In this case, chronic drought imposes a different challenge to soil microbial communities. The lack of water diminishes soil homogenization, isolating the microbial community in smaller compartments, which promotes niche formation (Carson et al. 2010), species differentiation (Dumbrell et al. 2010), and an increase in microbial metabolic dependency (Morris, Lenski, and Zinser 2012). The absence of water also leaves space for more air and therefore more oxygen (Preece, Farré-Armengol, and Peñuelas 2020), increasing the access of the soil microbial community to other gaseous and volatile substrates (Insam and Seewald 2010), such as methane. The extra oxygen and new substrate availability can then be used as high energy sources for soil bacteria to explore less available and more diverse energy sources (Fest et al. 2017; Hartmann, Buchmann, and Niklaus 2011).

As above mentioned, we could not observe a significant effect of the increasing plant richness gradient over soil bacterial alpha diversity, but over soil microbial composition. We did, however, identified a strong effect of the drought treatment on the soil bacterial community diversity. Against our initial expectations, bacterial diversity positively reacted to the drought treatment, increasing in comparison to the control plots (average Shannon Alpha diversity was 4.1 for control plots and 4.2 for drought plots, with standard deviation of 0.023 in both cases). We observed that drought exposed plots consistently presented higher bacterial diversity in both Shannon and Inverse Simpson diversity indexes, regardless of the plant richness level. More interestingly, we observed that plant biomass was not significantly different between drought exposed and control plots, which highlights the strong adaptation soil microbial communities displayed in this case.

Taking in consideration that soil samples were collected 1 year after the termination of the drought treatment and 10 years after the beginning of the experiment, we can conclude a community adaptation took place on the drought exposed plots, displaying a clear legacy effect of drought on the soil bacterial community. The overall soil bacterial composition was similar across the treatments, being Gemmytimonadota, Verrucomicrobiota, Patescibacteria, Myxococcota, Bacteroidota, Chloroflexi, Acidobacteriota, Actinobacteriota, Proteobacteria, and Planctomycetota the most predominant phyla, present in all treatments and all plant diversity levels. The difference however could be observed when specific taxa were associate with drought and control samples across the plant richness gradient. 1-species diversity had both increasing and decreasing ASVs belonging to Acidobacteriota phylum, both assigned to the Vicinamibacteria class, while 1 ASVs assigned to the Myxococcota phylum. Diversity levels 2 and 8 both had only significantly decreased ASVs, assigned to Bacteroidota and Acidobacteriota phyla, respectively. In the other hand, 4-species diversity level only presented a single ASV with significantly response to the drought treatment, increasing in abundance. This ASV was assigned to the Actiboacteriota phylum. In the 16-species plots, we observed an increase in Actinobacteriota and a decrease in Gemmatimonadota and Plantomycetota, and Bacteroidota had both increasing and decreasing ASVs, belonging to the uncultivated order AKYH767 (Mauch, Serra Moncadas, and Andrei 2022). The 60-species diversity level was the one with higher variance in terms of significantly increased/decreased ASVs, with 32 taxa (compared to 3 from levels 1, 2 and 4 and 10 from levels 8 and 6 on level 16). The increasing ASVs were assigned to the Planctomycetota, Latescibacterota, Actinobacteriota and Patescibacteria phyla, while ASVs belonging to Proteobacteriota, Planctomycetota and Chloroflexi were severely reduced. Among those, Actinobacteria and Chloroflexi are usually reported heaving increased abundance in dry exposed soil, being enriched due their specific traits that increase fitness under dry conditions (Naylor et al. 2017; Taketani et al. 2017), including sporulation and monoderm morphology (Taketani et al. 2017). Our data did not associate as specific taxa/ASV with the drought treatment. In this case, the affected bacterial taxa were particular to each of the analyzed diversity levels, without a consistent bacterial taxon being affected by the drought treatment consistently.

Although contrary to our initial expectations, those findings are supported by available literature. A study by Preece *et. al.* (2019) also identified an increase in soil bacterial diversity after exposure to chronic drought in a oak forest. A possible mechanism to explain this observation comes from the ecological role of water as a solute, not just a resource. The lack of water contributes to the isolation of microbial communities in soil, diminishing the homogenization of metabolites and nutrient sources. Low soil pore connectivity (Carson et al. 2010) has already been show to promote soil microbial diversity, which corroborates our findings. Treves et al. (2002) demonstrated that less dominant taxa

have a better chance of establishing as soil moisture decreases (Treves et al. 2003), with the competition between highly abundant taxa and less abundant taxa being more even under this condition. In this scenario, drought can reduce the nutrient availability to fast-growing taxa, allowing the growth of fastidious, less abundant microbes. Similarly, Carson et al. (2010) demonstrated that bacterial diversity increased in soils with low water content in comparison with the same soils with higher moisture.

Therefore, we speculate that the chronic aspect of the drought treatment implemented in our design, alongside with the seasonal intervals between the simulated drought (plots where only exposed to drought during summer) contributed to a slow but steady adaptation of soil microbial community to the lack of precipitation. This observation clearly demonstrates the legacy effect of drought on the soil bacterial community composition, highlighting how disturbance events can reverberate on the ecosystem functioning long after its termination. Data on acute drought indicate that initial response to drought events involve a drop on microbial diversity (Q. Wan et al. 2023). An experiment by Tóth *et. al.* (2017) identified a strong impact of acute drought on soil bacterial composition and litter decomposition, which however could not be observed 6 months after the treatment was terminated. In this case, we can estimate that the increase in diversity on the drought exposed samples are result of ling term adaptation to drought, which cannot be observed on acute drought models.

Early publications on the same experiment also observed contrasting effects, in comparison with our data. Albracht et al. (2023) working on the same experiment design, investigated the effects of both plant richness increase and drought treatment on the diversity and composition of arbuscular mycorrhiza (AMF) and total soil fungal community. They reported a significant impact of plant richness gradient on the diversity and composition of AMF and total fungal community but did not observe any significant effect of drought treatment on the same variables. Wagg et al. (2017) observed a reduction on plant biomass on drought treated plots in comparison to control plots, however reported a less pronounced biomass loss as the plant richness increases. In line with our findings, interestingly, Vogel et al. (2013) observed a significant effect of drought treatment on the litter decomposition, however irrespective of plant diversity. In this context, we also could not observe a direct interaction between increasing plant richness and the drought treatment itself, being the responde to drougth unique in each diversity level observed.

The effects of increasing plant diversity in both seed and soil, as well as a the diversity effects on drought treatments and the changes on core seed microbiome are summarized in figure 7.

In summary, those findings can indicate that the adaptation of soil bacterial community to drought pressure might be a delayed effect, which might take a few cycles to be perceptible. Also, the contrast

between acute and chronic drought might play a key role on the result observed during sampling. Sampling right after the drought chock might observe a drop on soil diversity, since the preset microbes would not be able to adapt to osmotic pressure, causing the loss of certain taxa (J. P. Schimel 2018). Long term sampling might access signs of adaptation and succession on soil microbial communities, embracing both drought and rewetting stress.

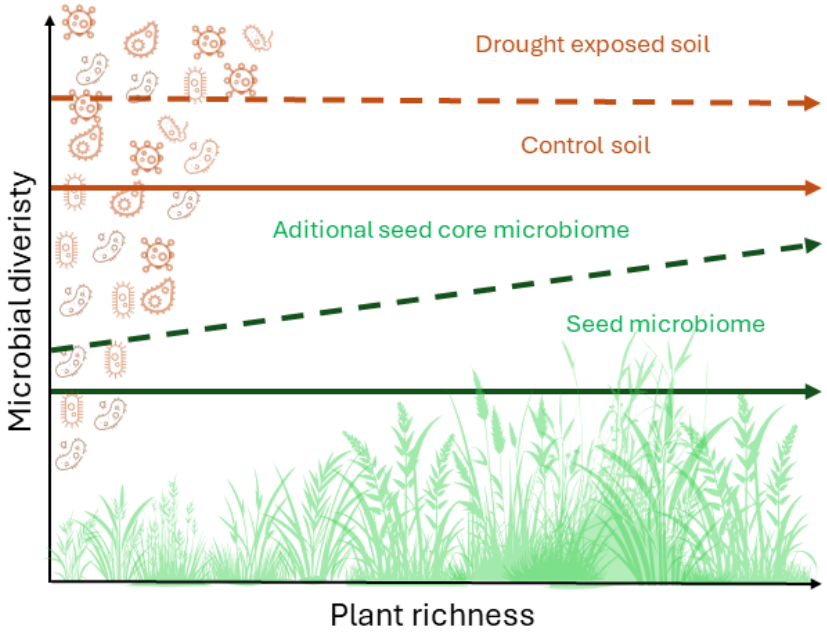


Figure 7: Effects of increasing plant richness. Soil bacterial diversity increases in soil exposed to long-term drought in comparison to control soil. The increase in microbial diversity is not linearly correlated to the increase in plant richness. The same is true for the diversity of seed endophytic bacterial community of Plantago lanceolata*, which had a stable bacterial diversity along the plant richness gradient. We did observe, however, an increase in the number of additional ASVs on the core microbiome of each observed diversity level as the plant richness increases. The scale between the types of samples is merely illustrative.*

4.2.2. Soil microbial diversity and stress recovery patterns

The last aspect of biodiversity approached in this thesis consists of the recovery of soil microbial communities after stress and how deterministic the development is according to the initial diversity load. We approached this question by running a microcosm experiment (**P1**) where soil disturbed by consecutive autoclavation cycles was re-inoculated with different microbial loads. We accessed original microbial abundance and composition and tracked the community development over the time, comparing the different inoculation loads to the non-inoculated control and with the original inoculum.

This research question approached fundamental aspects of microbial ecology as well as technical limitations of the study of soil microbial communities. Ecologically speaking, the patterns governing microbial community establishment are still poorly understood and posed additional challenges when comparted to plant and animal communities. The selection of suitable species colonizing a new environment is governed by both deterministic and stochastic factors and interactions (Dumbrell et al. 2010). The deterministic share of this process, also called 'niche-based' mechanisms includes environmental filtering, which is the interaction between the individuals, the abiotic environment, and the interspecific interactions and trade-offs, while the stochastic aspect is governed by "neutral processes", including unpredictable disturbances, life and death events, random dispersal and colonization, extinctions and ecological drift for example (Chase et al. 2011; Dumbrell et al. 2010). In this context, it has been previously demonstrated that the successful invasion of non-native species relies on the number of available niches (Van Elsas et al. 2012; Y. Li et al. 2014) and is negatively correlated with the diversity of the ecosystem, which is known as the diversity–invasiveness relationship (Nemergut et al. 2013). In technical terms, soil inoculation strategies with foreign microbes has being widely used as forms of bioremediation (Atuchin et al. 2023) and plant promoting techniques (de Souza, Ambrosini, and Passaglia 2015), where the enrichment of beneficial microbes would promote quicker environment purification and plant growth, respectively. However, the efficiency of those inoculation strategies and the factors ruling the successful establishment of inoculated microbes is still unclear. Therefore, the investigation of the factors governing the establishment of new microbial communities, especially after stress events, has extreme relevant for both practical and theoretical reasons.

Our data identified interesting patters during the soil colonization. First of all, qPCR data identified that bacterial diversity was quickly recovered and overcame the abundance on the inoculum soil after 1 week of incubation, while archaeal abundance did not fully recover over during the experiment´s duration (4 weeks). In contrast with the abundance data, bacterial and archaeal diversity did not recover over the experiment´s duration, indicating that the growth bloom after soil inoculation was dominated by a few microbial taxa. We also demonstrated the changes on the colonization patters by tracking the proposition of ASVs each inoculated sample coming from the inoculum soil, and how those proportions changes over time. Initially, inoculated samples ranged from 80% of ASVs coming from the inoculum samples in the 1:10 soil dilution to 50% in the 1:50 dilution and 40% in the 1:100 dilution at T0. At time 4 weeks, all diluted samples converged on the proportion of shared ASVs at 40%. This observation was confirmed when the taxonomical composition on soil samples were analysed. The first week of community development in inoculated soils was further by Cyclobacterieacea, Pseuomonadaceae and Burkholderiaceae. Cyclobacterieacea which are abundant in soil environments

and successful in processing a wide range of polysaccharides due to a rich repertoire of carbohydrate-active enzymes (Larsbrink and McKee 2020). Initially, the *Streptomyces* genus was highly dominant on the first two weeks with a drop in abundance on the following two. We estimate that the initial dominance of *Streptomyces* sp. might be explained by the production of antibiotics (de Lima Procópio et al. 2012) and the denitrification capacity of this genus (Zhang et al. 2021), which might have made use of nitrogen prevenient from microbial biomass in the autoclaved soil. Moreover, we identified a significant effect of both time and dilution treatment on the soil bacterial diversity, but didn´t identify a significant difference between the 3 inoculated treatments after the incubation period. This result indicates that the inoculation loads did not significantly impacts the final community composition. A conceptual schematic representation of the biodiversity development on inoculated soils over time is presented on figure 6.

This data has important ecological implications. Firstly, it indicates that as long as enough diversity is provided, the environmental filter prevails over stochastic processes during the community establishments. In this case, samples with higher microbial loads had their abundance equalized with samples with lower input, respecting environmental constrains as nutrient availability and species competition. Secondly, this phenomenon is time sensitive, being the observations along the weeks displaying different results, highlighting the importance of time-scale observations in adaptation studies.

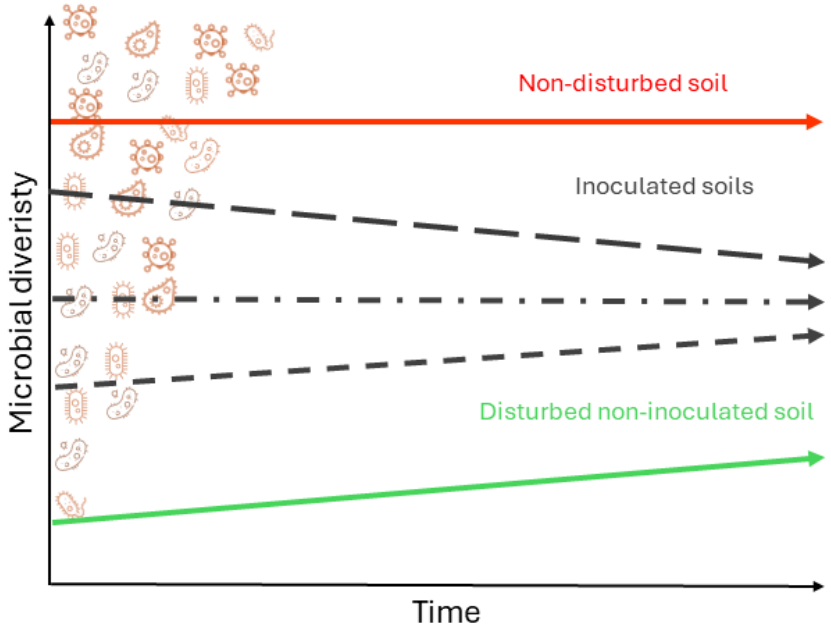


Figure 8: Schematic representation of inoculation effects on disturbed soil samples over time. Non-disturbed soil presented a stable bacterial and archaeal diversity over the experiment duration, while non-inoculated, disturbed presented a drastic reduced diversity, with slightly recovery over the weeks. Inoculated disturbed soils, however, had different starting points according to the inoculation load, converging in diversity towards the end of the experiment.

Those findings can help to understand the patterns observed on the drought experiment (**P2**). The development of soil microbial communities during drought stress might have been different over time and deterministic development might have also taken place during the 9 years the experiment was conducted. Since our analyses are based on a single sampling point, the variation presents on the soil, represented by the plant richness gradient, might have been homogenised over the years and we observed the convergency point of those soil samples at the time of sampling. Also, the experiment time frame of almost a decade might have been enough to slowly select soil microbial communities to responded to the drought stress, instead of only plant community richness and composition.

The deterministic colonization of soil samples after inoculation has already been demonstrated in literature. A study from Delmont *et. al.* (2014) with sterilized soil inoculated with soil samples from different countries, demonstrated that after two months incubation period, soil communities converse in their composition, showing no significantly different communities. In this case, the same sterilized soil received two different inoculums, resulting in similar microbial community composition. This data demonstrates that environmental filtering overcomes the individual differences between the inoculum samples, which is complemented by our results, showing that the load of inoculations also does not affect the final microbial community assemble. Another study by Bundy, Paton and Campbell (2002) observed that soil microbial community from tree different soil types did not converge after diesel contamination. This data suggests that, even though an overwhelming carbon source is to be provided for the soil microbial community, individual responses are still observed if the soil physicochemical characteristics are considerably distinct.

In contrast to the non-significantly different soil microbial composition among the inoculated samples, we did observe a significant difference between the inoculated soils and both the non-inoculated and inoculum controls. These results suggest that, even though microbial composition could not be recovered after the inoculation, the provision of initial inoculate significantly affects the speed and quality of community recovery during stress. Inoculated samples had overall smaller diversity compared to non-sterilized soils (averaging 1000 ASVs for control samples, while inoculated samples had average 450 ASVs and the non-inoculated ones around 50 ASVs in average) but displayed higher diversity in comparison to non-inoculated soils. This result has important implications for the study of recovery in disturbed environments. If for one side the environmental physicochemical conditions, e.i. environmental filter, prevails over inoculum diversity on the community establishment after a disturbance event, the provision of an initial diversity seems to facilitate the process and “jump-start” the community recovery process. Recent data (Dadzie et al. 2023) demonstrates that the inoculation of enriched, native microorganisms can improve nutrient cycling and availability in degraded soils, which might facilitate environmental recovery in a long term. This data suggests that, even though the

microbial community present in the inoculum might not prevail in the final microbial composition, as observed with the *Streptomyces* genus in our study, the initial boost in diversity can be beneficial for the recovery after stress. In disturbed soils this improvement seems to be related to the mobilization of key nutrients (nitrogen, phosphorus, potassium and iron) and the improvement of soils aggregation and structure (Rashid et al. 2016), as promoted by the production of microbial exopolysaccharides (Cania et al. 2019).

4.3. Open questions and experimental limitations

In this thesis, we approached experimentally a few open questions related to the study of biodiversity. More specifically, we investigated the impact increasing biodiversity has on the composition of soil and seed microbiome and how microbial biodiversity responds to stress conditions. However, due to practical and technical limitations, some questions were left unanswered, which might require further research on the topics. Besides that, the findings on our study lead to new exiting questions which hopefully will be approached in the future.

We observed a rather consistent result when investigating the direct impact of plant richness on the composition of both soil and seed microbiome. The plant richness gradient was shown to influence the microbial composition in both soil and seed endophytic microbiome, however without being linearly correlated with microbial alpha diversity. In the case of seeds, this leads to the conclusion that the beneficial effects provided by increasing ecosystem diversity can be somehow carried over to the next generations, representing an important transgenerational link between parental plant and its offspring, as well as a clear legacy effect from plant biodiversity. The question now is how the complexification of seed endophytic microbiome can benefit seedlings during germination. Seed endophytic community seems to be especially important right after germination, being the microbes carried with the seeds the first ones to colonize the seedling (Abdelfattah et al. 2022; Jones and Lennon 2010). Experimental evidence indicates that seeds growing in sterile substrate are successfully colonized by microbial taxa present in their seeds and, when exposed to soil microbial community, have their microbiome complemented by soil microbes (Abdelfattah et al. 2021; Johnston-Monje, Gutiérrez, and Lopez-Lavalle 2021). Therefore, we can speculate that seeds coming from environments with higher diversity would benefit from more diverse microbiome, being more prepared in case of germination in adverse conditions. Further research could explore the growing fitness of seeds coming from different plant diversity environments, investigating how the initial microbial community affects the overall plant growth. Additionally, seed ASVs can be tracked from the seed into the adult plants,

so the plant colonization from seed microbes can be estimated. In parallel, it would be crucial to determine whether the same behaviours can be also observed in other plant species and, if possible, from different functional groups. Currently literature suggests that seed endophytic microbiome is species-specific, respecting individual needs from each plant species (Abdelfattah et al. 2021; Eyre et al. 2019; Walsh et al. 2021), however the effect of increasing plant diversity on the seed microbiome compositions across plant functional groups hasn´t been reported yet. This data could lead to important conclusions regarding to management practices, where the seed history is considered during the planting process.

In regard to the impact of plant richness on the soil microbial composition, a few questions are still open. We didn´t identify a strong linear relationship between the plant species richness and the soil bacterial diversity during the field drought experiment. As already discussed, the drought treatment consistently impacted soil microbial alpha diversity, but the effect seemed to be the same regardless of the plant richness. In this case, one can argue whether the effect we observed on the metabarcoding data might represent the stable picture of the already stabilized microbial community after 9 years of consistent stress and 1 year of recovery. As observed in out microcosm experiment (**P1**), even though community tends to stabilize over time after disturbance, the path to this stabilization might be different depending on the starting point. This raises the questions whether soil microbial community react differently along the plant diversity gradient over the years. This might be supported by the observation that different taxa were significantly increased/decreased in certain treatments after the drought treatment, indicating that either plant species richness or species composition and functional groups might lead to unique stabilization paths.

We can hypothesize that plots with higher plant diversity could reach an early stabilization point in contrast to monoculture and/or low diversity plots. In this case, plant richness would provide a more diverse inoculum and potentialize the soil microbiome adaptation, as observed with the inoculation soil in the microcosm experiment. In addition to this, another interesting question would be to investigate the recovery path *after* the termination of the drought treatment. Since we could still observe a strong effect on drought on the soil microbial composition, even after 1 year since the drought treatment was interrupted, we can estimate a deep and consistent adaptation of soil microbial community to the drought stress, which might take a long time to be undetectable. An interesting question would be to investigate whether the drought adaptation effect disappears evenly along the diversity gradient or if there is a specific trend following the experimental design. We understand that such experiment is extremely laborious and expensive, however the research question is open, and the results seem extremely exiting. Current literature in the topic clearly demonstrates that changes on soil microbial community are directly related to the adaptation of soil to drought stress (Canarini et

al. 2021; Evans and Wallenstein 2012) being nutrient dynamics completely unbalanced after drought (Deng et al. 2021), leading to the question if it´s even possible to recover pre-drought conditions over time and weather the drought adapted soi communities are more prone to rewetting stress.

About our **P1** project, some interest questions were left open to further research. Taking in consideration that we demonstrated the importance of inoculation to soil recovery after stress and the deterministic behaviour of soil community development, now the factors determining those patterns are left to be described. The studies of Delmont *et. al.* (2014) and Bundy, Paton and Campbell (2002) nicely complement our research. If we consider that different inoculation loads (as demonstrated by our study) and different inoculations diversities (by *Deltmont* et al) both lead to similar final diversity and considering that the provision of a unified carbon source leads to different microbial communities in different soils (showed Bundy, Paton and Campbell 2002) indicates that the fundamental factor community development is present in the inoculated soil itself. The niche-based interactions responsible for deterministic community development are well known in ecology, corresponding to ecological selection as the driving force of community assembly (Dumbrell et al. 2010), as nutrient availability and species competition (Pavoine and Bonsall 2011; De Vrieze et al. 2020). Therefore, a more comprehensive study in which extend soil nutrients determine the selection process and which ecological interaction between the components of this community are key for the ecological succession. Additionally, the question regarding the diversity provided in the inoculum is also important. A study by Maron *et. al.* (2018) manipulated soil microbial diversity inoculating gamma irradiated soils using a dilution to extinction approach. They identified a significant effect of soil inoculation on the carbon sources decomposition according to soil dilutions. It´s important to notice that the dilution in this case was done until 1:1000 and 1:100000, while we used soil dilutions of 1:10, 1:50 and 1:100. This contrasting data suggests that the inoculation done in the sterilized soils during our experiment was still able to carry enough initial diversity to colonize the inoculated soils, even after environmental filtering. An interesting question now would be to determine the threshold where inoculation diversity would be low enough to significantly impact final community composition, also determining which keystone taxa/role are essential for the successful community colonization (Banerjee, Schlaeppi, and van der Heijden 2018). This data would help to clarify the importance of inoculation for soil recovery after disturbance and how the colonization of disturbed soils take place.

4.4. Outlook and conclusions

On this thesis we demonstrate the interaction between plant and microbial diversity in normal conditions and also during stress. We demonstrated the increasing plant richness modifies the stable core microbiome of our model plant, *Plantago lanceolata*, increasing the number of bacterial ASVs as the plant species richness increases. In regard of soil microbiome, the plant species gradient did not linearly affect the soil microbiome diversity, but significantly changed the soil community composition. The increasing plant diversity did not seem to have a strong effect on the adaptation of soil bacterial community to drought stress, which saw a subtle but steady increase in diversity after the exposure to intermittent, long-term drought. We also successfully demonstrated the soil microbial community recovery after stress respond to deterministic processes governed by soil structure and physicochemical conditions instead of stochastic processes. The provision of initial inoculum was shown to significantly affect the colonization processes, boosting the community recovery in both abundance and diversity. We also demonstrated that colonization follows a different path according to initial inoculum, however leading to similar communities after incubation period.

In regard to our initial hypothesis, we can concluded that; 1) Plant richness does affect soil and seed microbial community composition, but the increase in plant richness is not linearly correlated with the increase in microbial diversity, being the changes concentrated in special recruitment of microbial taxa according to individual features on each diversity level; 2) We did not identified either diversity loss or protective effect of increasing plant diversity on the mitigation of long term stress represented by drought, identifying however a significant effect of the drought treatment on the soil microbial composition and; 3) the main factors determining the microbial colonization after stress are the ones provided by the soil, prevailing over the initial diversity offer on final soil community composition. The inoculation however was essential for a quicker recovery in soil microbial abundance and diversity.

Altogether, our data bring some clarity on important topics on the matter of microbial ecology, the relationship between microbial community, its hosts and the process of recovery and adaptation after environmental stress. Study the whole of plant and microbial community on ecosystem functioning is essential for ecosystem and biodiversity preservation. In a world where human population increasing threatens the biodiversity and the sustainable use of natural resources, the investigation of alternative ways to restore damaged environments, as well as the key factors for ecosystem production, are crucial for a sustainable growth of human population.

5. Bibliography

Aanderud, Zachary T. et al. 2018. “Stoichiometric Shifts in Soil C:N:P Promote Bacterial Taxa Dominance, Maintain Biodiversity, and Deconstruct Community Assemblages.” *Frontiers in Microbiology* 9(JUL).

Abalos, Diego et al. 2021. “Manipulating Plant Community Composition to Steer Efficient N-Cycling in Intensively Managed Grasslands.” *Journal of Applied Ecology* 58(1): 167–80.

Abdelfattah, Ahmed et al. 2022. “From Seed to Seed: The Role of Microbial Inheritance in the Assembly of the Plant Microbiome.” *Trends in Microbiology* xx(xx): 1–10. https://doi.org/10.1016/j.tim.2022.10.009.

Abdelfattah, Ahmed, Michael Wisniewski, Leonardo Schena, and Ayco J.M. Tack. 2021. “Experimental Evidence of Microbial Inheritance in Plants and Transmission Routes from Seed to Phyllosphere and Root.” *Environmental Microbiology* 23(4): 2199–2214.

Abedini, Davar, Sébastien Jaupitre, Harro Bouwmeester, and Lemeng Dong. 2021. “Metabolic Interactions in Beneficial Microbe Recruitment by Plants.” *Current Opinion in Biotechnology* 70: 241–47.

Afzal, Imran, Zabta Khan Shinwari, Shomaila Sikandar, and Shaheen Shahzad. 2019. “Plant Beneficial Endophytic Bacteria: Mechanisms, Diversity, Host Range and Genetic Determinants.” *Microbiological Research* 221(April 2018): 36–49.

Ahmed, Moddassir, Muhammad Rauf, Zahid Mukhtar, and Nasir Ahmad Saeed. 2017. “Excessive Use of Nitrogenous Fertilizers: An Unawareness Causing Serious Threats to Environment and Human Health.” *Environmental Science and Pollution Research* 24(35): 26983–87.

Albracht, Cynthia et al. 2023. “Effects of Recurrent Summer Droughts on Arbuscular Mycorrhizal and Total Fungal Communities in Experimental Grasslands Differing in Plant Diversity and Community Composition.”

Alizon, S., A. Hurford, N. Mideo, and M. Van Baalen. 2009. “Virulence Evolution and the Trade-off Hypothesis: History, Current State of Affairs and the Future.” *Journal of Evolutionary Biology* 22(2): 245–59.

Apprill, Amy, Sean Mcnally, Rachel Parsons, and Laura Weber. 2015. “Minor Revision to V4 Region SSU RRNA 806R Gene Primer Greatly Increases Detection of SAR11 Bacterioplankton.” *Aquatic Microbial Ecology* 75(2): 129–37.

Atuchin, Victor V. et al. 2023. “Microorganisms for Bioremediation of Soils Contaminated with Heavy Metals.” *Microorganisms* 11(4): 1–19.

Babu, Anam Giridhar et al. 2015. “Penicillium Menonorum: A Novel Fungus to Promote Growth and Nutrient Management in Cucumber Plants.” *Mycobiology* 43(1): 49–56.

Bach, H. J., J. Tomanova, M. Schloter, and J. C. Munch. 2002. “Enumeration of Total Bacteria and Bacteria with Genes for Proteolytic Activity in Pure Cultures and in Environmental Samples by Quantitative PCR Mediated Amplification.” *Journal of Microbiological Methods* 49(3): 235–45.

Baedke, Jan, Alejandro Fábregas-Tejeda, and Abigail Nieves Delgado. 2020. “The Holobiont Concept before Margulis.” *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 334(3): 149–55.

Baker, Edward. 2015. “The Worldwide Status of Phasmids (Insecta: Phasmida) as Pests of Agriculture

and Forestry, with a Generalised Theory of Phasmid Outbreaks." *Agriculture and Food Security* 4(1): 1–19.

Balvanera, Patricia et al. 2014. "Linking Biodiversity and Ecosystem Services: Current Uncertainties and the Necessary next Steps." *BioScience* 64(1): 49–57.

Banerjee, Samiran, Klaus Schlaeppi, and Marcel G.A. van der Heijden. 2018. "Keystone Taxa as Drivers of Microbiome Structure and Functioning." *Nature Reviews Microbiology* 16(9): 567–76. http://dx.doi.org/10.1038/s41579-018-0024-1.

Bar-On, Yinon M., Rob Phillips, and Ron Milo. 2018. "The Biomass Distribution on Earth." *Proceedings of the National Academy of Sciences of the United States of America* 115(25): 6506–11.

Barnes, Andrew D. et al. 2018. "Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning." *Trends in Ecology and Evolution* 33(3): 186–97.

Barnett, David, Ilja Arts, and John Penders. 2021. "MicroViz: An R Package for Microbiome Data Visualization and Statistics." *Journal of Open Source Software* 6(63): 3201.

Bartelt-Ryser, Janine et al. 2005. "Soil Feedbacks of Plant Diversity on Soil Microbial Communities and Subsequent Plant Growth." *Perspectives in Plant Ecology, Evolution and Systematics* 7(1): 27–49.

Baslam, Marouane, Idoia Garmendia, and Nieves Goicoechea. 2011. "Plant Genotype, Microbial Recruitment and Nutritional Security." *Journal of Agricultural and Food Chemistry* 59(10): 5504–15.

Berendse, Frank, Jasper van Ruijven, Eelke Jongejans, and Saskia Keesstra. 2015. "Loss of Plant Species Diversity Reduces Soil Erosion Resistance." *Ecosystems* 18(5): 881–88. http://dx.doi.org/10.1007/s10021-015-9869-6.

Berg, Gabriele, and Jos M. Raaijmakers. 2018. "Saving Seed Microbiomes." *ISME Journal* 12(5): 1167–70. http://dx.doi.org/10.1038/s41396-017-0028-2.

van Bergeijk, Doris A., Barbara R. Terlouw, Marnix H. Medema, and Gilles P. van Wezel. 2020. "Ecology and Genomics of Actinobacteria: New Concepts for Natural Product Discovery." *Nature Reviews Microbiology* 18(10): 546–58. http://dx.doi.org/10.1038/s41579-020-0379-y.

Bergna, Alessandro et al. 2018. "Tomato Seeds Preferably Transmit Plant Beneficial Endophytes." *Phytobiomes Journal* 2(4): 183–93.

Berihu, Maria et al. 2023. "A Framework for the Targeted Recruitment of Crop-Beneficial Soil Taxa Based on Network Analysis of Metagenomics Data." *Microbiome* 11(1): 1–21. https://doi.org/10.1186/s40168-022-01438-1.

Bessler, Holger et al. 2009. "Aboveground Overyielding in Grassland Mixtures Is Associated with Reduced Biomass Partitioning to Belowground Organs." *Ecology* 90(6): 1520–30.

Bever, James D., Scott A. Mangan, and Helen M. Alexander. 2015. "Maintenance of Plant Species Diversity by Pathogens." *Annual Review of Ecology, Evolution, and Systematics* 46(September): 305–25.

Bramble, De Shorn E. et al. 2024. "Formation of Mineral-Associated Organic Matter in Temperate Soils Is Primarily Controlled by Mineral Type and Modified by Land Use and Management Intensity." *Global Change Biology* 30(1): 1–19.

Branco, Sara, Annie Schauster, Hui Ling Liao, and Joske Ruytinx. 2022. "Mechanisms of Stress

Tolerance and Their Effects on the Ecology and Evolution of Mycorrhizal Fungi." *New Phytologist* 235(6): 2158–75.

Bray, R. J., and J.T. Curtis. 1957. "An Ordination of the Upland Forest Communities of Southern Wisconsin." *Ecological Society of America* 27(4): 325–49.

Bulgarelli, Davide et al. 2013. "Structure and Functions of the Bacterial Microbiota of Plants." *Annual Review of Plant Biology* 64: 807–38.

Bundy, J. G., G. I. Paton, and C. D. Campbell. 2002. "Microbial Communities in Different Soil Types Do Not Converge after Diesel Contamination." *Journal of Applied Microbiology* 92(2): 276–88.

Callahan, Benjamin J, Amy Jo A , Paul J McMurdie, Michael J Rosen, Andrew W Han, and and Susan P Holmes Johnson. 2016. "Dada2: High Resolution Sample Inference from Illumina Amplicon Data." *Nature Methods* 13(7): 1–7.

Canarini, Alberto et al. 2021. "Ecological Memory of Recurrent Drought Modifies Soil Processes via Changes in Soil Microbial Community." *Nature Communications* 12(1): 1–14. http://dx.doi.org/10.1038/s41467-021-25675-4.

Cania, Barbara et al. 2019. "A Long-Term Field Experiment Demonstrates the Influence of Tillage on the Bacterial Potential to Produce Soil Structure-Stabilizing Agents Such as Exopolysaccharides and Lipopolysaccharides." *Environmental Microbiomes* 14(1): 1–14.

Capdevila-Cortada, Marcal. 2019. "Electrifying the Haber–Bosch." *Nature Catalysis* 2(12): 1055. http://dx.doi.org/10.1038/s41929-019-0414-4.

Cardinale, Bradley J. et al. 2012. "Biodiversity Loss and Its Impact on Humanity." *Nature* 486(7401): 59–67.

Carini, Paul et al. 2016. "Relic DNA Is Abundant in Soil and Obscures Estimates of Soil Microbial Diversity." *Nature Microbiology* 2(December 2016).

Carlson, C. A., and J. L. Ingraham. 1983. "Comparison of Denitrification by Pseudomonas Stutzeri, Pseudomonas Aeruginosa, and Paracoccus Denitrificans." *Applied and Environmental Microbiology* 45(4): 1247–53.

Carson, Jennifer K. et al. 2010. "Low Pore Connectivity Increases Bacterial Diversity in Soil." *Applied and Environmental Microbiology* 76(12): 3936–42.

Cesarz, Simone et al. 2017. "Plant Species Richness Sustains Higher Trophic Levels of Soil Nematode Communities after Consecutive Environmental Perturbations." *Oecologia* 184(3): 715–28.

Chaparro, Jacqueline M., Amy M. Sheflin, Daniel K. Manter, and Jorge M. Vivanco. 2012. "Manipulating the Soil Microbiome to Increase Soil Health and Plant Fertility." *Biology and Fertility of Soils* 48(5): 489–99.

Chase, Jonathan M et al. 2011. "Disentangling the Importance of Ecological Niches from Stochastic Processes across Scales Disentangling the Importance of Ecological Niches from Stochastic Processes across Scales." : 2351–63.

Chen, Youhua, and Tsung Jen Shen. 2017. "A General Framework for Predicting Delayed Responses of Ecological Communities to Habitat Loss." *Scientific Reports* 7(1): 1–11. http://dx.doi.org/10.1038/s41598-017-01070-2.

Chen, Yuxin et al. 2022. "Drought-Exposure History Increases Complementarity between Plant Species in Response to a Subsequent Drought." *Nature Communications* 13(1): 1–11.

Civitello, David J. et al. 2015. “Biodiversity Inhibits Parasites: Broad Evidence for the Dilution Effect.” *Proceedings of the National Academy of Sciences of the United States of America* 112(28): 8667–71.

Cong, Wen Feng et al. 2014. “Plant Species Richness Promotes Soil Carbon and Nitrogen Stocks in Grasslands without Legumes.” *Journal of Ecology* 102(5): 1163–70.

Contreras-Cornejo, Hexon Angel, Lourdes Macías-Rodríguez, Carlos Cortés-Penagos, and José López-Bucio. 2009. “Trichoderma Virens, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis.” *Plant Physiology* 149(3): 1579–92.

Cornwell, William K. et al. 2008. “Plant Species Traits Are the Predominant Control on Litter Decomposition Rates within Biomes Worldwide.” *Ecology Letters* 11(10): 1065–71.

Crawford, K. M., and K. D. Whitney. 2010. “Population Genetic Diversity Influences Colonization Success.” *Molecular Ecology* 19(6): 1253–63.

Crous, Kristine Y. 2019. “Plant Responses to Climate Warming: Physiological Adjustments and Implications for Plant Functioning in a Future, Warmer World.” *American Journal of Botany* 106(8): 1049–51.

Csonka, L N. 1989. “Physiological and Genetic Responses of Bacteria to Osmotic Stress.” *Microbiological Reviews* 53(1): 121–47.

Cuddington, Kim. 2011. “Legacy Effects: The Persistent Impact of Ecological Interactions.” *Biological Theory* 6(3): 203–10.

Dadzie, Frederick A. et al. 2023. “Inoculating Native Microorganisms Improved Soil Function and Altered the Microbial Composition of a Degraded Soil.” *Restoration Ecology* 32(2): 1–11.

Davis, Nicole M. et al. 2018. “Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data.” *Microbiome* 6(1): 1–14.

Decaëns, T. et al. 2006. “The Values of Soil Animals for Conservation Biology.” *European Journal of Soil Biology* 42(SUPPL. 1).

Dell’Anno, Antonio et al. 2012. “High Bacterial Biodiversity Increases Degradation Performance of Hydrocarbons during Bioremediation of Contaminated Harbor Marine Sediments.” *Environmental Pollution* 167: 85–92. http://dx.doi.org/10.1016/j.envpol.2012.03.043.

Delmont, Tom O. et al. 2014. “Microbial Community Development and Unseen Diversity Recovery in Inoculated Sterile Soil.” *Biology and Fertility of Soils* 50(7).

DeLong, Edward F., and Norman R. Pace. 2001. “Environmental Diversity of Bacteria and Archaea.” *Systematic Biology* 50(4): 470–78.

Deng, Lei et al. 2021. “Drought Effects on Soil Carbon and Nitrogen Dynamics in Global Natural Ecosystems.” *Earth-Science Reviews* 214(September 2020): 103501. https://doi.org/10.1016/j.earscirev.2020.103501.

Dhankhar, Neha, and Jagdeep Kumar. 2023. “Impact of Increasing Pesticides and Fertilizers on Human Health: A Review.” *Materials Today: Proceedings* (xxxx). https://doi.org/10.1016/j.matpr.2023.03.766.

Dias, Teresa, Angela Dukes, and Pedro M. Antunes. 2014. “Accounting for Soil Biotic Effects on Soil Health and Crop Productivity in the Design of Crop Rotations.” *Journal of the Science of Food and Agriculture* 95(3): 447–54.

Ding, Tao, and Ulrich Melcher. 2016. “Influences of Plant Species, Season and Location on Leaf Endophytic Bacterial Communities of Non-Cultivated Plants.” *PLoS ONE* 11(3): 1–13.

Dogan, Gokhan, and Bilgin Taskin. 2021. “Hydrolytic Enzymes Producing Bacterial Endophytes of Some Poaceae Plants.” *Polish Journal of Microbiology* 70(3): 297–304.

Dorn-In, Samart et al. 2015. “Specific Amplification of Bacterial DNA by Optimized So-Called Universal Bacterial Primers in Samples Rich of Plant DNA.” *Journal of Microbiological Methods* 113: 50–56. http://dx.doi.org/10.1016/j.mimet.2015.04.001.

Downs, Diana M. 2006. “Understanding Microbial Metabolism.” *Annual Review of Microbiology* 60: 533–59.

Dumbrell, Alex J et al. 2010. “Relative Roles of Niche and Neutral Processes in Structuring a Soil Microbial Community.” *The ISME Journal*: 337–45.

Eisenhauer, Nico et al. 2017. “Root Biomass and Exudates Link Plant Diversity with Soil Bacterial and Fungal Biomass.” *Scientific Reports* 7: 1–8.

Elbasiouny, Heba et al. 2022. “Plant Nutrition under Climate Change and Soil Carbon Sequestration.” *Sustainability (Switzerland)* 14(2): 1–20.

Van Elsas, Jan Dirk et al. 2012. “Microbial Diversity Determines the Invasion of Soil by a Bacterial Pathogen.” *Proceedings of the National Academy of Sciences of the United States of America* 109(4): 1159–64.

Eriyagama, Nishadi, Vladimir Smakhtin, and Nilantha Gamage. 2009. IWMI Research Report *Mapping Drought Patterns and Impacts: A Global Perspective In Ternational Water Management Institute*.

Estendorfer, Jennifer et al. 2017. “The Influence of Land Use Intensity on the Plant-Associated Microbiome of Dactylis Glomerata L.” *Frontiers in Plant Science* 8(June): 1–10.

Evans, Sarah E., and Matthew D. Wallenstein. 2012. “Soil Microbial Community Response to Drying and Rewetting Stress: Does Historical Precipitation Regime Matter?” *Biogeochemistry* 109(1–3): 101–16.

Eyre, Alexander W., Mengying Wang, Yeonyee Oh, and Ralph A. Dean. 2019. “Identification and Characterization of the Core Rice Seed Microbiome.” *Phytobiomes Journal* 3(2): 148–57.

Fadiji, Ayomide Emmanuel, and Olubukola Oluranti Babalola. 2020. “Exploring the Potentialities of Beneficial Endophytes for Improved Plant Growth.” *Saudi Journal of Biological Sciences* 27(12): 3622–33. https://doi.org/10.1016/j.sjbs.2020.08.002.

Fahrig, Lenore. 2003. “Effects of Habitat Fragmentation on Biodiversity.” *Annual Review of Ecology, Evolution, and Systematics* 34: 487–515.

Farrelly, Damien J., Colm D. Everard, Colette C. Fagan, and Kevin P. McDonnell. 2013. “Carbon Sequestration and the Role of Biological Carbon Mitigation: A Review.” *Renewable and Sustainable Energy Reviews* 21: 712–27. http://dx.doi.org/10.1016/j.rser.2012.12.038.

Fest, Benedikt et al. 2017. “Soil Methane Uptake Increases under Continuous Throughfall Reduction in a Temperate Evergreen, Broadleaved Eucalypt Forest.” *Ecosystems* 20(2): 368–79.

Fierer, Noah, Mark A. Bradford, and Robert B. Jackson. 2007. “Toward an Ecological Classification of Soil Bacteria.” *Ecology* 88(6): 1354–64.

Fluegge, Keith, and Kyle Fluegge. 2017. “Air Pollution and Risk of Hospitalization for Epilepsy: The

Role of Farm Use of Nitrogen Fertilizers and Emissions of the Agricultural Air Pollutant, Nitrous Oxide." *Arquivos de Neuro-Psiquiatria* 75(9): 614–19.

Fowler, A. M., and K. J. Hennessy. 1995. "Potential Impacts of Global Warming on the Frequency and Magnitude of Heavy Precipitation." *Natural Hazards* 11(3): 283–303.

Freschet, Grégoire T. et al. 2021. "Root Traits as Drivers of Plant and Ecosystem Functioning: Current Understanding, Pitfalls and Future Research Needs." *New Phytologist* 232(3): 1123–58.

Galindo-Solís, Juan M., and Francisco J. Fernández. 2022. "Endophytic Fungal Terpenoids: Natural Role and Bioactivities." *Microorganisms* 10(2): 1–22.

Geisen, Stefan, Diana H. Wall, and Wim H. van der Putten. 2019. "Challenges and Opportunities for Soil Biodiversity in the Anthropocene." *Current Biology* 29(19): R1036–44. https://doi.org/10.1016/j.cub.2019.08.007.

Geisseler, Daniel, and Kate M. Scow. 2014. "Long-Term Effects of Mineral Fertilizers on Soil Microorganisms - A Review." *Soil Biology and Biochemistry* 75: 54–63. http://dx.doi.org/10.1016/j.soilbio.2014.03.023.

Geng, S. M. et al. 2015. "Effects of Drought Stress on Agriculture Soil." *Natural Hazards* 75(2): 1997–2011.

Goldstein, Ellie J.C., Diane M. Citron, Victoria A. Peraino, and Sally A. Cross. 2003. "Desulfovibrio Desulfuricans Bacteremia and Review of Human Desulfovibrio Infections." *Journal of Clinical Microbiology* 41(6): 2752–54.

Goyal, Nishu, Zhi Zhou, and Iftekhar A. Karimi. 2016. "Metabolic Processes of Methanococcus Maripaludis and Potential Applications." *Microbial Cell Factories* 15(1): 1–19.

Grant, Kirsten N., Merrin L. Macrae, Fereidoun Rezanezhad, and W. Vito Lam. 2019. "Nutrient Leaching in Soil Affected by Fertilizer Application and Frozen Ground." *Vadose Zone Journal* 18(1): 1–13.

Gregorich, E. G., C. F. Drury, and J. A. Baldock. 2001. "Changes in Soil Carbon under Long-Term Maize in Monoculture and Legume-Based Rotation." *Canadian Journal of Soil Science* 81(1): 21–31.

Groussin, Mathieu, Florent Mazel, and Eric J. Alm. 2020. "Co-Evolution and Co-Speciation of Host-Gut Bacteria Systems." *Cell Host and Microbe* 28(1): 12–22. https://doi.org/10.1016/j.chom.2020.06.013.

van de Guchte, Maarten, Hervé M. Blottière, and Joël Doré. 2018. "Humans as Holobionts: Implications for Prevention and Therapy." *Microbiome* 6(1): 81.

Guenay, Yasemin et al. 2013. "Transgressive Overyielding of Soil Microbial Biomass in a Grassland Plant Diversity Gradient." *Soil Biology and Biochemistry* 60: 122–24. http://dx.doi.org/10.1016/j.soilbio.2013.01.015.

Habibullah, Muzafar Shah, Badariah Haji Din, Siow Hooi Tan, and Hasan Zahid. 2022. "Impact of Climate Change on Biodiversity Loss: Global Evidence." *Environmental Science and Pollution Research* 29(1): 1073–86.

Haines-young, Roy. 2009. "Land Use and Biodiversity Relationships." *Land Use Policy*: 178–86.

Handa, I. Tanya et al. 2014. "Consequences of Biodiversity Loss for Litter Decomposition across Biomes." *Nature* 509(7499): 218–21.

Hanson, China A., Jed A. Fuhrman, M. Claire Horner-Devine, and Jennifer B.H. Martiny. 2012.

"Beyond Biogeographic Patterns: Processes Shaping the Microbial Landscape." *Nature Reviews Microbiology* 10(7): 497–506.

Hartman, Kyle, and Susannah G. Tringe. 2019. "Interactions between Plants and Soil Shaping the Root Microbiome under Abiotic Stress." *Biochemical Journal* 476(19): 2705–24.

Hartmann, Adrian A., Nina Buchmann, and Pascal A. Niklaus. 2011. "A Study of Soil Methane Sink Regulation in Two Grasslands Exposed to Drought and N Fertilization." *Plant and Soil* 342(1–2): 265–75.

Hawkes, Christine V., James J. Bull, and Jennifer A. Lau. 2020. "Symbiosis and Stress: How Plant Microbiomes Affect Host Evolution." *Philosophical Transactions of the Royal Society B: Biological Sciences* 375(1808).

Hector, A. et al. 1999. "Plant Diversity and Productivity Experiments in European Grasslands." *Science* 286(5442): 1123–27.

Helfrich, Eric J.N., Geng Min Lin, Christopher A. Voigt, and Jon Clardy. 2019. "Bacterial Terpene Biosynthesis: Challenges and Opportunities for Pathway Engineering." *Beilstein Journal of Organic Chemistry* 15: 2889–2906.

Higginbottom, Thomas P, and Elias Symeonakis. 2014. "Assessing Land Degradation and Desertification Using Vegetation Index Data: Current Frameworks and Future Directions." (I): 9552–75.

Hoffmann, K. et al. 2014. *Klimauntersuchungen in Jena Für Die Anpassung an Den Klimawandel Und Seine Erwarteten Folgen. Offenbach Am Main: Selbstverlag Des Deutschen Wetterdienstes.*

Hoiss, Bernhard et al. 2013. "Combined Effects of Climate and Management on Plant Diversity and Pollination Type in Alpine Grasslands." *Diversity and Distributions* 19(4): 386–95.

Hu, Ruifa et al. 2015. "Long- and Short-Term Health Effects of Pesticide Exposure: A Cohort Study from China." *PLoS ONE* 10(6): 1–13.

Huang, Yuanyuan et al. 2023. "Enhanced Stability of Grassland Soil Temperature by Plant Diversity." *Nature geoscience*.

Hubálek, Z. 2000. "Measures of Species Diversity in Ecology: An Evaluation." *Folia Zoologica* 49(4): 241–60.

Hughes, A. Randall et al. 2008. "Ecological Consequences of Genetic Diversity." *Ecology Letters* 11(6): 609–23.

Humphreys, Aelys M. et al. 2019. "Global Dataset Shows Geography and Life Form Predict Modern Plant Extinction and Rediscovery." *Nature Ecology and Evolution* 3(7): 1043–47.

Huntington, Thomas G. 2006. "Evidence for Intensification of the Global Water Cycle: Review and Synthesis." *Journal of Hydrology* 319(1–4): 83–95.

Insam, Heribert, and Martin S.A. Seewald. 2010. "Volatile Organic Compounds (VOCs) in Soils." *Biology and Fertility of Soils* 46(3): 199–213.

Isbell, Forest et al. 2015. "Biodiversity Increases the Resistance of Ecosystem Productivity to Climate Extremes." *Nature* 526(7574): 574–77.

Jaacks, Lindsay M., and Lisa R. Staimez. 2015. "Association of Persistent Organic Pollutants and Non-Persistent Pesticides with Diabetes and Diabetes-Related Health Outcomes in Asia: A Systematic Review." *Environment International* 76: 57–70.

http://dx.doi.org/10.1016/j.envint.2014.12.001.

Jaccard, Paul. 1912. “The Distribution of the Flora in the Alpine Zone.” *New Phytologist* 11(2): 37–50.

Janssen, Peter H. 2006. “Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S RRNA and 16S RRNA Genes.” *Applied and Environmental Microbiology* 72(3): 1719–28.

Jansson, Janet K., and Kirsten S. Hofmockel. 2020. “Soil Microbiomes and Climate Change.” *Nature Reviews Microbiology* 18(1): 35–46. http://dx.doi.org/10.1038/s41579-019-0265-7.

Jha, S, and K S Bawa. 2006. “Population Growth , Human Development , and Deforestation in Biodiversity Hotspots.” 20(3): 906–12.

Johnson, Christopher N. et al. 2017. “Biodiversity Losses and Conservation Responses in the Anthropocene.” *Science* 356(6335): 270–75.

Johnston-Monje, David, Janneth P. Gutiérrez, and Luis Augusto Becerra Lopez-Lavalle. 2021. “Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes.” *Frontiers in Microbiology* 12(October).

Jones, Stuart E., and Jay T. Lennon. 2010. “Dormancy Contributes to the Maintenance of Microbial Diversity.” *Proceedings of the National Academy of Sciences of the United States of America* 107(13): 5881–86.

Jr, F A, A The Origin, and New Haven. 2000. “Genetic Diversity and Disease Control in Rice.” 406(August): 718–22.

Kaisermann, Aurore, Franciska T. de Vries, Robert I. Griffiths, and Richard D. Bardgett. 2017. “Legacy Effects of Drought on Plant–Soil Feedbacks and Plant–Plant Interactions.” *New Phytologist* 215(4): 1413–24.

Kakouridis, Anne et al. 2022. “Routes to Roots: Direct Evidence of Water Transport by Arbuscular Mycorrhizal Fungi to Host Plants.” *New Phytologist* 236(1): 210–21.

Kembel, Steven W. et al. 2010. “Picante: R Tools for Integrating Phylogenies and Ecology.” *Bioinformatics* 26(11): 1463–64.

Kennedy, A. C., and K. L. Smith. 1995. “Soil Microbial Diversity and the Sustainability of Agricultural Soils.” *Plant and Soil* 170(1): 75–86.

Khare, Ekta, Jitendra Mishra, and Naveen Kumar Arora. 2018. “Multifaceted Interactions between Endophytes and Plant: Developments and Prospects.” *Frontiers in Microbiology* 9(NOV): 1–12.

King, K. C., and C. M. Lively. 2012. “Does Genetic Diversity Limit Disease Spread in Natural Host Populations.” *Heredity* 109(4): 199–203.

Kirkegaard, J. A., and J. R. Hunt. 2010. “Increasing Productivity by Matching Farming System Management and Genotype in Water-Limited Environments.” *Journal of Experimental Botany* 61(15): 4129–43.

Knights, Dan et al. 2011. “Bayesian Community-Wide Culture-Independent Microbial Source Tracking.” *Nature Methods* 8(9): 761–65.

Koops, H. P. et al. 1991. “Classification of Eight New Species of Ammonia-Oxidizing Bacteria: Nitrosomonas Communis Sp. Nov., Nitrosomonas Ureae Sp. Nov., Nitrosomonas Aestuarii Sp. Nov., Nitrosomonas Marina Sp. Nov., Nitrosomonas Nitrosa Sp. Nov., Nitrosomonas Eutropha Sp. Nov., N.” *Journal of General Microbiology* 137(7): 1689–99.

Kopittke, Peter M. et al. 2019. “Soil and the Intensification of Agriculture for Global Food Security.”

Environment International 132(August): 105078. https://doi.org/10.1016/j.envint.2019.105078.

Kostenko, Olga, and T. Martijn Bezemer. 2020. "Abiotic and Biotic Soil Legacy Effects of Plant Diversity on Plant Performance." *Frontiers in Ecology and Evolution* 8(April): 1–12.

Kowalchuk, George A. et al. 2002. "Effects of Above-Ground Plant Species Composition and Diversity on the Diversity of Soil-Borne Microorganisms." *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* 81(1–4): 509–20.

Kruskal, Joseph B., and Roger N. Shepard. 1974. "A Nonmetric Variety of Linear Factor Analysis." *Psychometrika* 39(2): 123–57.

Lal, Rattan, and William C. Moldenhauer. 1987. "Effects of Soil Erosion on Crop Productivity." *Critical Reviews in Plant Sciences* 5(4): 303–67.

Lange, Markus et al. 2014. "Biotic and Abiotic Properties Mediating Plant Diversity Effects on Soil Microbial Communities in an Experimental Grassland." *PLoS ONE* 9(5).

———. 2015. "Plant Diversity Increases Soil Microbial Activity and Soil Carbon Storage." *Nature Communications* 6.

Lata, R., S. Chowdhury, S. K. Gond, and J. F. White. 2018. "Induction of Abiotic Stress Tolerance in Plants by Endophytic Microbes." *Letters in Applied Microbiology* 66(4): 268–76.

Lawrence, Corey R., Jason C. Neff, and Joshua P. Schimel. 2009. "Does Adding Microbial Mechanisms of Decomposition Improve Soil Organic Matter Models? A Comparison of Four Models Using Data from a Pulsed Rewetting Experiment." *Soil Biology and Biochemistry* 41(9): 1923–34. http://dx.doi.org/10.1016/j.soilbio.2009.06.016.

Leek, Jeffrey T. et al. 2012. "The SVA Package for Removing Batch Effects and Other Unwanted Variation in High-Throughput Experiments." *Bioinformatics* 28(6): 882–83.

Lehmann, Anika, Weishuang Zheng, and Matthias C. Rillig. 2017. "Soil Biota Contributions to Soil Aggregation." *Nature Ecology and Evolution* 1(12): 1828–35. http://dx.doi.org/10.1038/s41559-017-0344-y.

Lekli, Istvan, Diptarka Ray, and Dipak K. Das. 2010. "Longevity Nutrients Resveratrol, Wines and Grapes." *Genes and Nutrition* 5(1): 55–60.

Leo, Lahti. "Microbiome R Package." http://microbiome.github.io.

Letourneau, Deborah K., and Sara G. Bothwell. 2008. "Comparison of Organic and Conventional Farms: Challenging Ecologists to Make Biodiversity Functional." *Frontiers in Ecology and the Environment* 6(8): 430–38.

Li, Sheng Jin et al. 2014. "Microbial Communities Evolve Faster in Extreme Environments." *Scientific Reports* 4: 1–9.

Li, Yuanyuan, Hongyu Wen, Longqian Chen, and Tingting Yin. 2014. "Succession of Bacterial Community Structure and Diversity in Soil along a Chronosequence of Reclamation and Re-Vegetation on Coal Mine Spoils in China." *PLoS ONE* 9(12).

de Lima Procópio, Rudi Emerson et al. 2012. "Antibiotics Produced by *Streptomyces*." *Brazilian Journal of Infectious Diseases* 16(5): 466–71. http://dx.doi.org/10.1016/j.bjid.2012.08.014.

Lin, Huang, and Shyamal Das Peddada. 2020. "Analysis of Compositions of Microbiomes with Bias Correction." *Nature Communications* 11(1): 1–11. http://dx.doi.org/10.1038/s41467-020-17041-7.

Lipiec, J., C. Doussan, A. Nosalewicz, and K. Kondracka. 2013. “Effect of Drought and Heat Stresses on Plant Growth and Yield: A Review.” *International Agrophysics* 27(4): 463–77.

Liu, Lan, Kai Zhu, Nina Wurzburger, and Jian Zhang. 2020. “Relationships between Plant Diversity and Soil Microbial Diversity Vary across Taxonomic Groups and Spatial Scales.” *Ecosphere* 11(1).

Liu, Yonghong, Wenting Liu, and Zongsuo Liang. 2015. “Endophytic Bacteria from Pinellia Ternata, a New Source of Purine Alkaloids and Bacterial Manure.” *Pharmaceutical Biology* 53(10): 1545–48.

Liu, Zidi et al. 2023. “Abiotic Factors and Endophytes Co-Regulate Flavone and Terpenoid Glycoside Metabolism in Glycyrrhiza Uralensis.” *Applied Microbiology and Biotechnology* 107(7–8): 2671–88. https://doi.org/10.1007/s00253-023-12441-3.

Lively, Curtis M. 2010. “The Effect of Host Genetic Diversity on Disease Spread.” *American Naturalist* 175(6): 2–5.

Long, Hoang Hoa, Dominik D. Schmidt, and Ian T. Baldwin. 2008. “Native Bacterial Endophytes Promote Host Growth in a Species-Specific Manner; Phytohormone Manipulations Do Not Result in Common Growth Responses.” *PLoS ONE* 3(7).

Loreau, M. et al. 2001. “Ecology: Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges.” *Science* 294(5543): 804–8.

Loreau, Michel, and Claire de Mazancourt. 2013. “Biodiversity and Ecosystem Stability: A Synthesis of Underlying Mechanisms.” *Ecology Letters* 16(SUPPL.1): 106–15.

Ludwig-Müller, Jutta. 2011. “Auxin Conjugates: Their Role for Plant Development and in the Evolution of Land Plants.” *Journal of Experimental Botany* 62(6): 1757–73.

Ludwig, B. et al. 2011. “Effects of Fertilization and Soil Management on Crop Yields and Carbon Stabilization in Soils. A Review.” *Agronomy for Sustainable Development* 31(2): 361–72.

Lueders, Tillmann, Mike Manefield, and Michael W. Friedrich. 2004. “Enhanced Sensitivity of DNA- and RRNA-Based Stable Isotope Probing by Fractionation and Quantitative Analysis of Isopycnic Centrifugation Gradients.” *Environmental Microbiology* 6(1): 73–78.

Manzoni, S., and G. Katul. 2014. “Invariant Soil Water Potential at Zero Microbial Respiration Explained by Hydrological Discontinuity in Dry Soils.” *Geophysical Research Letters* 41(20): 7151–58.

Manzoni, S., F. Moyano, T. Kätterer, and J. Schimel. 2016. “Modeling Coupled Enzymatic and Solute Transport Controls on Decomposition in Drying Soils.” *Soil Biology and Biochemistry* 95(January): 275–87.

Marhuenda-muñoz, Mar et al. 2019. “Microbial Phenolic Metabolites : WWhich Molecules Actually Have an Effect on Human Health?” *Nutrients* 11: 1–15.

Maron, Pierre Alain et al. 2018. “High Microbial Diversity Promotes Soil Ecosystem Functioning.” *Applied and Environmental Microbiology* 84(9).

Mauch, Emanuel, Lucas Serra Moncadas, and Adrian-Stefan Andrei. 2022. “Complete Genome Sequence of an Uncultivated Freshwater Bacteroidota Lineage.” *Microbiology Resource Announcements* 11(10): 7–8.

McDonald-Madden, Eve, Michael C. Runge, Hugh P. Possingham, and Tara G. Martin. 2011. “Optimal Timing for Managed Relocation of Species Faced with Climate Change.” *Nature Climate Change* 1(5): 261–65. http://dx.doi.org/10.1038/nclimate1170.

McMurdie, Paul J., and Susan Holmes. 2013. “Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data.” *PLoS ONE* 8(4).

Mead, A. 1992. “Review of the Development of Multidimensional Scaling Methods.” *The Statistician* 41(1): 27.

Mellado-Vázquez, Perla Griselle et al. 2016. “Plant Diversity Generates Enhanced Soil Microbial Access to Recently Photosynthesized Carbon in the Rhizosphere.” *Soil Biology and Biochemistry* 94: 122–32.

Miliute, Inga, Odeta Buzaite, Danas Baniulis, and Vidmantas Stanys. 2015. “Bacterial Endophytes in Agricultural Crops and Their Role in Stress Tolerance: A Review.” *Zemdirbyste* 102(4): 465–78.

Mitchell, Ruth J., Martin H.D. Auld, Michael G. Le Duc, and Marrs H. Robert. 2000. “Ecosystem Stability and Resilience: A Review of Their Relevance for the Conservation Management of Lowland Heaths.” *Perspectives in Plant Ecology, Evolution and Systematics* 3(2): 142–60.

Moreno-Mateos, David et al. 2017. “Anthropogenic Ecosystem Disturbance and the Recovery Debt.” *Nature Communications* 8: 8–13.

Morris, J. Jeffrey, Richard E. Lenski, and Erik R. Zinser. 2012. “The Black Queen Hypothesis: Evolution of Dependencies through Adaptive Gene Loss.” *mBio* 3(2).

El Moujahid, Lamiae et al. 2017. “Effect of Plant Diversity on the Diversity of Soil Organic Compounds.” *PLoS ONE* 12(2): 1–16.

Mraja, Anne et al. 2011. “Plant Community Diversity Influences Allocation to Direct Chemical Defence in Plantago Lanceolata.” *PLoS ONE* 6(12).

Nakagawa, Tatsunori, and Reiji Takahashi. 2015. “Nitrosomonas Stercoris Sp. Nov., a Chemoautotrophic Ammonia-Oxidizing Bacterium Tolerant of High Ammonium Isolated from Composted Cattle Manure.” *Microbes and Environments* 30(3): 221–27.

Nanda, Sonil, Sivamohan N. Reddy, Sushanta K. Mitra, and Janusz A. Kozinski. 2016. “The Progressive Routes for Carbon Capture and Sequestration.” *Energy Science and Engineering* 4(2): 99–122.

Naylor, Dan, Stephanie Degraaf, Elizabeth Purdom, and Devin Coleman-derr. 2017. “Drought and Host Selection Influence Bacterial Community Dynamics in the Grass Root Microbiome.” 11(12): 2691–2704. http://dx.doi.org/10.1038/ismej.2017.118.

Nelson, Eric B. 2018. “The Seed Microbiome: Origins, Interactions, and Impacts.” *Plant and Soil* 422(1–2): 7–34.

Nemecek, Thomas et al. 2012. “Estimation of the Variability in Global Warming Potential of Worldwide Crop Production Using a Modular Extrapolation Approach.” *Journal of Cleaner Production* 31: 106–17. http://dx.doi.org/10.1016/j.jclepro.2012.03.005.

Nemergut, Diana R. et al. 2013. “Patterns and Processes of Microbial Community Assembly.” *Microbiology and Molecular Biology Reviews* 77(3): 342–56.

Nicol, Graeme W., L. Anne Glover, and James I. Prosser. 2003. “Molecular Analysis of Methanogenic Archaeal Communities in Managed and Natural Upland Pasture Soils.” *Global Change Biology* 9(10): 1451–57.

Nishihara, H., Y. Igarashi, and T. Kodama. 1991. “Hydrogenovibrio Marinus Gen. Nov., Sp. Nov., a Marine Obligately Chemolithoautotrophic Hydrogen-Oxidizing Bacterium.” *International Journal of Systematic Bacteriology* 41(1): 130–33.

Oksanen, r Jari et al. 2022. “Title Community Ecology Package.” *Vegan: Community Ecol Package*.

Oldroyd, Giles E.D. 2013. “Speak, Friend, and Enter: Signalling Systems That Promote Beneficial Symbiotic Associations in Plants.” *Nature Reviews Microbiology* 11(4): 252–63.

Osborne, Tom, Gillian Rose, and Tim Wheeler. 2013. “Variation in the Global-Scale Impacts of Climate Change on Crop Productivity Due to Climate Model Uncertainty and Adaptation.” *Agricultural and Forest Meteorology* 170: 183–94. http://dx.doi.org/10.1016/j.agrformet.2012.07.006.

Ostad-Ali-Askari, Kaveh, Hossein Ghorbanizadeh Kharazi, Mohammad Shayannejad, and Mohammad Javad Zareian. 2020. “Effect of Climate Change on Precipitation Patterns in an Arid Region Using GCM Models: Case Study of Isfahan-Borkhar Plain.” *Natural Hazards Review* 21(2): 04020006.

Ostfeld, Richard S., and Felicia Keesing. 2000. “Biodiversity and Disease Risk: The Case of Lyme Disease.” *Conservation Biology* 14(3): 722–28.

Oteino, Nicholas et al. 2015. “Plant Growth Promotion Induced by Phosphate Solubilizing Endophytic Pseudomonas Isolates.” *Frontiers in Microbiology* 6(JUL): 1–9.

Ouzari, Hadda et al. 2008. “Diversity of Auxin-Producing Bacteria Associated to Pseudomonas Savastanoi-Induced Olive Knots.” *Journal of Basic Microbiology* 48(5): 370–77.

Parada, Alma E., David M. Needham, and Jed A. Fuhrman. 2016. “Every Base Matters: Assessing Small Subunit RRNA Primers for Marine Microbiomes with Mock Communities, Time Series and Global Field Samples.” *Environmental Microbiology* 18(5): 1403–14.

Park, Inmyoung, Young Su Seo, and Mohamed Mannaa. 2023. “Recruitment of the Rhizo-Microbiome Army: Assembly Determinants and Engineering of the Rhizosphere Microbiome as a Key to Unlocking Plant Potential.” *Frontiers in Microbiology* 14.

Pascale, Alberto, Silvia Proietti, Iakovos S. Pantelides, and Ioannis A. Stringlis. 2020. “Modulation of the Root Microbiome by Plant Molecules: The Basis for Targeted Disease Suppression and Plant Growth Promotion.” *Frontiers in Plant Science* 10(January): 1–23.

Pavoine, S., and M. B. Bonsall. 2011. “Measuring Biodiversity to Explain Community Assembly: A Unified Approach.” *Biological Reviews* 86(4): 792–812.

Pérez Castro, Sherlynette et al. 2019. “Soil Microbial Responses to Drought and Exotic Plants Shift Carbon Metabolism.” *ISME Journal* 13(7): 1776–87. http://dx.doi.org/10.1038/s41396-019-0389-9.

Pons, Simon et al. 2020. “Phytohormone Production by the Arbuscular Mycorrhizal Fungus Rhizophagus Irregularis.” *PLoS ONE* 15(10 October): 1–18.

Porter, Stephanie S. et al. 2020. “Beneficial Microbes Ameliorate Abiotic and Biotic Sources of Stress on Plants.” *Functional Ecology* 34(10): 2075–86.

Potts, Malcolm. 2001. “Desiccation Tolerance: A Simple Process?” *Trends in Microbiology* 9(11): 553–59.

Powlson, D. S. 1993. “Understanding the Soil Nitrogen Cycle.” *Soil Use and Management* 9(3): 86–93.

Preece, Catherine et al. 2019. “Effects of Past and Current Drought on the Composition and Diversity of Soil Microbial Communities.” *Soil Biology and Biochemistry* 131: 28–39. https://doi.org/10.1016/j.soilbio.2018.12.022.

Preece, Catherine, Gerard Farré-Armengol, and Josep Peñuelas. 2020. “Drought Is a Stronger Driver

of Soil Respiration and Microbial Communities than Nitrogen or Phosphorus Addition in Two Mediterranean Tree Species." *Science of the Total Environment* 735: 139554. https://doi.org/10.1016/j.scitotenv.2020.139554.

Price, Samantha A., and John L. Gittleman. 2007. "Hunting to Extinction: Biology and Regional Economy Influence Extinction Risk and the Impact of Hunting in Artiodactyls." *Proceedings of the Royal Society B: Biological Sciences* 274(1620): 1845–51.

Prober, Suzanne M. et al. 2015. "Plant Diversity Predicts Beta but Not Alpha Diversity of Soil Microbes across Grasslands Worldwide." *Ecology Letters* 18(1): 85–95.

Pugh, Brittany E. et al. 2022. "A Possible Role for River Restoration Enhancing Biodiversity through Interaction with Wildfire." *Global Ecology and Biogeography* 31(10): 1990–2004.

Quast, Christian et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41(D1): 590–96.

Rashid, Muhammad Imtiaz et al. 2016. "Bacteria and Fungi Can Contribute to Nutrients Bioavailability and Aggregate Formation in Degraded Soils." *Microbiological Research* 183: 26–41. http://dx.doi.org/10.1016/j.micres.2015.11.007.

Reed, David H., and Richard Frankham. 2003. "Correlation between Fitness and Genetic Diversity." *Conservation Biology* 17(1): 230–37.

Renard, Delphine, Lucie Mahaut, and Frederik Noack. 2023. "Crop Diversity Buffers the Impact of Droughts and High Temperatures on Food Production." *Environmental Research Letters* 18(4).

Roscher, Christiane et al. 2004. "The Role of Biodiversity for Element Cycling and Trophic Interactions: An Experimental Approach in a Grassland Community." *Basic and Applied Ecology* 5(2): 107–21.

Roscher, Christiane, Bernhard Schmid, and Ernst Detlef Schulze. 2009. "Non-Random Recruitment of Invader Species in Experimental Grasslands." *Oikos* 118(10): 1524–40.

Saeid, Agnieszka, Ewelina Prochownik, and Justyna Dobrowolska-Iwanek. 2018. "Phosphorus Solubilization by Bacillus Species." *Molecules* 23(11): 1–18.

Salam, Md Tareq Bin, and Ryota Kataoka. 2023. "Changes in the Endophytic Bacterial Community of Brassica Rapa after Application of Systemic Insecticides." *International Journal of Molecular Sciences* 24(20).

Santos, Mariana Sanches, Marco Antonio Nogueira, and Mariangela Hungria. 2019. "Microbial Inoculants: Reviewing the Past, Discussing the Present and Previewing an Outstanding Future for the Use of Beneficial Bacteria in Agriculture." *AMB Express* 9(1). https://doi.org/10.1186/s13568-019-0932-0.

Santoyo, Gustavo. 2022. "How Plants Recruit Their Microbiome? New Insights into Beneficial Interactions." *Journal of Advanced Research* 40: 45–58. https://doi.org/10.1016/j.jare.2021.11.020.

Sapoukhina, Natalia, Yuri Tyutyunov, Ivan Sache, and Roger Arditi. 2010. "Spatially Mixed Crops to Control the Stratified Dispersal of Airborne Fungal Diseases." *Ecological Modelling* 221(23): 2793–2800. http://dx.doi.org/10.1016/j.ecolmodel.2010.08.020.

Sardans, Jordi, and Josep Pen. 2008. "Mediterranean Forest." 54(5): 513–22.

Scherber, Christoph et al. 2010. "Bottom-up Effects of Plant Diversity on Multitrophic Interactions in a Biodiversity Experiment." *Nature* 468(7323): 553–56.

Schimel, Joshua, Teri Balser, and Matthew Wallenstein. 2007. “Microbial Stress-Response Physiology and Its Implications.” *Ecology* 88(6): 1386–94.

Schimel, Joshua P. 2018. “Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes.” *Annual Review of Ecology, Evolution, and Systematics* 49(August): 409–32.

Schimel, Joshua P., and Sean M. Schaeffer. 2012. “Microbial Control over Carbon Cycling in Soil.” *Frontiers in Microbiology* 3(SEP): 1–11.

Schmid, B. et al. 2004. “The Role of Biodiversity for Element Cycling and Trophic Interactions: An Experimental Approach in a Grassland Community.” *Basic and Applied Ecology* 5(2): 107–21.

Schmid, Marc W. et al. 2021. “Effects of Plant Community History, Soil Legacy and Plant Diversity on Soil Microbial Communities.” *Journal of Ecology* 109(8): 3007–23.

Schöler, Anne et al. 2017. “Analysis of Soil Microbial Communities Based on Amplicon Sequencing of Marker Genes.” *Biology and Fertility of Soils* 53(5): 485–89.

Schubert, Mikkel, Stinus Lindgreen, and Ludovic Orlando. 2016. “AdapterRemoval v2: Rapid Adapter Trimming, Identification, and Read Merging.” *BMC Research Notes* 9(1): 1–7.

Schultz, J. E., and P. F. Weaver. 1982. “Fermentation and Anaerobic Respiration by Rhodospirillum Rubrum and Rhodopseudomonas Capsulata.” *Journal of Bacteriology* 149(1): 181–90.

Schulz, S. et al. 2013. “Diversity Pattern of Nitrogen Fixing Microbes in Nodules of Trifolium Arvense (L.) at Different Initial Stages of Ecosystem Development.” *Biogeosciences* 10(2): 1183–92.

Sohoulande Djebou, Dagbegnon C., and Vijay P. Singh. 2016. “Impact of Climate Change on Precipitation Patterns: A Comparative Approach.” *International Journal of Climatology* 36(10): 3588–3606.

Somers, E. et al. 2005. “Azospirillum Brasilense Produces the Auxin-like Phenylacetic Acid by Using the Key Enzyme for Indole-3-Acetic Acid Biosynthesis.” *Applied and Environmental Microbiology* 71(4): 1803–10.

de Souza, Rocheli, Adriana Ambrosini, and Luciane M.P. Passaglia. 2015. “Plant Growth-Promoting Bacteria as Inoculants in Agricultural Soils.” *Genetics and Molecular Biology* 38(4): 401–19.

Spaepen, Stijn, and Jos Vanderleyden. 2011. “Auxin and Plant-Microbe Interactions.” : 1–14.

Spain, Anne M., Lee R. Krumholz, and Mostafa S. Elshahed. 2009. “Abundance, Composition, Diversity and Novelty of Soil Proteobacteria.” *ISME Journal* 3(8): 992–1000.

Spohn, Marie et al. 2023. “The Positive Effect of Plant Diversity on Soil Carbon Depends on Climate.” *Nature Communications* 14(1): 1–10.

Stegen, James C, Xueju Lin, Allan E Konopka, and James K Fredrickson. 2012. “Stochastic and Deterministic Assembly Processes in Subsurface Microbial Communities.” 6(9): 1653–64. http://dx.doi.org/10.1038/ismej.2012.22.

Steinauer, Katja et al. 2016. “Convergence of Soil Microbial Properties after Plant Colonization of an Experimental Plant Diversity Gradient.” *BMC Ecology* 16(1): 1–9.

Strecker, Tanja, Odette González Macé, Stefan Scheu, and Nico Eisenhauer. 2016. “Functional Composition of Plant Communities Determines the Spatial and Temporal Stability of Soil Microbial Properties in a Long-Term Plant Diversity Experiment.” *Oikos* 125(12): 1743–54.

Taketani, Rodrigo Gouvêa et al. 2017. “Dry Season Constrains Bacterial Phylogenetic Diversity in a Semi-Arid Rhizosphere System.” *Microbial Ecology*: 153–61. http://dx.doi.org/10.1007/s00248-

016-0835-4.

Tardy, Vincent et al. 2014. “Stability of Soil Microbial Structure and Activity Depends on Microbial Diversity.” *Environmental Microbiology Reports* 6(2): 173–83.

Thompson, Janelle R., Hanny E. Rivera, Collin J. Closek, and Mónica Medina. 2014. “Microbes in the Coral Holobiont: Partners through Evolution, Development, and Ecological Interactions.” *Frontiers in Cellular and Infection Microbiology* 4(DEC): 1–20.

Tilman, David. 1996. “Biodiversity: Population versus Ecosystem Stability.” *Ecology* 77(2): 350–63.

Tilman, David, and John A. Downing. 1994. “Biodiversity and Stability in Grasslands.” *Nature* 367(6461): 363–65.

Tilman, David, Forest Isbell, and Jane M. Cowles. 2014. “Biodiversity and Ecosystem Functioning.” *Annual Review of Ecology, Evolution, and Systematics* 45: 471–93.

Torsvik, V., J. Goksoyr, and F. L. Daae. 1990. “High Diversity in DNA of Soil Bacteria.” *Applied and Environmental Microbiology* 56(3): 782–87.

Tóth, Z. et al. 2017. “Extreme Effects of Drought on Composition of the Soil Bacterial Community and Decomposition of Plant Tissue.” *European Journal of Soil Science* 68(4): 504–13.

Treves, D. S., B. Xia, J. Zhou, and J. M. Tiedje. 2003. “A Two-Species Test of the Hypothesis That Spatial Isolation Influences Microbial Diversity in Soil.” *Microbial Ecology* 45(1): 20–28.

Trimble, Stanley W. 2020. “Surface Water: Pollution by Nitrogen Fertilizers.” *Encyclopedia of Water Science, Second Edition* (January 2003): 1185–90.

Trivedi, Pankaj, Bruna D. Batista, Kathryn E. Bazany, and Brajesh K. Singh. 2022. “Plant–Microbiome Interactions under a Changing World: Responses, Consequences and Perspectives.” *New Phytologist* 234(6): 1951–59.

Tucker, Caroline M. et al. 2017. “A Guide to Phylogenetic Metrics for Conservation, Community Ecology and Macroecology.” *Biological Reviews* 92(2): 698–715.

Tyc, Olaf et al. 2020. “The Ecological Role of Bacterial Seed Endophytes Associated with Wild Cabbage in the United Kingdom.” *MicrobiologyOpen* 9(1): 1–19.

Ungar Irwin A. 1987. “Population Ecology of Halophyte Seeds.” *The Botanical Review* 53(3): 301–34.

V., Anju M., Rekha R. Warrier, and C. Kunhikannan. 2022. “Significance of Soil Seed Bank in Forest Vegetation—A Review.” *Seeds* 1(3): 181–97.

Valdés, Jorge et al. 2008. “Acidithiobacillus Ferrooxidans Metabolism: From Genome Sequence to Industrial Applications.” *BMC Genomics* 9: 1–24.

Valletta, Alessio, Lorenzo Maria Iozia, and Francesca Leonelli. 2021. “Impact of Environmental Factors on Stilbene Biosynthesis.” *Plants* 10(1): 1–40.

Vives-Peris, Vicente, Carlos de Ollas, Aurelio Gómez-Cadenas, and Rosa María Pérez-Clemente. 2020. “Root Exudates: From Plant to Rhizosphere and Beyond.” *Plant Cell Reports* 39(1): 3–17. https://doi.org/10.1007/s00299-019-02447-5.

Vogel, Anja, Thomas Fester, et al. 2013. “Separating Drought Effects from Roof Artifacts on Ecosystem Processes in a Grassland Drought Experiment.” *PLoS ONE* 8(8).

Vogel, Anja, Nico Eisenhauer, Alexandra Weigelt, and Michael Scherer-Lorenzen. 2013. “Plant Diversity Does Not Buffer Drought Effects on Early-Stage Litter Mass Loss Rates and Microbial

Properties." *Global Change Biology* 19(9): 2795–2803.

Vogel, Anja, Michael Scherer-Lorenzen, and Alexandra Weigelt. 2012. "Grassland Resistance and Resilience after Drought Depends on Management Intensity and Species Richness." *PLoS ONE* 7(5).

De Vrieze, Jo et al. 2020. "Stochasticity in Microbiology: Managing Unpredictability to Reach the Sustainable Development Goals." *Microbial Biotechnology* 13(4): 829–43.

Wagg, Cameron et al. 2017. "Plant Diversity Maintains Long-Term Ecosystem Productivity under Frequent Drought by Increasing Short-Term Variation." *Ecology* 98(11): 2952–61.

Wagi, Shabana, and Ambreen Ahmed. 2019. "Bacillus Spp.: Potent Microfactories of Bacterial IAA." *PeerJ* 2019(7).

Walsh, Corinne M., Isadore Becker-Uncapher, Madeline Carlson, and Noah Fierer. 2021. "Variable Influences of Soil and Seed-Associated Bacterial Communities on the Assembly of Seedling Microbiomes." *ISME Journal* 15(9): 2748–62. http://dx.doi.org/10.1038/s41396-021-00967-1.

Wan, Qian et al. 2023. "Different and Unified Responses of Soil Bacterial and Fungal Community Composition and Predicted Functional Potential to 3 Years' Drought Stress in a Semiarid Alpine Grassland." *Frontiers in Microbiology* 14(March): 1–14.

Wan, Xiaohua, Xinli Chen, Zhiqun Huang, and Han Y.H. Chen. 2021. "Contribution of Root Traits to Variations in Soil Microbial Biomass and Community Composition." *Plant and Soil* 460(1–2): 483–95.

Wang, Guangzhou et al. 2019. "Soil Microbiome Mediates Positive Plant Diversity-Productivity Relationships in Late Successional Grassland Species." *Ecology Letters* 22(8): 1221–32.

Wang, Jichen et al. 2021. "Arbuscular Mycorrhizal Fungi and Plant Diversity Drive Restoration of Nitrogen-Cycling Microbial Communities." *Molecular Ecology* 30(16): 4133–46.

Wang, Xiao-yan et al. 2021. "Evenness Alters the Positive Effect of Species Richness on Community Drought Resistance via Changing Complementarity." *Ecological Indicators* 133: 108464. https://doi.org/10.1016/j.ecolind.2021.108464.

Wang, Zhipeng et al. 2020. "Sensitivity of Terrestrial Carbon Cycle to Changes in Precipitation Regimes." *Ecological Indicators* 113(February): 106223. https://doi.org/10.1016/j.ecolind.2020.106223.

Wang, Zhonghua et al. 2022. "Screening of Phosphate-Solubilizing Bacteria and Their Abilities of Phosphorus Solubilization and Wheat Growth Promotion." *BMC Microbiology* 22(1): 1–15. https://doi.org/10.1186/s12866-022-02715-7.

Wardle, David A. 2006. "The Influence of Biotic Interactions on Soil Biodiversity." *Ecology Letters* 9(7): 870–86.

Waters, Colin N. et al. 2016. "The Anthropocene Is Functionally and Stratigraphically Distinct from the Holocene." *Science* 351(6269).

Weisser, Wolfgang W. et al. 2017. "Biodiversity Effects on Ecosystem Functioning in a 15-Year Grassland Experiment: Patterns, Mechanisms, and Open Questions." *Basic and Applied Ecology* 23: 1–73.

Whittaker, R. H. 1960. "Vegetation of the Siskiyou Mountains , Oregon and California." *Ecological Monographs* 30(3): 279–338.

Wickham, Hadley et al. 2019. “Welcome to the Tidyverse.” *Journal of Open Source Software* 4(43): 1686.

Willis, Amy D., and Bryan D. Martin. 2022. “Estimating Diversity in Networked Ecological Communities.” *Biostatistics* 23(1): 207–22.

Woodwell, George M, and Robert H Whittaker. 1968. “Primary Production in Terrestrial Ecosystems Used in Maintaining the Plant — Energy " Burned " in Respiration . The Relationship Applies to Any Green Plant or Any Commu- Nity of Green Plants and Can Be Summarized Most Simply by This Formula : Where NPP Z .” 30: 19–30.

Wright, Alexandra J. et al. 2015. “Flooding Disturbances Increase Resource Availability and Productivity but Reduce Stability in Diverse Plant Communities.” *Nature Communications* 6: 1–6.

Wrighton, Kelly C. et al. 2008. “A Novel Ecological Role of the Firmicutes Identified in Thermophilic Microbial Fuel Cells.” *ISME Journal* 2(11): 1146–56.

Wu, Wei et al. 2021. “Beneficial Relationships Between Endophytic Bacteria and Medicinal Plants.” *Frontiers in Plant Science* 12(April): 1–13.

Yachi, Shigeo, and Michel Loreau. 1999. “Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis.” *Proceedings of the National Academy of Sciences of the United States of America* 96(4): 1463–68.

Yang, Gaowen, Julien Roy, Stavros D. Veresoglou, and Matthias C. Rillig. 2021. “Soil Biodiversity Enhances the Persistence of Legumes under Climate Change.” *New Phytologist* 229(5): 2945–56.

Yang, Yi, David Tilman, George Furey, and Clarence Lehman. 2019. “Soil Carbon Sequestration Accelerated by Restoration of Grassland Biodiversity.” *Nature Communications* 10(1): 1–7. http://dx.doi.org/10.1038/s41467-019-08636-w.

Zeigler, Daniel R. 2014. “The *Geobacillus* Paradox: Why Is a Thermophilic Bacterial Genus so Prevalent on a Mesophilic Planet?” *Microbiology (United Kingdom)* 160(PART 1): 1–11.

Zeppel, M. J.B., J. V. Wilks, and J. D. Lewis. 2014. “Impacts of Extreme Precipitation and Seasonal Changes in Precipitation on Plants.” *Biogeosciences* 11(11): 3083–93.

Zhang, Haihan, Ben Ma, Tinglin Huang, and Yinjie Shi. 2021. “Nitrate Reduction by the Aerobic Denitrifying Actinomycete *Streptomyces Sp.* XD-11-6-2: Performance, Metabolic Activity, and Micro-Polluted Water Treatment.” *Bioresource Technology* 326(December 2020): 124779. https://doi.org/10.1016/j.biortech.2021.124779.

Zhu, Feng et al. 2018. “Species-Specific Plant–Soil Feedbacks Alter Herbivore-Induced Gene Expression and Defense Chemistry in Plantago Lanceolata.” *Oecologia* 188(3): 801–11. https://doi.org/10.1007/s00442-018-4245-9.

6. Acknowledgments

Completing this PhD is, by far, the most adventurous endeavour I ever committed to. This title is the result of a decade of bitter-sweet work and dedication to science and personal improvement. The path however, wasn´t taken alone, and I wouldn't be able to walk it without the support and motivation of many people who I feel like thanking to.

I´d like to thank firstly my parents, Monica and Manoel, who sacrificed their youth, health, and sanity in order to provide me and my siblings with the education and comfort themselves never had. This title is as mine as it is theirs and I´ll be forever grateful for everything you did. The same can be said about my siblings, Allan, Yara and Allana, who were always a great source of motivation and encouragement. The ambition and nonconformity we share is what makes us different, facing the adversities and striving for a better future. Thank you all for standing by my side, I love you all. Thanks also to my long-term friends, Rubens Akamine, Claudia Groposo, Guilherme Braga and Rafael Galvão, for being present and keeping an eye on me from afar.

Many thanks also to my supervisors, Prof. Michael Schloter and Dr. Stefanie Schulz. Thank you for the trust, patience and for the opportunity to develop my scientific skills in such high-level environment. A special thanks to Steffi, for helping me to develop my confidence and for always be so accessible. No doubt what I learned at COMI will pavement my future career and I´m proud to have your names in my CV from now on.

I´m also extremely grateful for my work colleagues at COMI, who shared the journey with me. Thank you for listening to my complaints and for supporting me during the though journey. I need to say your encouragement was essential and made everything easier. Special thanks to Roberto, Zhongjie, Seun, Juliette and Sarah. You guys are great and one day we´ll meet and remember the crazy rollercoaster the graduate school was. Many thanks also to my students, Nida Tasneem and Moritz Klessinger, for making my days in the lab much happier.

I would like also to express my deep appreciation for all the staff at the Jena Experiment, including (but not limited) to Prof. Dr. Nico Eisenhauer, Dr. Anne Ebeling, Dr. Yuanyuan Huang and Prof. Dr. Wolfgang Weisser, for organizing this amazing experiment and providing all the support I could need to successfully conclude my research. The same can be said to my fellow PhD students, especially Pamela Medina van Berkum, Akanksha Rai and Marcel Solbach who I had the happiness to work directly with. Many thanks to my co-authors for their contribution to the studies presented in this thesis. I am profoundly grateful for their hard work and insightful feedback.

Lastly, I´d like to thank my little one, Fabien Behner, for being so supportive on this final step of my PhD. Thank you for the deep conversations (in English even) and for showing me a new life for a new angle. You´re a grateful surprise the PhD brought me and I'm lucky to have you by my side.

7. Attachments

7.1. Publication I

Microbial Ecology
https://doi.org/10.1007/s00248-023-02285-9

RESEARCH

Deterministic Development of Soil Microbial Communities in Disturbed Soils Depends on Microbial Biomass of the Bioinoculum

Yuri Pinheiro Alves de Souza[1,2] · Michael Schloter[1,2] · Wolfgang Weisser[3] · Stefanie Schulz[1]

Received: 4 August 2023 / Accepted: 4 August 2023

Abstract
Despite its enormous importance for ecosystem services, factors driving microbial recolonization of soils after disturbance are still poorly understood. Here, we compared the microbial recolonization patterns of a disturbed, autoclaved soil using different amounts of the original non-disturbed soil as inoculum. By using this approach, we manipulated microbial biomass, but did not change microbial diversity of the inoculum. We followed the development of a new soil microbiome after reinoculation over a period of 4 weeks using a molecular barcoding approach as well as qPCR. Focus was given on the assessment of bacteria and archaea. We could show that 1 week after inoculation in all inoculated treatments bacterial biomass exceeded the values from the original soil as a consequence of high dissolved organic carbon (DOC) concentrations in the disturbed soil resulting from the disturbance. This high biomass was persistent over the complete experimental period. In line with the high DOC concentrations, in the first 2 weeks of incubation, copiotrophic bacteria dominated the community, which derived from the inoculum used. Only in the disturbed control soils which did not receive a microbial inoculum, recolonization pattern differed. In contrast, archaeal biomass did not recover over the experimental period and recolonization was strongly triggered by amount of inoculated original soil added. Interestingly, the variability between replicates of the same inoculation density decreased with increasing biomass in the inoculum, indicating a deterministic development of soil microbiomes if higher numbers of cells are used for reinoculation.

Keywords Soil microbiome · Metabarcoding · Soil disturbance · Recolonization, βNTI

Introduction

Soil is one of the most diverse environments on the planet [1]. Its heterogeneity leads to complex three-dimensional spaces where microbes interact and compete for nutrients and niche formation, promoting species diversification and interaction [2]. The microbial colonization and community assembly of soils is driven by four main processes, which are diversification, dispersal, selection, and drift [3]. Among those, diversification and dispersal are related to the introduction of new species, while drift and selection determine the relative abundance and turnover of species [4]. The latter is strongly related to the nutrient status of the soil and thus the growth rate of microbes.

Species selection is governed by both deterministic and stochastic factors and interactions [5]. The deterministic share of this process, also called "niche-based" mechanisms includes environmental filtering, which is the interaction between individuals, the surounding abiotic environment and interspecific interactions and trade-offs, while the stochastic aspect, is governed by "neutral processes", including unpredictable disturbances, life and death events, random dispersal and colonization, extinctions, and ecological drift [5, 6]. Regarding dispersal, Nemergut et al. (2013) clarified that dispersal and migration must be clearly distinguished, since the chance to reach a site (dispersal) does not guarantee the successful establishment on the site (migration). Thus, rather environmental filters and niche competition, which are related to the process of selection [7], might determine the successful establishment of microbes.

✉ Stefanie Schulz
stefanie.schulz@helmholtz-muenchen.de

[1] Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany

[2] Technische Universität München, TUM School of Life Science, Chair of Environmental Microbiology, Freising, Germany

[3] Technische Universität München, TUM School of Life Science, Chair of Terrestrial Ecology, Freising, Germany

Published online: 25 August 2023

In this context, it has been demonstrated previously that successful invasion of non-native species relies on the number of available niches [8, 9] and is negatively correlated with diversity, which is known as the diversity–invasiveness relationship [3]. Chiba et al. (2021) demonstrated that litter born microbes were only able to invade soil, if microbial diversity of the soil community was low, as achieved by autoclaving. Additionally, the colonization sequence of different microbial taxa seems to be a critical factor for further community development [9]. Causes for such divergence include facilitation or inhibition through microbial interaction [3], or changes of environmental conditions [10] like depletion of easily available nutrients. Delmont et al. (2014) [11] demonstrated that environmental filtering determines microbial colonization more than the composition of the inoculum community. They performed a cross-incubation experiment where they inoculated sterilized soil with its own initial microbial community or that from another soil origin. In general, in the case of sterilized soils, an endogenous heterotrophic succession can be expected [12] where nutrients released from dead cells will feed copiotrophic bacteria in the early development stages.

The knowledge about microbial community assembly and colonization is used in different field applications as well as laboratory setups including reclamation strategies, where microbial communities of natural soils from the same area are used as bioinoculum to direct the soil reclamation towards the previous natural situation [13]. Recently, it has been also suggested to inoculate microbiota from highly diverse soil ecosystems into soils, which are degraded as a result of intensive agricultural practice, to re-establish ecosystem services at such sites [14]. In several laboratory experiments often sterile soils have been used as substrate, which have been re-inoculated with differently diverse communities [15, 16]. Often differences in microbial diversity are achieved by using a distinction by dilution technique [8, 12], which is in most cases confounded by differences in microbial biomass of the inoculum. In addition, the ratio between soil and inoculum might determine the dynamics of community assembly and the likelihood of successful invasion of alien species. The question of what drives community development in disturbed soils between the diversity of inoculated microbiota and the inoculum microbial biomass is still not fully answered.

To test the consequences of inoculum amount (microbial biomass of the inoculum) for the recovery of microbial communities, we performed an incubation experiment with disturbed soil, which was achieved by successive autoclaving. The disturbed soil was either inoculated with 10%, 2%, or 1% of the original soil community (treatments) or left without an inoculum (non-inoculated control). Thus, the introduced microbial diversity was comparable between treatments and only the microbial biomass differed. These treatments were incubated 5 weeks under constant water content (50% maximal water holding capacity) and temperature (20 °C). The development of bacterial and archaeal biomass, diversity and community assembly were compared with the original soil (original soil control), which was incubated alongside. We combined quantitative PCR measurements, diversity analysis using metabarcoding approaches and community assembly analysis based on the calculation of nearest taxon index [17], which is a measure of phylogenetic relatedness using the 16S rRNA gene as a marker.

We hypothesized that (1) differences in microbial biomass of the inoculum will significantly impact microbial colonization pattern of disturbed soils. (2) Increasing biomass will induce more deterministic development of the soil microbiome whereas lower diversity and cell numbers in the inoculum will result in stochastic development of the soil microbiome. (3) The new established microbial community will be dominated by copiotroph bacteria, possibly spore forming bacteria, making use of necromass nutrients present in soil and differ from the community composition of the original soil.

Material and Methods

Soil Sampling and Sterilization

Soil samples were taken in June 2020 from "The Jena Experiment" field side (Roscher et al. 2004; Weisser et al. 2017 - http://the-jena-experiment.de/), which is located in Jena (Thuringia, Germany, 50°55′N, 11°35′E, 130 m a.s.l.) on the floodplain of the Saale river (altitude 130 m a.s.l.). The mean annual air temperature is 9.9 °C (1980–2010), and mean annual precipitation is 610 mm [18]. The Jena Experiment is composed of 82 plots with dimensions of 20 × 20 m where plant diversity has been manipulated since more than 20 years. The soil for our experiment was taken outside the treatment plots, reflecting the original soil where the experiment has been built on. The soil is classified as an Eutric Fluvisol (World Reference Base for Soil Resources 2015 [19]) with a pH value range from 7.1 to 8.4 and C_{org} 5–33 g C kg^{-1} [20]. A total of 50 kg fresh soil was sampled from the top 20 cm of a 1 m^2 area, using a shovel, and transported to the lab. For homogenization, the soil was sieved to 5 mm. The soil was split into two parts: one part to be disturbed and the other part to be used as control for the natural soil as well as to generate the inoculum. Soils were kept at 4 °C until further processing.

Disturbed soil was obtained by autoclaving. Autoclaving was done at 130 °C and 1.5 ATM for 1 h. After autoclaving the soil was incubated at 4 °C for 1 week to allow for potential spore germination and tested for successful sterilization. Therefore, 0.5 g soil and 100 µL of 0.8% NaCl sterile saline buffer were mixed. The obtained soil slurry was diluted 1:10, 1:100, and 1:1000, and 100µL of each dilution was plated on R2A [18] agar plates. Plates were incubated for a week at room

temperature, and growth was evaluated at the end of this period. This cycle of autoclaving, incubation and testing for sterility was repeated four times until no microbial growth on R2A medium was observed (Fig. S1)." After four rounds of autoclaving, no microbial growth on R2A medium was observed anymore.

Experimental Design

To evaluate the soil microbial recolonization after autoclaving, disturbed soil was reinoculated with original soil in three different proportions: 1:10 (10% inoculation), 1:50 (2%), and 1:100 (1%) by mixing. The amount of inoculum was calculated based on soil dry weight (w/w). In addition, both non-autoclaved soil (termed *Original*) and the autoclaved non-inoculated soil (termed *No inoculum*) served as controls alongside the experiment. Per treatment 15 g of the soil mixtures were incubated in open, 50 ml Falcon Tubes (Universal Medical-Germany) at 20 °C in the dark for 4 weeks. During the incubation, soil moisture was kept constant at 50% of maximum water holding capacity (mWHC) by watering every second day. Samples were taken at the beginning (T0) and after one (T1), two (T2), and 4 weeks (T4) of incubation from three independent replicates per treatment. Samples were immediately frozen at −20 °C for DNA analysis. Overall, 60 samples (5 treatments × 3 replicates × 4 samplings) were analyzed.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) was extracted by incubating 4 g of soil in 20 ml of 0.01 M $CaCl_2$ solution for 40 min on an overhead shaker [21]. Afterwards, samples were filtered using cellulose filter papers (595 ½ filter papers, Whatman–Germany). The DOC concentration of the extracts was measured with a DIMA-TOC 100 (Dima Tec, Germany).

DNA Extraction

Soil DNA was extracted from 0.5 g of fresh soil following a Phenol/Chloroform/Isoamyl alcohol method (modified from Pommerenke and Friedrich, 2007). The sample lysis was done using Lysing Matrix E tubes (MP Biomedicals™, Germany). The bead beating was done using the TissueLyser II bead beater (QIAGEN®, Germany) at a frequency of 15 Hz during 2 min. Resulting DNA was quantified by Qubit fluorometric system (Thermo Fisher Scientific, Germany) using the broad range assay kit. The DNA quality was checked using the Nanodrop photometric system (Thermo Fisher Scientific, Germany) and by agarose gel electrophoresis. The extracted DNA was stored at −20 C° until usage. To exclude contaminations during DNA extraction a blank control without soil was included.

In addition to the normal DNA extraction, a smaller test was performed to estimate how much DNA in the original soil derived from intact cells. We used propidium monoazide (PMA), a photo-reactive DNA-binding dye which, when exposed to light, degrades extracellular DNA [22]. Therefore, a set of parallel DNA extractions was performed. Those comprise the original soil (−PMA), original soil treated with PMA (+PMA), a "dead" soil control (dead−PMA) and a "dead" soil treated with PMA (dead+PMA). The "dead" soil control was included to determine the capability of PMA to remove DNA from the samples, being used to calculate the efficiency of extracellular DNA removal. The "dead" soil samples were heated at 90 °C for 1 h prior to DNA extraction, in order to kill vegetative cells on the samples. The test was conducted in triplicate for each treatment. Soil samples with PMA (0.5 g) were incubated in the dark with 25 µM of PMA (Biotium-Germany) for 10 min, and then exposed to white light for 25 min. DNA was extracted as described above. The percentage of DNA coming from dead and/or damaged cells was calculated by follows:

$$\text{ExtracelularDNA}\% = \left[\left(1 - \left(\frac{\text{dead} + \text{PMA}}{\text{dead} - \text{PMA}}\right)\right) \times \left(1 - \left(\frac{+\text{PMA}}{-\text{PMA}}\right)\right)\right] \times 100$$

Quantitative PCR of Bacteria and Archaea

Bacterial and archaeal abundance was determined by a SybrGreen based absolute quantification using a 7300 Real-Time PCR System (Applied Biosystems, Germany). To quantify bacteria, the primer pair FP 16S and RP 16S [23] was used; for archaea the primer pair rSAf(i) [24] and 958r (Bano et al. 2004). Each PCR reactions contained 12.5 µL Power SYBR™ Green PCR Master Mix (Thermo Fisher Scientific, Germany), 1 µL of each primer with 10 pmol μL^{-1}, 0.5 µL of 3% BSA, 2 µL of extracted DNA, and 8.0 µL of DEPC treated water. Thermal profiles are summarized in Table 1. As standard curve serial dilutions (10^3 to 10^9 copies per μL^{-1}) of plasmids containing the 16S rRNA gene fragment of *Pseudomonas putida* for bacteria and *Methanobacterium* sp. for archaea were used in three technical replications. Besides three replicates per standard, three no template controls were included in each run. The obtained copy number was subtracted from samples and controls.

To exclude any inhibitory effects of co-extracted substances, a dilution test was performed, which identified a 1:32 dilution as sufficient. The specificity of the PCR

Table 1 Thermal profiles for qPCR of the 16S rRNA gene of bacteria and archaea

Target Gene	Thermal profile	No cycles
16S rRNA bacteria	95 °C—10 min	1
	95 °C—45 s/58 °C; 45 s/ 72 °C—45 s	35
16S rRNA archaea	95 °C—10 min	1
	95 °C—20 s/55 °C; 60 s/72 °C—60 s	5 (touchdown: −1 °C per cycle)
	95 °C—20 s/58 °C; 60 s/72 °C—60 s	40

product was ensured by doing a melting curve analysis as well as an agarose gel at 1.5%, running for 40 min at 120 V and 400 mA. Final copy numbers were calculated by normalizing the number of copies per µL to g of dry soil. The respective qPCR efficiency (calculated with the formula $\text{Eff} = [10^{(1/\text{slope})}-1]$) and R^2 for bacteria and archaea ranged between R^2 0.997 and 0.982 and efficiency 76.47% and 71.12% for bacteria and; R^2 0.998 and 0.997 and efficiency 84% and 85.4% for archaea.

Amplicon Sequencing and Bioinformatics

To analyze bacterial and archaeal diversity, a metabarcoding approach was used following the "16S Metagenomic Sequencing Library Preparation" protocol (Illumina, San Diego, CA, USA) and quality guidelines recommended by Schöler et al. (2017). Bacteria and archaea were targeted together by using the universal primer pair 515FB and 806RB [25, 26] with Illumina adapters. Each reaction contained 12.5 µL NEB Next High-Fidelity Master Mix (Thermo, Germany), 0.5 µL of each primer at 10 pmol μL^{-1}, 2.5 µL of 3% BSA, 1 µL of 5 ng μL^{-1} diluted DNA, and 8 µL of DEPC treated water. The thermal profile was the following: 98°C for 1 min, followed by 23 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s, ended by final extension of 72 °C for 5 min. Afterwards, samples were purified with the MagSi-NGSprep Plus Beads (ratio 0.8 beads: 1 sample); according to the manufacturers protocol and quantity of the PCR, product was measured using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Germany). Purified samples were indexed using Nextera® XT Index Kit v2 (Illumina, San Diego, CA, USA) and again purified with MagSi-NGSprep Plus Beads (ratio 0.8 beads: 1 sample). Quality assessment and final quantification of the indexed fragments was done via Fragment Analyser (Agilent, Germany). High-quality DNA was diluted to 4 nM and sequenced on Illumina MiSeq using MiSeq Reagent v3 (600 Cycle) kit. 5 pM, 20% PhiX was loaded alongside the samples.

Raw sequences were demultiplexed based on the associated barcodes and adapters were removed using AdapterRemoval version 2.3.1 [27]. Afterwards datasets were processed using Qiime2 version 2021.2 and the plugin DADA 2 version 1.18 [28]. The sample processing includes denoising using denoise-paired option with the following parameters: --p-trim-left-f 20; --p-trim-left-r 20; --p-trunc-len-f 240; --p-trunc-len-r 200; --p-max-ee-f 4; --p-max-ee-r 4. Taxonomic assignments were done against SILVA database (version 138) using *classify-consensus-blast* option in default parameters. The raw sequencing reads were uploaded to NCBI sequencing read archive under the bioproject number PRJNA937438.

Statistical Analysis

The table of amplified sequence variants (ASVs) was exported to R, where Phyloseq version 1.42.0 [29] and Vegan version 2.6-2 packages were used to construct rarefaction curves to observe sequencing coverage, to normalize the number of reads in each sample using median sequencing depth, plot relative abundance and ordination plots (NMDS with Bray-Curtis dissimilarity distance). Furthermore, the packages were used to calculate alpha diversity metrics (Observed ASVS, Shannon diversity and Evenness). Those metrics were used as response variable for experimental design and statistical significances across the treatment was calculated via ANOVA. Pairwise comparison was done using Tukey post hoc test for multiple comparisons of means. Additionally, to get values for bacteria and archaea separately, bacterial and archaeal reads were filtered manually from a phyloseq object and analyzed the same way. From a total of 7820 ASVs, distributed in 3.224.377 reads, 7764 ASVs (3.185.379 reads) were assigned as bacteria and 56 ASVs (38.998 reads) were from archaea. We also used PERMANOVA to calculate the effect size and significance over beta diversity, using as input a Bray-Curtis dissimilarity matrix. Prior to statistical testing, data was checked for normality via the Shapiro-Wilk tests and Q-Q plots.

The Qiime2 plugin SourceTracker version 1.0.1 [30] was used with default parameters to track the origin of ASVs in different treatments. The original soil at T0 was treated here as source. The sink was the re-inoculated soils.

The normalized dataset was used to plot the taxonomical composition of the most abundant taxa in a form of a

heatmap using the pheatmap package (version 1.0.12). ASVs with abundance of 5% or greater in at least one of the samples were filtered using the *filter taxa* function on phyloseq package. Dendrograms were drawn using Ward's Hierarchical Clustering.

To assess the phylogenetic relationship, we calculated the mean-nearest-taxon-distance (MNTD) and the nearest-taxon-index (NTI) [17] using "mntd" and "ses.mntd" implemented in the package "picante" version 1.8.2 [31]. This procedure allows to determine whether a given community (input is a phylogenetic tree generated by Qiime2) is more phylogenetic related than expected by chance, when compared to a random version of this same community (*null model*) [17]. We use this metric to estimate whether microbial communities' assembly stochastically (NTI less than −2) or deterministically (NTI greater than +2). To generate the underlying phylogenetic tree, Qiime2 was used by applying the *alignment mafft* function for alignment, *alignment mask* to mask ambiguously aligned regions, *fasttree* for tree generation, and *phylogeny midpoint-root* for tree rooting.

Results

Recovery of Bacterial and Archaeal Biomass After Reinoculation of Disturbed Soil

Bacterial abundance was significantly influenced by time (bacteria: $p = 0.003$, $F = 5.247$) while archaeal abundance not ($p = 0.184$, $F = 1.673$). The abundances of both groups were significantly impacted by the dilution treatment (bacteria: $p = 0.00745$, $F = 3.918$; archaea: $p = 1.79e\text{-}15$, $F = 40.927$). The Tukey post hoc test (Table S1) indicates that bacterial abundance significantly differed between T0 and T1 ($p = 0.009$) and T0 and T4 ($p = 0.019$). Across all treatments, significant differences of bacterial abundance were found when samples with no-inoculum and inoculation level 1:10 ($p = 0.016$) were compared, while for archaea the differences were between no-inoculum samples and inoculation level 1:10 ($p = 0.0092348$) as well as between original soil and all inoculation levels, being 1:10 ($p < 0.00001$), 1:50 ($p < 0.00001$), 1:100 ($p < 0.00001$), and non-inoculated soils ($p < 0.00001$).

Bacterial and archaeal abundance in original soils remained stable over the entire period of the experiment ranging between $1.2* 10^8$ to $9* 10^8$ copies g^{-1} of dry soil for bacteria and $9* 10^5$ to $2.3* 10^6$ copies g^{-1} of dry soil for archaea (Fig. 1A and B). Already after 1 week of incubation (T1), bacterial abundance of the inoculated treatments was significantly higher than that of the original soil and remained stable until the end of the experiment, at average $1.8* 10^9$ copies g^{-1} of dry soil, independent from inoculation level. In contrast, in the non-inoculated disturbed soil treatment, bacterial abundance was lowest at T0 ($7.8* 10^4$ copies g^{-1} of dry soil), indicating that some microorganisms or their DNA remained in the soil after 4 steps of autoclaving. However, recovery of microbial biomass in non-inoculated soils was much slower compared to the inoculated treatments and reached comparable values only after 3 weeks of incubation (T3).

Abundance of archaea was at least 2 orders of magnitude lower than the bacterial counterpart over the complete

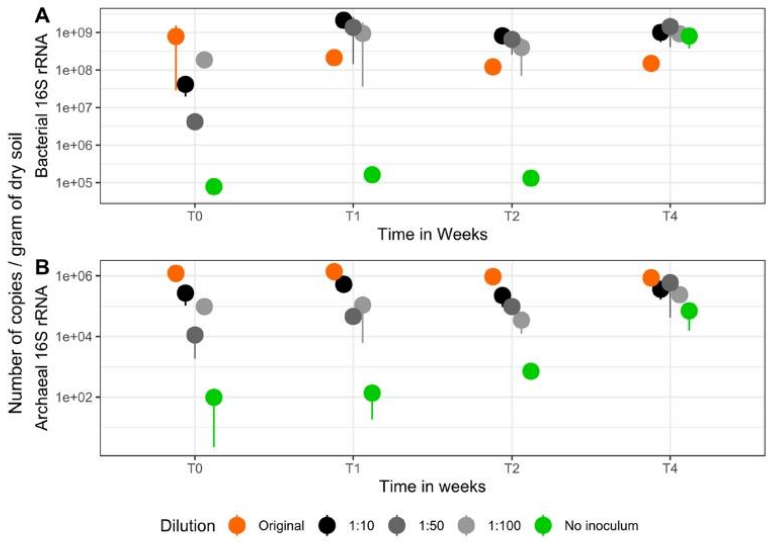


Fig. 1 Mean values of the absolute quantification of 16S rRNA gene copies per g^{-1} dry soil for bacteria (**A**) and archaea (**B**). Treatments are color coded as follows: original soil (orange), the reinoculated dilutions 1:10 dilution (black), 1:50 dilution (dark grey), 1:100 dilution (light grey), and the soil without inoculum (green) soil across the experimental period (T0–T4). Error bars are standard deviation ($n = 3$). *Y*-axis is presented in logarithmic scale

duration of the experiment in all treatments. In contrast to bacteria, we observed that the amount of inoculated soil positively affected the community recovery since the 1:10 dilutions lead to a consistently higher number of copies (average 5.5e+05 copies g^{-1} of dry soil) compared to the treatment level 1:50 (average 2.0e+05 copies g^{-1} of dry soil) and 1:100 (average 1.5 e+05 copies g^{-1} of dry soil). Values in the original soil were always higher independent from the biomass of the inoculum (average 1.4 e+06 copies g^{-1} of dry soil) (Fig. 1B).

The viability PCR (PMA PCR) indicated that 23.9% of DNA present in the original soil samples was derived from non-intact cells (see Fig. S5).

Recovery of Prokaryotic Alpha Diversity After Reinoculation of Disturbed Soil

The 16S rRNA amplicon sequencing yielded a total of 3.325.064 high quality reads. After filtering, denoising, merging, and chimeral removal 3.267.439 reads remained, which were normalized to 54.515 reads per sample. Rarefaction analysis revealed that the number of reads was enough to cover bacterial and archaeal diversity in the samples (Fig. S3).

As shown in Fig. 2A, both time and treatment had significant effects on alpha diversity (ANOVA $p = 2.81*10^{9}$ for dilution effects and $p = 5.68*10^{5}$ for time effects). As observed for the abundance, alpha diversity of prokaryotes expressed as number of observed ASVs was stable in the original soil and ranged between 1676 and to 913. The post hoc test indicated significant differences between T0 and T2 ($p = 0.0061539$) as well as treatment specific differences between the original soil and all other treatments ($p < 0.00001$ – Table S2). Diversity was significantly lower in all inoculation treatments compared to original soil ranging from 240 at T2 to 413 at T4 independent from the biomass of the inoculum. In the non-inoculated treatment, the number of observed ASVs was lowest after 4 weeks (T4) of incubation and reached a value of 68 observed ASVs only, which was accompanied by a strong additional drop in Evenness values (Fig. S4). The diversity patters can by mostly attributed to the bacteria community, which composed 98.8% of the assigned reads, while archaea had 1.2%. The diversity pattern can, however, also be observed in the archaeal community (Fig. S6 and Table S3).

Recovery of Prokaryotic Community Composition After Reinoculation of Sterilized Soil

The Sourcetracker based mapping of ASVs (Fig. 2B) from the original soil at T0 towards the disturbed and re-inocculated soils demonstrated that ASVs in the inoculated disturbed soils derived from the imoculum. Interestingly, differences in shared ASVs were observed in response to the different inoculation treatments used. For example, at T0, the 1:10 treatment shared 78% of ASVs with the original

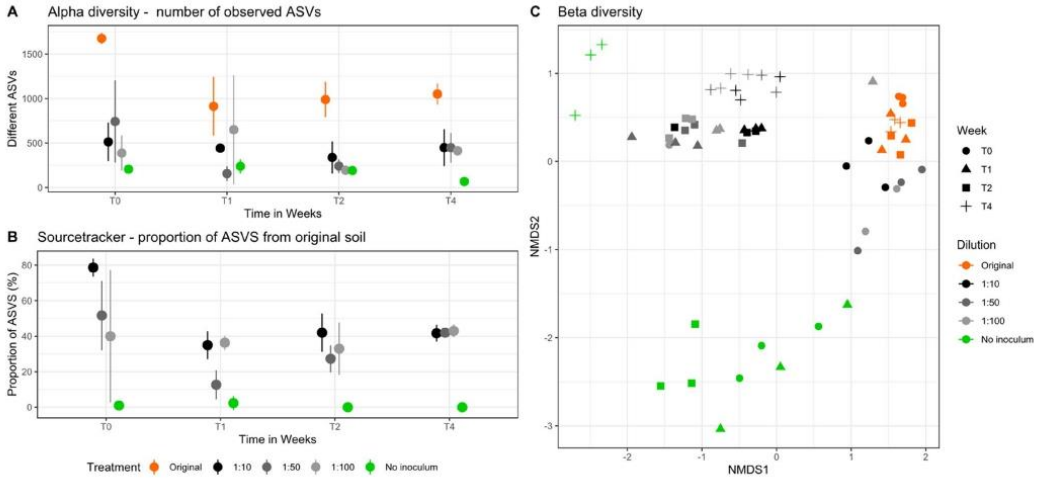


Fig. 2 **A** Alpha diversity of prokaryotes shown as the number of observed ASVs at T0, T1, T2 and T4. **B** Sourcetracker analysis showing the percentage of ASVs in the diluted samples coming from the original soil over the experimental period (T0–T4). Shown are mean values of three replicates and error bars represent standard deviations. **C** NMDS plot based on Bray-Curtis dissimilarity matrix based on ASVs. Shown is the original soil (orange), 1:10 dilution (black), 1:50 dilution (dark grey), 1:100 dilution (light grey), and non-inoculated (green) soils

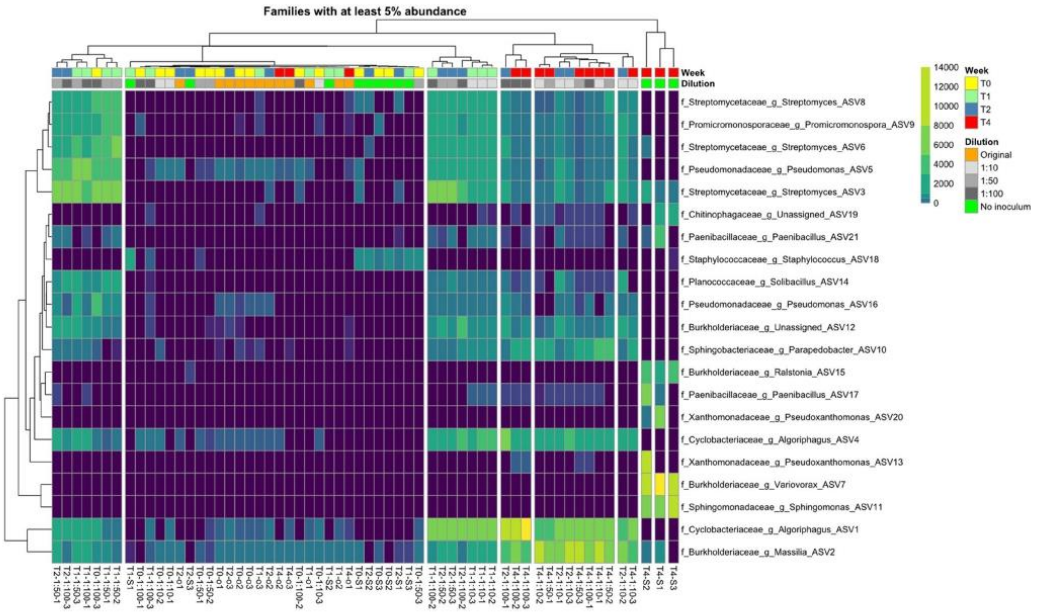


Fig. 3 Heatmap and dendrogram showing the relative abundance of dominant ASVs annotated at family and genus level. Depicted ASVs needed to exceed 5% relative abundance in at least one sample. The dendrogram was achieved by applying the Ward's Hierarchical Clustering. The color codes depict absolute abundance of reads assigned to the given ASVs

soil, the 1:50 treatment shared 52%, and the 1:100 treatment shared 40%, while the non-inoculated soil showed no overlap. This result was further confirmed by beta-diversity analysis (Fig. 2C) and hierarchical clustering methods (Fig. 3). The NMDS plot indicated, that at T0, the original soil and the soil inoculation treatments were most similar, except for the non-inoculated soil treatment, which separates from all other T0 samples. The initial load of inoculum impacted the reproducibility of the initial colonization. The 1:10 treatment showed a standard deviation of 5.0% at week 0 based on Sourcetracker analysis, while the 1:50 and 1:100 treatments showed standard deviations of 19.5% and 37.2%, respectively. At T4, all dilution levels converged to a similar share of 42% of ASVs deriving from original soil. The composition of all communities in the inoculated soils significantly differed at T4 from the community composition at T0 (PERMANOVA $p = 0.006$), but no clear pattern in response to the different inoculation densities were observed.

The hierarchical clustering of communities shown in the dendrogram of Fig. 3 further supports that observation. One cluster is represented by samples of the non-inoculated treatment, which is dominated by ASVs assigned to different genera of *Burkholderiaceae* namely *Variovorax* sp. and *Ralstonia* sp., and to specific genera of *Sphingomonadaceae*, *Chitinophagaceae*, *Paenibacillaceae*, and *Xanthomonadaceae*, which are *Sphinogmonas* sp., *Paenibacillus* sp., and *Pseudoxanthomonas* sp. However, this community significantly differs from the other two clusters. Cluster 2 represented samples of the dilution treatments at T1 and the original soils, which showed an even distribution of ASVs. The dilution samples at T1 are dominated by ASVs assigned to *Cyclobacteriaceae* genus *Algoriphagus* sp., *Promicromonosporaceae* genus *Promicromonospora* sp., *Pseudomonadacaea* genus *Pseudomonas*, and *Streptomycetaceae* genus *Streptomyces* sp. The third cluster includes all dilution treatments at T4, but also those from T2 and is characterized by samples, which have a high relative abundance of ASVs assigned to *Burkholderiaceae*, *Cyclobacteriaceae* genus *Algoriphagus* sp. and *Sphingobacteriaceae* genus *Parapedobacter* sp., *Paenibaciliaceae* genus *Paenibacillus* sp. Compared to cluster 1 another genus of *Burkholderiaceae* was identified in cluster 2, which was *Massilia* sp. The dominance of those families indicates an initial bloom of copiotrophic bacteria, which decline during the subsequent incubation period. The results of NTI calculation revealed that changes in community composition are mostly deterministic, as all samples had an NTI value > 2 (Fig. 4), which indicates "niche-based" deterministic mechanisms.

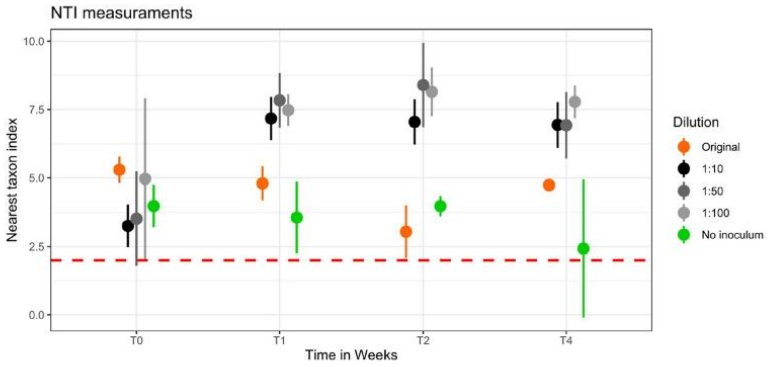


Fig. 4 Nearest taxon index measurements built over phylogenetic tree from sequenced samples. The results show that all the samples were clustered above the threshold of 2 (red dashed line), indicating strong phylogenetic signal on all the samples over time. Diluted samples show higher NTI values.

Nevertheless, the diluted samples always exceeded the non-inoculated soil samples, indicating a stronger phylogenetic clustering as a result of the inoculation.

Discussion

Biomass Recovery Is Driven by Few Taxa

Our data indicated that the recovery of microbial biomass (Fig. 1) was different for bacteria and archaea and was driven by few taxa, which quickly increased in relative abundance (Fig. 3). Although the initial microbial load was consistent to the dilution (highest abundance in original soil, followed by 1:10, 1:50, 1:100, and non-inoculated soil treatments), bacterial abundance recovered quicker than the archaeal abundance, while diversity recovery followed similar pattern. Previous data [32] indicate that colonization is driven for bacteria in nutrient-rich habitats, but for archaea more under oligotrophic conditions [32]. The soil in our study can be considered as nutrient rich, as the process of autoclaving releases dissolved nutrients from dead cells into the soil. The DOC concentrations after autoclaving (Fig. S5) revealed a significant increase, which was also been previously observed [33]. The flush of carbon was most likely derived from dead cells [34]. Microbial necromass was already shown to be an important player for soil nutrient turnover [35]. Furthermore, the Jena Experiment is built on a previous agricultural site, which was frequently fertilized [20]. This in combination with the release of organic carbon as a result of autoclaving might have favored fast-growing bacteria.

In our study, the most dominant bacteria has been classified as copiotroph for example Burkholderiaceae, Sphingomonadaceae, and Xanthomonadaceae [36] able to use organic carbon and nitrogen for quick growth. In contrast to bacteria, archaea recovered slower and never exceeded the abundance of the original soil. Interestingly, this was not reflected by the diversity development of bacteria and archaea, which revealed a similar pattern, indicating that the abundance effects is most likely dedicated to slower growth of the archaeal species.

The analysis of the community composition revealed that prokaryotic growth was not a uniform response over the whole community, but only of some fast-growing taxa mostly dominated by bacteria. We had originally hypothesized that the initial weeks are dominated by spore forming microbes, which are widely spread as spore bank in soil [33]. However, this could be only partly verified. In the non-inoculated soil, *Paenibacillus* sp. was indeed highly dominant but surprisingly only at T4. In the dilution treatments an initial *Streptomyces* bloom was observed at T1. This observation indicates that even though spores might be able to survive extreme conditions as extreme heat, their increase in abundance also depends on interactions with other microbes. In this respect, *Streptomyces* sp. might be especially successful, as they are well known as producers of different antibiotics [34], which displays a selective advantage during colonization of new habitats and occupation of empty niches. Moreover, this family comprises many metabolic versatile taxa [35, 37]. The combination of carbon consumption, antibiotic production, and denitrification is likely responsible for successful colonization of inoculated soils by *Streptomyces* sp. The dominance of Actinobacteria, to which *Streptomyces* belong, was also reported in a study of Delmond et al. (2014) as being highly abundant after soil sterilization and reinoculation and being positively correlated with carbon availability, which was also increased in our study (DOC in Fig. S6). Additionally, the first week of community development in inoculated soils was further dominated by *Cyclobacteriaceae, Pseudomonadaceae*, and *Burkholderiaceae*. *Cyclobacteriaceae* are abundant in soil environments and successful in processing a wide range of polysaccharides due to a rich repertoire of carbohydrate-active enzymes [38].

Burkholderiaceae consist of a metabolic diverse range of taxa [39] including denitrifying [40] and plant growth promoting strains.

Denitrification has been often observed in nutrient-rich environments [37], as is the case of soil used in our study, indicating a very early establishment of microbiota driving key processes of N cycling including mineralization of N and nitrification, resulting in the needed nitrate for denitrification. The abundance *Massilia* and *Streptomyces* genera in the inoculated soils indicates that microbial community on these treatments sustained a better metabolization of complex carbon forms in the soil, as chitin [41] and cellulose, speeding up the assimilation of carbon by the community. Additionally, *Pseudomonas* sp. was highly abundant. This genus is present in a variety of environments from soil to plant and animal tissue [42] and contains both plant pathogens and plant growth promoting bacteria. The distinction of plant growth promoting or pathogenic takes often place on strain level [42].

If no inoculum was applied, soil recolonization was much slower, much more random and resulted in an altered community composition. This difference in community composition and recovery rate stresses the positive effect of inoculation to re-establish original community even in small amounts, i.e., 1:100 dilution. Evidence shows that rarefying microbial communities can impact ecosystem functions and only extreme dilutions can lead to the inability from microbial community to establish over time [12].

Community Composition Converges if Inoculum Is Given

The beta diversity analysis and Sourcetracker results demonstrated that inoculation of sterilized soils resulted in a prokaryotic community similar to the original soil and that the degree of overlap was further modulated by amount of inoculum. Considering the proportion of relic DNA in the original soil, the overlap might be even higher [22]. However, the following recovery of prokaryotic biomass caused a drop in alpha diversity (Fig. 2A). The diversity of original soil was also never exceeded during the incubation by any of the treatments, which underlines that initial microbial bloom in the dilutions was dominated by a few microbial groups, as discussed in previous sections. Diversity seemed to randomly fluctuate over time on non-inoculated soils and on soils which received less inoculate biomass (1:50 and 1:100). Nevertheless, after 4 weeks all microbial communities of the soils receiving an inoculum converged and were not significantly different from each other anymore (PERMANOVA $p = 0.317$). However, original community composition was only partly resembled; indicating that regardless of initial inoculation load, the inoculated soils had the potential to reproducibly recolonize the non-inoculated one, but original diversity was not reached at least during the incubation period. All the inoculated soils stabilized in an intermediary stage in terms of diversity and community composition between original and non-inoculated disturbed soil (Fig. 2A). These findings are strongly supported by the NTI measurements, which clearly points to microbial community's deterministic adaptation. This was further demonstrated by the convergent behavior of communities regardless of inoculation amount. Interestingly, the NTI values tripled from week 0 to week 1 in the dilutions, while it remained relatively stable not just in the original soil, but also in the non-inoculated soil samples (Fig. 3). This data shows that microbial community present in the inoculum actively reacts to environmental filtering, strengthening the phylogenetic signal. These findings corroborate the theory that phylogenetically closer taxa would prefer similar habitats and perform similar ecological processes [43], here selected by its ability to utilize easily available carbon and other nutrient sources and quickly occupy empty niches.

The effect of soil inoculation on recovering microbial diversity has already been reported in a similar study [11]. In this study, 2 g of soil from different countries was inoculated in 30 g of sterilized soil (1:15 proportion), leading to similar final microbial communties after an incubaction period of 6 months. Those findings corroborate with our results, in a way that soil as inoculum harbor enough microbial diversity to populate new environments according to environmental filtering [1, 44]. In this sense, the community establishment limitation is not the diversity in the initial inoculum, as stated by Deltmont et al. (2014) or the load of the inoculum, which we tested in our experiment, but rather the environmental filter imposed to this community. In terms of microbial diversity recovery, the initial loss of diversity and subsequencial recovery was also oberved in chronosequence studies by Li et al. [8] and Jurburg et al. [45], where microbial diveristy decreased after an initial disturbance, being dominted by a few surviving taxa. Jurburg et al. [45] also reported microbial community development to be ruled by niche processes.

When we studied the natural development of microbial communities in non-inoculated soil, the recovery was much slower, less reproducible, and resulted in an altered community composition. The competition with indigenous microbes is lower in autoclaved treatment, thus empty niches can be randomly colonized. Potential sources for the initial community might be prokaryotes resistant to soil autoclaving procedure as it might be the case for *Paenibacillaceae* or airborne prokaryotes. In previous studies, Firmicutes, Proteobacteria, and Bacteriodetes were frequently detected in different bioaerosols [46, 47]. However, their settlement might be random, in low abundance and thus causing high fluctuation in community dynamics.

Interestingly, the Sourcetracker mapping of shared ASVs indicated that initial microbial load followed the dilution

pattern, being 1:10 dilution, the one with higher percentage of shared ASVs (79%), followed by 1:50 (52%), and 1:100 (40%) (Fig. 2B). Considering that even for 1:100 dilution, 0.15 g of original soil was added, which was similar in bacterial and archaeal diversity compared to original soil and the other two dilutions, this result is surprising, but indicates that the amount of applied microbiota mainly for the less abundant ones is an important factor for recolonization. The numbers of shared ASVs might be even higher, considering the amount of relic DNA present in the original soil [22], which in our case was approximately 23%.

Conclusion

Despite the study of deterministic and stochastic colonization being a well-known field in ecology and fairly well covered in microbial ecology [6, 48, 49], proofs of principle and case studies are still poorly explored. Currently, literature indicate that environmental filtering pressure might vary when physicochemical parameters change [50] and deterministic community development seems to be predominant over stochastic ones [17, 48, 51]. Together with the data from Delmont et al. (2014), our study demonstrated that soil physicochemical conditions overshadow initial inoculum load and composition as determining factor for community establishment. Such knowledge has to be taken in consideration during the design of inoculation experiments [52, 53] and indicated that soil management might be a clever approach to enrich certain microbial taxa [54, 55]

In summary, our data indicates that microbial community assembly in sterilized and re-inocculated soils occurs in deterministic ways, being ruled by "niche-based" interactions. In agreement with our expectations, microbial inoculation drives soil colonization, however unable to fully recover initial microbial composition and diversity, leading in the end to the dominance of different microbial taxa. Colonization was clearly driven by bacteria compared to a stable archaeal community. The changes in environmental filtering can be mostly attributed to physicochemical changes in the soil after autoclaving. Although still being ruled by niche processes, the non-inoculated soil did not have a clear colonization pattern, reinforcing the importance of inoculation to stochastic colonization. Overall, our findings may help to better understand the process of microbial establishment in soil communities, as well as the limitations of soil microbiome manipulation, which can have important practical implications during soil restoration.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-023-02285-9.

Acknowledgements We would like to thank the Jena Experiment coordination team, Nico Eisenhauer, Anne Ebeling, and the field coordinator, Annette Jesch for the support on organizing the soil sampling and the access to the field site and the gardeners and technicians with the help during the soil sampling. We also would like to thank Gudrun Hufnagel for her valuable help on preparing the experiment and Aaron Fox for the proofreading. Furthermore, we thank the Deutsche Forschungsgemeinschaft (DFG) for funding the Jena Experiment (FOR 5000, SCHU2907/8-1, SCHL446/45-1, WE3081/39-1).

Author Contribution All authors contributed to the study conception and design. Wolfgang Weisser collected the soil material. Experimental execution, data collection and analysis were performed by Yuri Pinheiro. Data interpretation and visualization was performed by Yuri Pinheiro and Stefanie Schulz. The first draft of the manuscript was written by Yuri Pinheiro and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by Deutsche Forschungsgemeinschaft (DFG) by funding the Jena Experiment Research Unit FOR 5000. The authors MS, WW, and SS received the following grants: SCHL446/45-1, WE3081/39-1 SCHU2907/8-1.

Data Availability The sequencing datasets generated during the current study are available in the NCBI sequencing read archive under the bioproject number PRJNA937438.

Declarations

Competing Interests The authors declare no competing interests.

References

1. Kennedy AC, Smith KL (1995) Soil microbial diversity and the sustainability of agricultural soils. Plant Soil **170**:75–86. https://doi.org/10.1007/BF02183056
2. Morris JJ, Lenski RE, Zinser ER (2012) The black queen hypothesis: evolution of dependencies through adaptive gene loss. MBio **3**. https://doi.org/10.1128/mBio.00036-12
3. Nemergut DR, Schmidt SK, Fukami T et al (2013) Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. **77**:342–356. https://doi.org/10.1128/mmbr.00051-12
4. Dial R, Roughgarden J (1998) Theory of marine communities: the intermediate disturbance hypothesis. Ecology **79**:1412–1424. https://doi.org/10.1890/0012-9658(1998)079[1412:TOMCTI]2.0.CO;2
5. Dumbrell AJ, Nelson M, Helgason T et al (2010) Relative roles of niche and neutral processes in structuring a soil microbial community. ISME J **4**:337–345. https://doi.org/10.1038/ismej.2009.122
6. Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. Philos.

Trans. R. Soc. Lond. B Biol. Sci. **366**:2351–2363. https://doi.org/10.1098/rstb.2011.0063
7. Hargreaves SK, Williams RJ, Hofmockel KS (2015) Environmental filtering of microbial communities in agricultural soil shifts with crop growth. PLoS One **10**:1–14. https://doi.org/10.1371/journal.pone.0134345
8. Li Y, Wen H, Chen L, Yin T (2014) Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS One **9**. https://doi.org/10.1371/journal.pone.0115024
9. Van Elsas JD, Chiurazzi M, Mallon CA et al (2012) Microbial diversity determines the invasion of soil by a bacterial pathogen. Proc. Natl. Acad. Sci. U. S. A. **109**:1159–1164. https://doi.org/10.1073/pnas.1109326109
10. Parks DH, Imelfort M, Skennerton CT et al (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res. **25**:1043–1055. https://doi.org/10.1101/gr.186072.114
11. Delmont TO, Francioli D, Jacquesson S et al (2014) Microbial community development and unseen diversity recovery in inoculated sterile soil. Biol. Fertil. Soils **50**. https://doi.org/10.1007/s00374-014-0925-8
12. Maron PA, Sarr A, Kaisermann A et al (2018) High microbial diversity promotes soil ecosystem functioning. Appl. Environ. Microbiol. **84**. https://doi.org/10.1128/AEM.02738-17
13. Schmid B, Weisser WW, Schumacher J et al (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl. Ecol. **5**:107–121
14. Duell EB, O´Hare A, Wilson GWT, (2022) Inoculation with native soil improves seedling survival and reduces non-native reinvasion in a grassland restoration. Restoration Ecology 31:1061–2971
15. Baumann K, Dignac MF, Rumpel C et al (2013) Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry **114**:201–212. https://doi.org/10.1007/s10533-012-9800-6
16. Yang G, Roy J, Veresoglou SD, Rillig MC (2021) Soil biodiversity enhances the persistence of legumes under climate change. New Phytol. **229**:2945–2956. https://doi.org/10.1111/nph.17065
17. Stegen JC, Lin X, Konopka AE, Fredrickson JK (2012) Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J **6**:1653–1664. https://doi.org/10.1038/ismej.2012.22
18. Hoffmann K, Bivour W, Früh B et al (2014) Klimauntersuchungen in Jena für die Anpassung an den Klimawandel und seine erwarteten Folgen. Selbstverlag des Deutschen Wetterdienstes, Offenbach am Main
19. Weisser WW, Roscher C, Meyer ST et al (2017) Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. Basic Appl. Ecol. **23**:1–73. https://doi.org/10.1016/j.baae.2017.06.002
20. Roscher C, Schumacher J, Baade J et al (2004) The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl. Ecol. **5**:107–121. https://doi.org/10.1078/1439-1791-00216
21. Schulz S, Engel M, Fischer D et al (2013) Diversity pattern of nitrogen fixing microbes in nodules of Trifolium arvense (L.) at different initial stages of ecosystem development. Biogeosciences **10**:1183–1192. https://doi.org/10.5194/bg-10-1183-2013
22. Carini P, Marsden PJ, Leff JW et al (2016) Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nat Microbiol **2**:16242. https://doi.org/10.1038/nmicrobiol.2016.242
23. Bach HJ, Tomanova J, Schloter M, Munch JC (2002) Enumeration of total bacteria and bacteria with genes for proteolytic activity in pure cultures and in environmental samples by quantitative PCR mediated amplification. J. Microbiol. Methods **49**:235–245. https://doi.org/10.1016/S0167-7012(01)00370-0
24. Nicol GW, Glover LA, Prosser JI (2003) Molecular analysis of methanogenic archaeal communities in managed and natural upland pasture soils. Glob. Chang. Biol. **9**:1451–1457. https://doi.org/10.1046/j.1365-2486.2003.00673.x
25. Parada AE, Needham DM, Fuhrman JA (2016) Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. **18**:1403–1414. https://doi.org/10.1111/1462-2920.13023
26. Apprill A, Mcnally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. **75**:129–137. https://doi.org/10.3354/ame01753
27. Schubert M, Lindgreen S, Orlando L (2016) AdapterRemoval v2: rapid adapter trimming, identification, and read merging. BMC Res. Notes **9**:1–7. https://doi.org/10.1186/s13104-016-1900-2
28. Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. **41**:590–596. https://doi.org/10.1093/nar/gks1219
29. McMurdie PJ, Holmes S (2013) Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One **8**. https://doi.org/10.1371/journal.pone.0061217
30. Knights D, Kuczynski J, Charlson ES et al (2011) Bayesian community-wide culture-independent microbial source tracking. Nat. Methods **8**:761–765. https://doi.org/10.1038/nmeth.1650
31. Kembel SW, Cowan PD, Helmus MR, et al (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics **26**, 1463–1464. https://doi.org/10.1093/bioinformatics/btq166
32. French E, Kozlowski JA, Bollmann A (2021) Competition between ammonia-oxidizing archaea and bacteria from freshwater environments. Appl. Environ. Microbiol. **87**:1–11. https://doi.org/10.1128/AEM.01038-21
33. Zeigler DR (2014) The *Geobacillus* paradox: why is a thermophilic bacterial genus so prevalent on a mesophilic planet? Microbiology **160**:1–11. https://doi.org/10.1099/mic.0.071696-0
34. de Lima Procópio RE, da Silva IR, Martins MK et al (2012) Antibiotics produced by *Streptomyces*. Braz. J. Infect. Dis. **16**:466–471. https://doi.org/10.1016/j.bjid.2012.08.014
35. Zhang H, Ma B, Huang T, Shi Y (2021) Nitrate reduction by the aerobic denitrifying actinomycete *Streptomyces sp.* XD-11-6-2: performance, metabolic activity, and micro-polluted water treatment. Bioresour. Technol. **326**:124779. https://doi.org/10.1016/j.biortech.2021.124779
36. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology **88**:1354–1364. https://doi.org/10.1890/05-1839
37. Shoun H, Kano M, Baba I et al (1998) Denitrification by actinomycetes and purification of dissimilatory nitrite reductase and azurin from *Streptomyces thioluteus*. J. Bacteriol. **180**:4413–4415. https://doi.org/10.1128/jb.180.17.4413-4415.1998
38. Larsbrink J, McKee LS (2020) Bacteroidetes bacteria in the soil: glycan acquisition, enzyme secretion, and gliding motility1st edn. Elsevier Inc.
39. Baldani JI, Videira SS, dos Santos Teixeira KR, et al (2014) The Family Rhodospirillaceae BT - The Prokaryotes: Alphaproteobacteria and Betaproteobacteria
40. Hetz SA, Horn MA (2021) *Burkholderiaceae* are key acetate assimilators during complete denitrification in acidic cryoturbated peat circles of the arctic tundra. Front. Microbiol. **12**:1–18. https://doi.org/10.3389/fmicb.2021.628269
41. Du C, Li C, Cao P et al (2021) *Massilia cellulosiltytica* sp. nov., a novel cellulose-degrading bacterium isolated from rhizosphere soil of rice (Oryza sativa L.) and its whole genome analysis.

Antonie Van Leeuwenhoek **114**:1529–1540. https://doi.org/10.1007/s10482-021-01618-3
42. Dogan B, Boor KJ (2003) Genetic diversity and spoilage potentials among *Pseudomonas spp.* isolated from fluid milk products and dairy processing plants. Appl. Environ. Microbiol. **69**:130–138. https://doi.org/10.1128/AEM.69.1.130-138.2003
43. Pei N, Lian J, Erickson DL et al (2011) Exploring tree-habitat associations in a Chinese subtropical forest plot using a molecular phylogeny generated from DNA barcode Loci. PLoS One **6**:e21273. https://doi.org/10.1371/journal.pone.0021273
44. Thompson LR, Sanders JG, McDonald D et al (2017) A communal catalogue reveals Earth's multiscale microbial diversity. Nature **551**:457–463. https://doi.org/10.1038/nature24621
45. Jurburg SD, Nunes I, Stegen JC et al (2017) Autogenic succession and deterministic recovery following disturbance in soil bacterial communities. Sci. Rep. **7**:1–11. https://doi.org/10.1038/srep45691
46. Ruiz-Gil T, Acuña JJ, Fujiyoshi S et al (2020) Airborne bacterial communities of outdoor environments and their associated influencing factors. Environ. Int. **145**. https://doi.org/10.1016/j.envint.2020.106156
47. Cho BC, Hwang CY (2011) Prokaryotic abundance and 16S rRNA gene sequences detected in marine aerosols on the East Sea (Korea). FEMS Microbiol. Ecol. **76**:327–341. https://doi.org/10.1111/j.1574-6941.2011.01053.x
48. Zhou J (2017) Stochastic community assembly : does it matter in microbial ecology? Microbiol. Mol. Biol. Rev. **81**:1–32
49. Li W, Cheng JM, Yu KL et al (2015) Niche and neutral processes together determine diversity loss in response to fertilization in an alpine meadow community. PLoS One **10**:1–15. https://doi.org/10.1371/journal.pone.0134560
50. Lee KC, Caruso T, Archer SDJ et al (2018) Stochastic and deterministic effects of a moisture gradient on soil microbial communities in the McMurdo dry valleys of Antarctica. Front. Microbiol. **9**:1–12. https://doi.org/10.3389/fmicb.2018.02619
51. Zhao P, Bao J, Wang X et al (2019) Deterministic processes dominate soil microbial community assembly in subalpine coniferous forests on the Loess Plateau. PeerJ **7**:1–19. https://doi.org/10.7717/peerj.6746
52. Docherty KM, Gutknecht JLM (2019) Soil microbial restoration strategies for promoting climate-ready prairie ecosystems. Ecol. Appl. **29**. https://doi.org/10.1002/eap.1858
53. De Corato U (2020) Soil microbiota manipulation and its role in suppressing soil-borne plant pathogens in organic farming systems under the light of microbiome-assisted strategies. Chem. Biol. Technol. Agric. **7**:1–26. https://doi.org/10.1186/s40538-020-00183-7
54. Bailey D, Koney K (2014) Selection, succession, and stabilization of soil microbial consortia. Strateg Alliances among Heal Hum Serv Organ From Affil to Consol **4**:105–118. https://doi.org/10.4135/9781483328546.n7
55. Schöler A, Jacquiod S, Vestergaard G et al (2017) Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biol. Fertil. Soils **53**:485–489. https://doi.org/10.1007/s00374-017-1205-1

Supplementary Material

Deterministic development of soil microbial communities in disturbed soils depends on microbial biomass of the bioinoculum

revised version July 24th, 2022

Yuri Pinheiro [1, 2]; Michael Schloter [1, 2]; Wolfgang Weisser [3]; Stefanie Schulz [1*]

[1]Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany

[2]Technische Universität München, TUM School of Life Science, Chair of Environmental Microbiology, Freising, Germany

[3] Technische Universität München, TUM School of Life Science, Chair of Terrestrial Ecology, Freising, Germany

*corresponding author: stefanie.schulz@helmholtz-muenchen.de

ORCID IDs:

Yuri Pinheiro Alves de Souza: 0000-0001-9854-6383

Stefanie Schulz: 0000-0001-5520-8106

Wolfgang Weisser: 0000-0002-2757-8959

Michael Schloter: 0000-0003-1671-1125

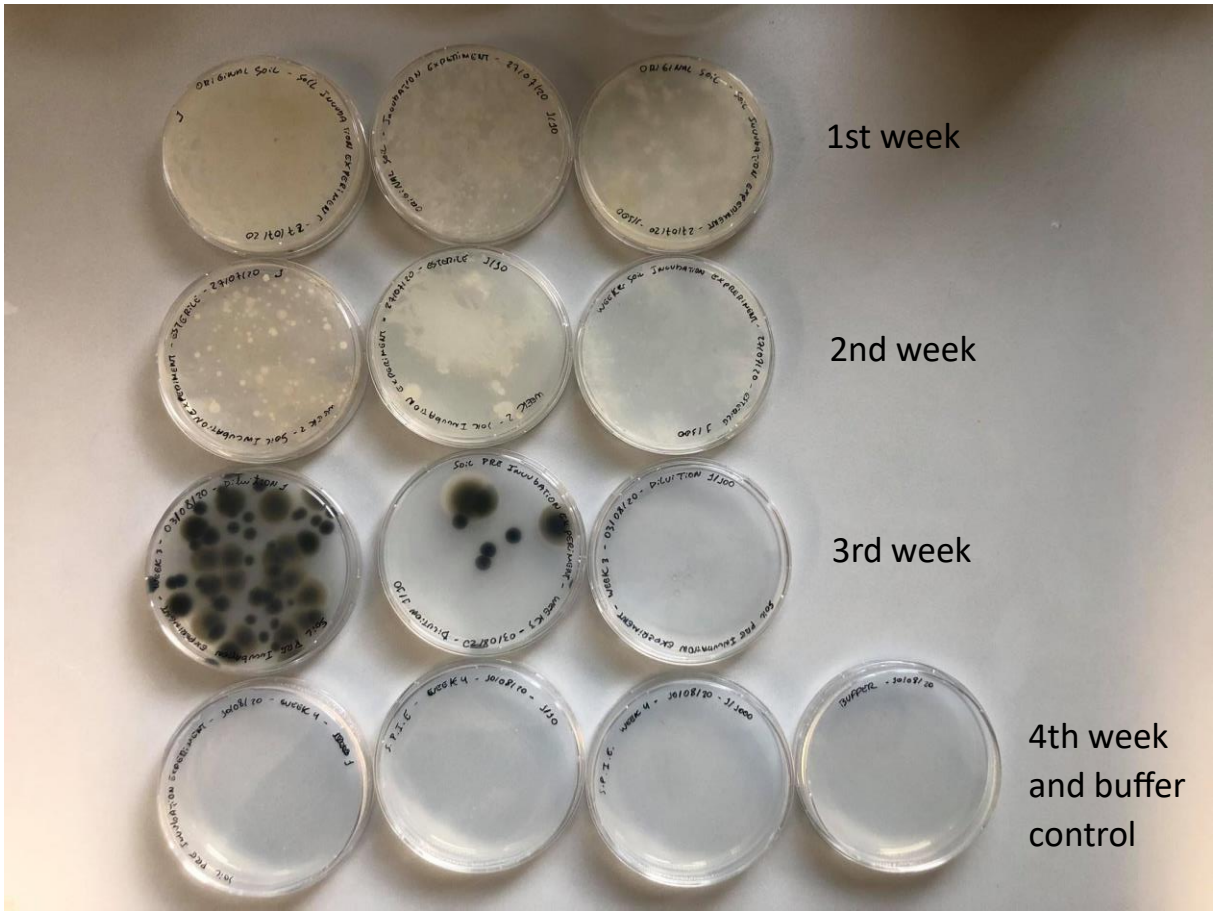


Fig. S1: Microbial growth during the soil autoclavation over the weeks. 1g of autoclaved soil was resuspended in 0.8% NaCl2 solution. 100 µl of resuspended soil was plated in R2A agar plates also in dilutions 1:10 and 1:100. In the picture we show the last dilution to have growth in each of the time points. At week 4, no microbial growth could be observed.

*Table S1: Table displaying the ANOVA and Post Hoc test (Tukey test) results for qPCR copy number for both bacterial and archaeal 16S rRNA gene. Fitting order for ANOVA was CopyNumber ~ Dilution + Time, using aov function in base R. Significant effects are labelled p < 0.05, p < 0.005 or p < 0.0005 are labelled with *, ** or ***.*

Anova											
Bacteria						**Archaea**					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Dilution	4	5,58E+21	1,39E+21	3.918	0.007**	Dilution	4	8,95E+15	2,24E+15	40.927	1.79e-15***
Time	3	5,60E+21	1,87E+21	5.247	0.003**	Time	3	2,74E+14	9,15E+13	1.673	0.184
Residuals	52	1,85E+22	3,56E+20			Residuals	52	2,84E+15	5,47E+13		
Tukey Post Hoc Test											
Bacteria						**Archaea**					
Dilution						**Dilution**					
	diff	lwr	upr	p adj			diff	lwr	upr	p adj	
1:100-1:10	-3,9E+08	-1,1E+09	3,03E+08	0.516		1:100-1:10	-227324.54	-497097.5	42448.42	0.137	
1:50-1:10	-1,3E+08	-8,2E+08	5,57E+08	0.983		1:50-1:10	-162008.58	-431781.5	107764.39	0.444	
No inoculum-1:10	-7,9E+08	-1,5E+09	-1,1E+08	0.016*		No inoculum-1:10	-330206.29	-599979.3	-60433.33	0.009*	
Original-1:10	-6,8E+08	-1,4E+09	10503657	0.056		Original-1:10	747822.45	478049.5	1017595.42	0.000***	
1:50-1:100	2,53E+08	-4,3E+08	9,42E+08	0.835		1:50-1:100	65315.96	-204457.0	335088.93	0.959	
No inoculum-1:100	-4,1E+08	-1,1E+09	2,79E+08	0.455		No inoculum-1:100	-102881.75	-372654.7	166891.21	0.817	
Original-1:100	-2,9E+08	-9,8E+08	3,96E+08	0.750		Original-1:100	975147.00	705374.0	1244919.96	0.000***	
No inoculum-1:50	-6,6E+08	-1,4E+09	25599410	0.064		No inoculum-1:50	-168197.71	-437970.7	101575.25	0.406	
Original-1:50	-5,5E+08	-1,2E+09	1,42E+08	0.181		Original-1:50	909831.03	640058.1	1179604.00	0.000***	
Original-No inoculum	1,17E+08	-5,7E+08	8,05E+08	0.989		Original-No inoculum	1078028.75	808255.8	1347801.71	0.000***	
Time						**Time**					
	diff	lwr	upr	p adj			diff	lwr	upr	p adj	
T1-T0	7,24E+08	1,46E+08	1,3E+09	0.009*		T1-T0	93064.98	-133566.58	319696.54	0.697	
T2-T0	1,94E+08	-3,8E+08	7,73E+08	0.809		T2-T0	-57261.20	-283892.76	169370.36	0.908	
T4-T0	6,58E+08	79487303	1,24E+09	0.019*		T4-T0	107013.97	-119617.59	333645.54	0.596	
T2-T1	-5,3E+08	-1,1E+09	48517645	0.084		T2-T1	-150326.18	-376957.74	76305.38	0.304	
T4-T1	-6,6E+07	-6,4E+08	5,12E+08	0.990		T4-T1	13948.99	-212682.57	240580.55	0.998	
T4-T2	4,63E+08	-1,1E+08	1,04E+09	0.158		T4-T2	164275.17	-62356.39	390906.73	0.231	

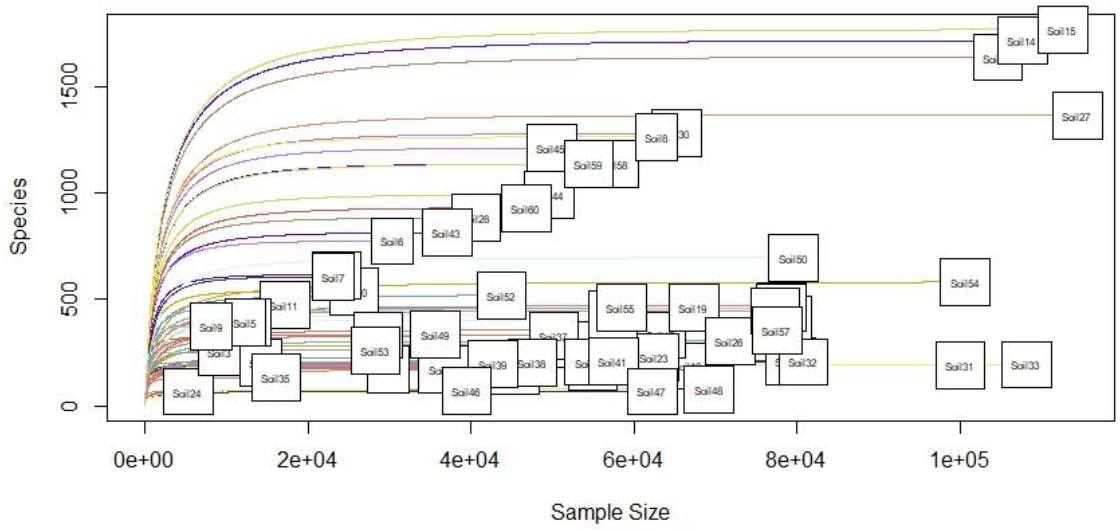


Fig. S2: Rarefaction curve built over the ASV table using rarecurve command from Vegan (version 2.6.2) package on R (step=50, cex=0.5).

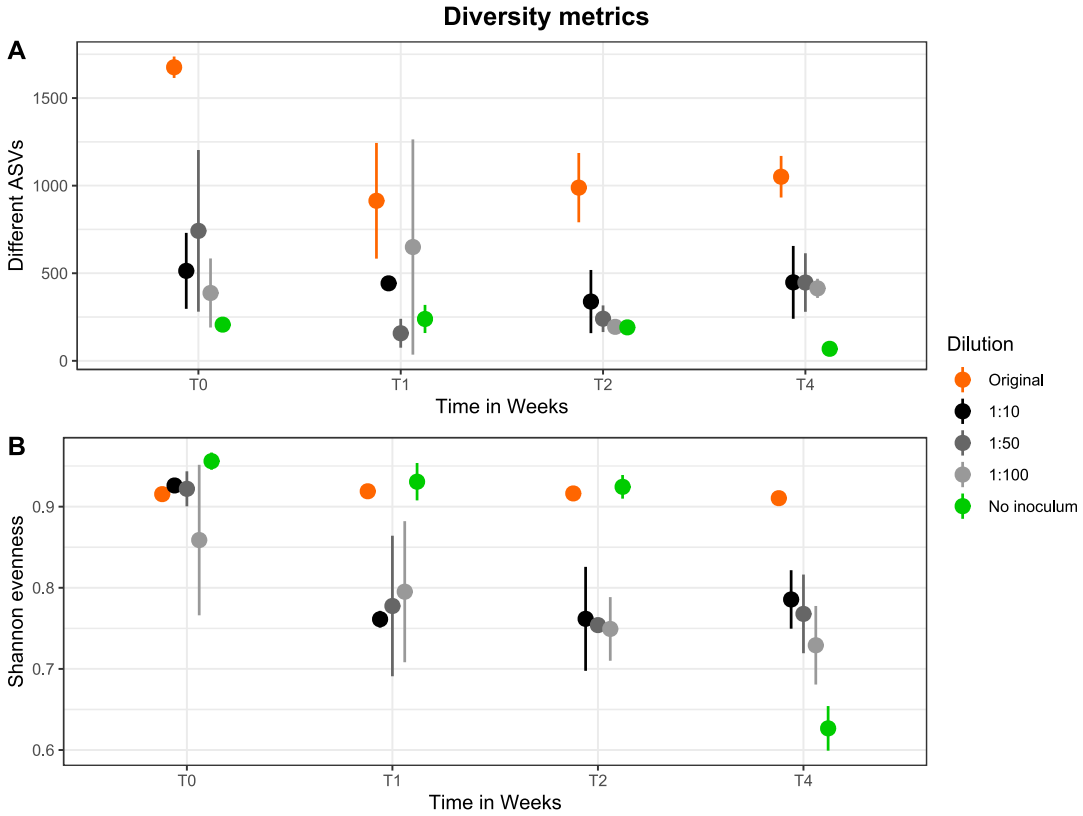


Fig. S3: Number of observed ASVS (A) and Shannon evenness (B) measurements calculated over the ASVs table from metabarcoding sequencing for prokaryotes.

*Table S2: Table displaying the ANOVA and Post Hoc test (Tukey test) results for alpha diversity (calculated based on the number of observed ASVs). Fitting order for PERMANOVA was CopyNumber ~ Dilution + Time, using aov function in base R. Significant effects are labelled p < 0.05, p < 0.005 or p < 0.0005 are labelled with *, ** or ***.*

Anova					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Dilution	4	31.98	7.996	17.848	2.81e-09 ***
Time	3	12.33	4.109	9.171	5.68e-05 ***
Residuals	52	23.30	0.448		
Tukey Post Hoc Test					
Dilution					
	diff	lwr	upr	p adj	
1:100-1:10	-2.441.667	-3.133.736	26.454.031	0.999	
1:50-1:10	-3.891.667	-3.278.736	25.004.031	0.995	
Original-1:10	72.175.000	4.327.930	101.070.697	0.000***	
Sterile-1:10	-25.925.000	-5.482.070	2.970.697	0.098	
1:50-1:100	-1.450.000	-3.034.570	27.445.697	0.999	
Original-1:100	74.616.667	4.572.097	103.512.364	0.000***	
Sterile-1:100	-23.483.333	-5.237.903	5.412.364	0.162	
Original-1:50	76.066.667	4.717.097	104.962.364	0.000***	
Sterile-1:50	-22.033.333	-5.092.903	6.862.364	0.213	
Sterile-Original	-98.100.000	-12.699.570	-69.204.303	0.000***	
Time					
diff	lwr	upr	p	p adj	
T1-T0	-224.733.333	-4.674.810	1.801.438	0.079	
T2-T0	-314.666.667	-5.574.144	-7.191.895	0.006*	
T4-T0	-219.466.667	-4.622.144	2.328.105	0.089	
T2-T1	-89.933.333	-3.326.810	15.281.438	0.759	
T4-T1	5.266.667	-2.374.810	24.801.438	0.999	
T4-T2	95.200.000	-1.475.477	33.794.771	0.726	

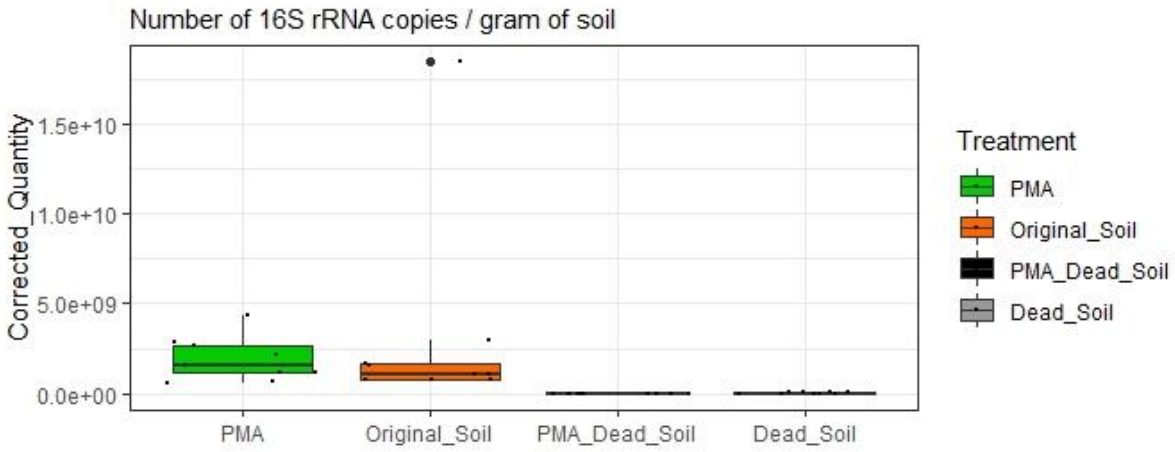


Fig. S4: PMA PCR performed with Original soil used on the inoculation. Boxes show both minimum and maximum values in both edges, 25th Percentile, Median and 75th percentile. PMA treatment had average number 16S rRNA copies of 1.905.079.047 (median value of 1.553.144.704 copies), while Original, untreated soil had 3.224.934.322 (median value of 1.088.152.000 copies). The "dead" soil control was meant to access the limitation of PMA to fully remove DNA from samples and how it impacts on the qPCR amplification. While the "dead" soil without PMA treatment had 21.401.234 reads in average, PMA treated "dead" soil had 7.254.347, indicating that 37% of the DNA remains in the soil. Considering an efficiency of 63%, and that the PMA treatment removed 38% of the DNA on the Original soil, we can estimate that 23,9% of the DNA present in the Original soil derivates from dead or damaged cells

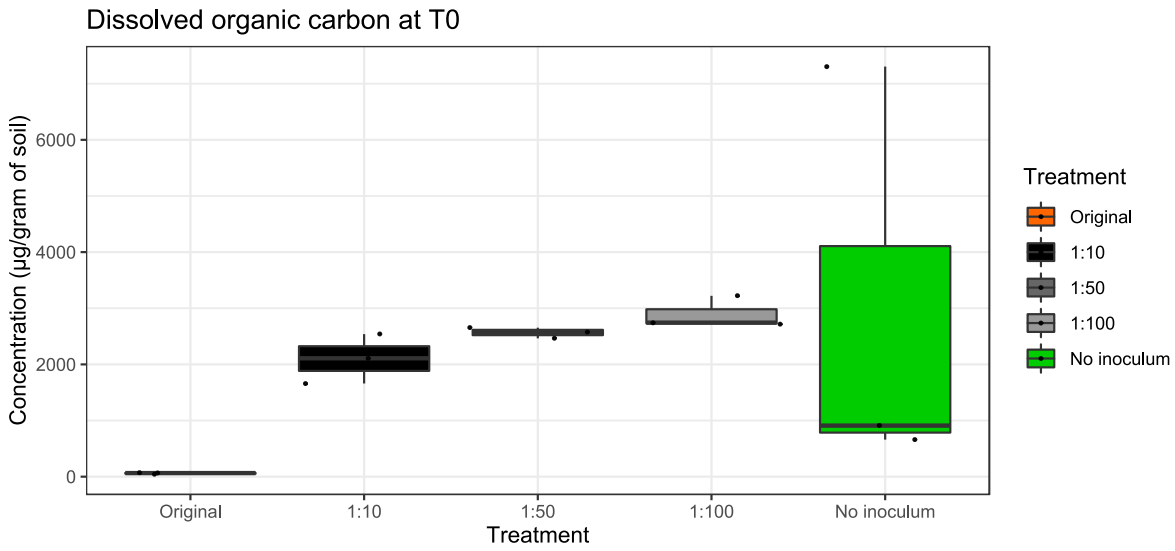


Fig. S5: Dissolved organic carbon at time point week 0.

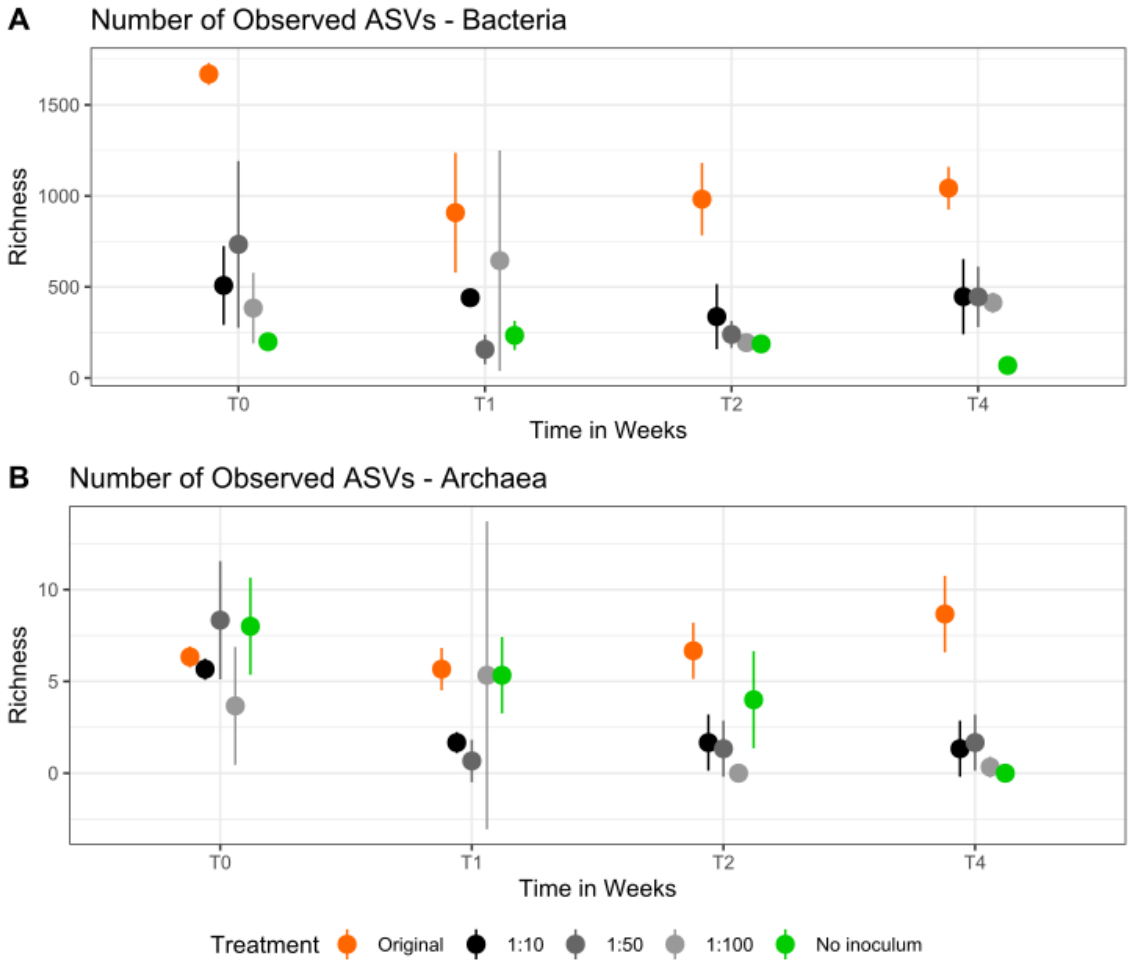


Fig. S6: Diversity (Number of Observed ASVs) of Bacterial and Archeal reads along the experiment´s duration. The primeir pair used in this study (The Earth Microbiom project´s Eukariotic primer) target both Archaea and Bacteria, so the bacterial and archaeal assinged reads were filtered out of the total dataset using phyloseq. From the 7820 ASVs in the dataset (distributed among 3.224.377 reads), 56 ASVs (38.998 reads) were assigned to Archaea, totalizing 1.2% of the annotated reads. We used the number of observed ASVs as alpha diversity metric. Overall diversity follows the patters of the combined community, with significant effects of Dilution and Time (see supplementary table S3 for statistical significances).

*Table S3: Table displaying the ANOVA and Post Hoc test (Tukey test) results for alpha diversity (calculated based on the number of observed ASVs) for bacterial and archaeal community individually. Fitting order for PERMANOVA was CopyNumber ~ Dilution + Time, using aov function in base R. Significant effects are labelled p < 0.05, p < 0.005 or p < 0.0005 are labelled with *, ** or ***.*

Anova											
	Bacteria						Archaea				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)		Df	Sum Sq	Mean Sq	F value	Pr(>F)
Dilution	4	6650023	1662506	26.835	4.2e-12***	Dilution	4	165.1	41.27	4.877	0.00205**
Week	3	785774	261925	4.228	0.0095**	Week	3	147.9	49.31	5.827	0.00165**
Residuals	52	3221549	61953			Residuals	52	440.0	8.46		
Tukey Post Hoc Test											
	Bacteria						Archaea				
Dilution	diff	lwr	upr	p adj		Dilution	diff	lwr	upr	p adj	
1:100-1:10	-2.416.667	-3.113.077	26.297.432	0.9992683		1:100-1:10	-0.2500000	-36.057.466	3.105.747	0.9995481	
1:50-1:10	-3.933.333	-3.264.743	24.780.766	0.9950984		1:50-1:10	0.4166667	-29.390.799	3.772.413	0.9966470	
No inoculum-1:10	-26.100.000	-5.481.410	2.614.099	0.0913721		No inoculum-1:10	17.500.000	-16.057.466	5.105.747	0.5838653	
Original-1:10	71.750.000	4.303.590	100.464.099	0.0000000		Original-1:10	42.500.000	0.8942534	7.605.747	0.0065186	
1:50-1:100	-1.516.667	-3.023.077	27.197.432	0.9998845		1:50-1:100	0.6666667	-26.890.799	4.022.413	0.9800060	
No inoculum-1:100	-23.683.333	-5.239.743	5.030.766	0.1516780		No inoculum-1:100	20.000.000	-13.557.466	5.355.747	0.4524360	
Original-1:100	74.166.667	4.545.257	102.880.766	0.0000000		Original-1:100	45.000.000	11.442.534	7.855.747	0.0034737	
No inoculum-1:50	-22.166.667	-5.088.077	6.547.432	0.2029099		No inoculum-1:50	13.333.333	-20.224.133	4.689.080	0.7938224	
Original-1:50	75.683.333	4.696.923	104.397.432	0.0000000		Original-1:50	38.333.333	0.4775867	7.189.080	0.0176278	
Original-No inoculum	97.850.000	6.913.590	126.564.099	0.0000000		Original-No inoculum	25.000.000	-0.8557466	5.855.747	0.2334345	
Time						Time	diff	lwr	upr	p adj	
T1-T0	-22.206.667	-4.632.888	1.915.547	0.0814537		T1-T0	-26.666.667	-5.485.771	0.1524375	0.0699752	
T2-T0	-31.100.000	-5.522.221	-6.977.786	0.0064944		T2-T0	-36.666.667	-6.485.771	-0.8475625	0.0059496	
T4-T0	-21.546.667	-4.566.888	2.575.547	0.0955736		T4-T0	-40.000.000	-6.819.104	-11.808.958	0.0023291	
T2-T1	-8.893.333	-3.301.555	15.228.880	0.7622247		T2-T1	-10.000.000	-3.819.104	18.191.042	0.7827191	
T4-T1	660.000	-2.346.221	24.782.214	0.9998608		T4-T1	-13.333.333	-4.152.438	14.857.708	0.5950701	
T4-T2	9.553.333	-1.456.888	33.675.547	0.7203382		T4-T2	-0.3333333	-3.152.438	24.857.708	0.9891691	

7.3. Publication II (Manuscript)

The effect of plant species richness on bacterial diversity maintenance during extended drought periods

Yuri Pinheiro [1,2]; Roberto Siani [1,2]; Cynthia Albracht [3,4], Yuanyuan Huang [5,6], Nico Eisenhauer [5,6], Anja Vogel [5,6], Cameron Wagg [7,8]; Michael Schloter [1,2]; Stefanie Schulz [1]

[1] Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany

[2] Technische Universität München, TUM School of Life Science, Chair of Environmental Microbiology, Neuherberg, Germany

[3]Swammerdam Institute of Life Sciences at University of Amsterdam, Amsterdam, the Netherlands

[4]Department Soil Ecology, Helmholtz Centre for Environmental Research – UFZ, Halle (Saale), Germany

[5] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[6] Institute of Biology, Leipzig University, Leipzig, Germany

[7] Department of Geography, Remote Sensing Laboratories, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland

[8] Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 95 Innovation Road, Post Office Box 20280, Fredericton, E3B 4Z7, NB, Canada

Abstract

Drought is a major stressor to soil microbial communities, and the intensification of climate change is predicted to exacerbate hydric stress worldwide in the coming decades. As a potential mitigating factor for the consequences of prolonged droughts, biodiversity can increase ecosystem resistance and resilience by enhancing metabolic redundancy and complementarity. Here, we investigated the interaction between plant diversity and summer drought on soil microbial communities, specifically bacterial communities. For that, we made use of a well-established biodiversity experiment (The Jena Experiment) to investigate the response in soil bacterial community diversity and compositionexposed to recurring summer drought over nine yearsalongside a plant diversity gradient ranging from one to 60 species. Our data indicate that bacterial diversity increased after exposure to drought. These data

indicate that long-term drought promotes soil biodiversity by increasing niche differentiation and that these effects can be potentiated by increasing plant diversity.

Introduction

As a result of the intensification of human activities over the past two centuries, Earth´s biosphere is facing unprecedented alterations. Increasing industrialization leads to alarming air pollution, which is predicted to intensify the worldwide rain pattern (Fowler and Hennessy 1995; Nemecek et al. 2012; Sohoulande Djebou and Singh 2016). As a consequence of the disturbances on the rain regime, many regions are now experiencing long periods of drought, suffering from insufficient precipitation or impairment of the water distribution of rivers, lakes, and other water bodies (Eriyagama, Smakhtin, and Gamage 2009). This hydric stress directly affects agricultural production, threatening the food supply chain (Osborne, Rose, and Wheeler 2013; Ostad-Ali-Askari et al. 2020; Zhipeng Wang et al. 2020), as well as the equilibrium of natural environments (Geng et al. 2015; Huntington 2006).

The consequences of hydric stress can be observed not only macroscopically but also on a microscopic scale. Bacterial communities respond very quickly to environmental stress caused by both physicochemical and biological factors, such as increasing temperature, lack of water, or a secondary response to a biotic factor responding to a physicochemical factor (Jansson and Hofmockel 2020). Bacterial communities play a crucial role in the maintenance of soil functioning, and changes in these soil characteristics can affect the soil bacterial community composition and the services provided by this community (Bartelt-Ryser et al. 2005). Currently, literature on the effects of drought on bacterial communities indicates that drought events are followed by an increase in gram-positive bacteria (such as *Actinobacteria*), which are capable of utilizing recalcitrant carbon sources and are highly present in arid, nutrient-poor soils (Connon et al., 2007) and are also capable to generate stress-resistant structures, like spores (Zeigler 2014). Gram-negative bacteria, on the other hand, prefer labile carbon compounds and organic nitrogen (Treseder et al., 2011), particularly in the form of plant root exudates, widely abundant in eutrophic, nutrient-rich soils (Balasooriya et al., 2014). Drought also seems to have a legacy effect, even after rewetting, on the soil bacterial community composition, reducing population richness in the long term (Meinsner et al, 2018). During water stress, bacterial cells increase intracellular solute production to achieve osmotic equilibrium with the environment, increasing energy demands for the cell. The same happens during rewetting, when cells release excess solute to, again, achieve balance (Csonka 1989). The lack of water also reduces bacterial motility and nutrient uptake (J. Schimel, Balser, and Wallenstein 2007), since the environment becomes less homogenous as water

concentrations diminish. In this sense, the intensification in both length and frequency of drought events can select drought-resistant microbes, changing soil microbial population fitness and composition (Kaisermann et al. 2017; J. P. Schimel 2018).

Taking into consideration ongoing climate change and its predicted impact on the global precipitation regime (Huntington 2006), investigating buffering factors of drought is extremely relevant (Huang et al. 2023). One of those factors is the plant community composition, which reportedly can influence and modulate the soil microbial community by recruiting and sustaining important microbial taxa (Abedini et al. 2021; Hartman and Tringe 2019). Previous work (M. W. Schmid et al. 2021; G. Wang et al. 2019) has already shown the beneficial effects of increasing plant diversity on ecosystem functions (Isbell et al. 2015) and bacterial community composition (Eisenhauer et al. 2017; Lange et al. 2015). Higher biodiversity promotes nutrient turnover, biomass production, and overall ecosystem resilience against stress and disturbances (Isbell et al. 2017; Roscher et al. 2004). Biodiverse environments also see an increase in carbon and nitrogen stocks in soil, which ultimately contributes to higher productivity and ecosystem quality (Weisser et al. 2017; G. Yang et al. 2021). In this regard, both plant and bacterial diversity are key to the maintenance of essential ecosystem functions, providing metabolic complementarity and stabilizing the overall community against stress (Vogel, Scherer-Lorenzen, and Weigelt 2012). Increasing plant diversity can increase bacterial activity (Bartelt-Ryser et al. 2005; Lange et al. 2015) and possibly work as a mitigator of long-term drought effects on the soil bacterial community. In regarding to drought, plant community diversity has already been shown to increase complementarity between plant species, with further adaptation to plant offspring after long periods of drought (Yuxin Chen et al. 2022). A similar study, the other hand, indicates that increasing plant diversity did not show any significant buffering effects on the soil fungal community, which significantly responded to long-term drought (Albracht et al. 2023). Although the effect of drought and shifts in precipitation regimes on plant communities has already been investigated (Zeppel, Wilks, and Lewis 2014), the complex interaction between plant diversity, prokaryotic communities, and drought is poorly understood. Investigating the potential buffering effects of plant communities on soil prokaryotic diversity can be crucial to maintain critical ecosystem functions as affected by climate change.

To investigate the potential of plant diversity in alleviating the impacts of repeated summer drought on prokaryotic diversity and community composition, we made use of an experimental gradient in plant species richness in a long-term summer drought simulation in the Jena Experiment (B. Schmid et al. 2004; Weisser et al. 2017), where plots were sheltered from environmental precipitation for over 9 years (Vogel, Eisenhauer, et al. 2013). Control plots were also sheltered to account for potential side effects of the roof infrastructure but received ambient levels of precipitation.

Soil samples were taken from 80 plots (160 subplots) with varying plant species richness, and metabarcoding sequencing was used to assess changes in bacterial and archaeal diversity and composition across the plant species diversity gradient. Our main hypotheses were that 1) summer drought will lead to significantly different bacterial communities in comparison to ambient precipitation (control) subplots and that 2) increasing plant diversity will buffer potential drought-induced soil microbial diversity loss.

Methods

Experimental design and sampling

The experiment was established as a sub-experiment on 80 plots of the Jena Experiment (B. Schmid et al. 2004; Weisser et al. 2017), which has been running since 2002. The experiment was established in the city of Jena, Germany (50°55'43.61"N, 11°35'23.64"E), on the floodplain of the Saale River (altitude 130 m a.s.l.). The mean annual air temperature is 9.9 °C (1980–2010), and the mean annual precipitation is 610 mm (Hoffmann et al. 2014). The soil is classified as an Eutric Fluvisol (World Reference Basef or Soil Resources 2015 (Weisser et al. 2017)) with a pH between 7.1 and 8.4 and soil organic carbon 5–33 g C kg^{-1} (Roscher et al. 2004). The field site contains 80 large plots of 20 x 20 m containing different plant community compositions varying in both plant species richness (1, 2, 4, 8, 16 and 60 plant species) and number of plant functional groups (1-4 groups: grasses, small herbs, tall herbs, and legumes). The plots are distributed in four blocks to account for spatial variations in edaphic properties (including soil texture and water-holding capacity), which are related to the distance of the plots to the adjacent river Saale. Each block contained a similar number of plots at each plant species richness level (except 60-species plots), covering the range of functional groups (Roscher et al. 2004).

The drought experiment was established in 2008 as a sub-experiment nested inside the existing plots of the Main Experiment (Vogel, Eisenhauer, et al. 2013). Prior to the second annual mowing in September, transparent rain shelters (wood and PVC sheets, 2.6 x 3 m) were installed for 6 weeks every year to induce a prolonged summer drought period over a span of 9 years (2008-2016) (Vogel, Eisenhauer, et al. 2013). Of the two sheltered subplots within each plot, one received no water after installation (the 'drought' treatment), and one received collected rain water as equivalent precipitation after rain events ('control'), thereby controlling for non-drought roofing effects such as altered light and temperature (Vogel, Fester, et al. 2013). The roof shelters reduced summer precipitation by an average of 42% in 2008-2014 (Wagg et al. 2017). In July 2013, the field experiment was completely flooded during a natural flood event of the Saale River, disrupting the drought treatment during the

flood period. The experiment and the treatments were continued after the flood recovered (Cesarz et al. 2017).

To assess the changes in the soil microbial community after successive periods of drought, soil was sampled in each of the 160 subplots of the drought experiment in August 2017, one year after the last drought period. The subplots of the drought experiment were not covered by roof construction during 2017. Approximately 50 g of bulk soil was taken by pooling 3-5 soil cores of 0-15 cm depth per subplot. The soil was sieved at 2 mm for homogenization and to remove bigger plant materials. Soil samples were stored at -80°C until processing for DNA extraction and next-generation sequencing.

Soil moisture and pH measurements

Soil moisture was measured by gravimetry. One gram of fresh soil was weighed (each sample was measured in duplicate) and left in the oven overnight at 104°C. After cooling, the soil was weighted several times until a stable weight was reached. The soil moisture is given in g of water/g of soil. pH was measured by adding 25 ml of 0.01 M calcium chloride to 10 g of air-dried soil, which was vigorously shaken. Samples were incubated at room temperature for 1 h, and then the pH was measured with a calibrated pH meter (Albracht et al. 2023).

DNA extraction and sequencing

DNA extraction was performed using the DNeasy Powersoil Kit (Qiagen- Germany). The resulting DNA was quantified by a Qubit fluorometric system (Thermo – Germany), and the quality was checked using a Nanodrop photometric system (Thermo - Germany) and by gel electrophoresis. As a control for DNA extraction, we included a blank extraction (DNA extraction without sample).

We performed Illumina next-generation amplicon sequencing targeting the 16S rRNA gene using the primer pair 515F (Parada, Needham, and Fuhrman 2016) and 806R (Apprill et al. 2015). Each reaction had 25 µL containing 12.5 µL NEB Next High-Fidelity Master Mix (Thermo - Germany), 0.5 µL of each primer at 10 pmol/µl, 2.5 µL of 3% BSA, 1 µl of 5 ng/µL diluted DNA, (for the negative control, 1 µl of DEPC-treated water instead) and 8 µL of DEPC-treated water. The amplification program was as follows: 98°C for 1 min, followed by 23 cycles of 98°C for 10 s, 55°C for 30 s and 72°C for 30 s, and a final extension at 72°C for 5 min. Samples were indexed using a Nextera® XT Index Kit v2 (Illumina - USA) and purified with MagSi-NGSprep Plus Beads (ratio 0.8 beads:1 sample) according to the

manufacturer's protocol, and quality assessment was performed via a Fragment Analyzer (Agilent - Germany). High-quality DNA was diluted to 4 nM and sequenced on an Illumina MiSeq using a MiSeq Reagent v3 (600 Cycle) kit. PhiX (5 pM, 20%) was loaded alongside the samples.

Bioinformatics

After sequencing, samples were uploaded to the European Galaxy server (https://usegalaxy.eu). The Cutadpat tool was used to remove adapters, and read quality was accessed via FastQC and with the dada2 version 1.16 (Callahan, , Paul J McMurdie, Michael J Rosen, Andrew W Han, and Johnson 2016) plotQualityProfile option. Trimming parameters were set to 220 bp for forward reads and 200 bp for reverse reads, and dada2 was used to trim the sequences without adapters. We also used dada2 to apply error rates, merge the read pairs and make a sequence table according to the default dada2 pipeline (https://benjjneb.github.io/dada2/tutorial.html). Taxonomy was assigned using assingTaxonomy and addSpecies function, aligning the Amplicon Sequencing Variants (ASVs) against the Silva database (Quast et al. 2013) version 138 The table with ASV counts and taxonomic assignments was downloaded, and we conducted all statistical analyses using the R environment (v4.2.2), by using the packages phyloseq v.1.4 (McMurdie and Holmes 2013), microbiome (v1.18) (Leo n.d.) and tidyverse (v1.3.1) (Wickham et al. 2019). We first removed nonbacterial ASVs along with any ASV assigned to chloroplasts and mitochondria. We removed exogenous ASVs present in the negative controls using the prevalence-based method from package decontam (v1.16) (Davis et al. 2018) and addressed batch effects from multiple sequencing runs using the "ComBat_seq" function from package sva (v3.44) (Leek et al. 2012). Finally, we preserved nonsingleton ASVs observed in at least 5 percent of the samples.

Statistics and data visualization

We estimated microbial diversity (Shannon, Inverse Simpson indexes) and richness in each sample by using the packages DivNet (v0.4) (Willis and Martin 2022) and breakaway (v4.7.9). We calculated the effects of the covariates on standardized estimates of alpha diversity by running a reduction analysis (RDA) using MicroViz package (v 0.10.10) (Barnett, Arts, and Penders 2021) and using the Bray-Curtis dissimilarity index (Bray and Curtis 1957). We visualized the distance across the samples by plotting the first and second components of a singular value decomposition of the count matrix, and we included the values of soil water content, pH, and number of functional groups as covariates. We analyzed the differential abundance of ASVs across control and drought samples for all

plant diversity levels using ANCOM-BC (v1.6) (Lin and Peddada 2020). The code and data used in these analyses are deposited in the GitHub repository https://github.com/rsiani/yuri_et_al_22.

For the statistical analyses, plant species richness and number of functional groups were log-transformed to improve distribution and reach linearity. We fit the transformed data to a linear mixed-effects model using the lme function in the package *nlme* to investigate the effects of block, plant diversity (plant species richness/number of functional groups), drought treatment, as well as the interaction of plant diversity and drought treatmenton the measured variables (Shannon, Inverse Simpson and Observed Richness metrics for alpha diversity). Plot was fitted as a random variable. The drought treatment was randomized at subplot level, while plant diversity was randomized at plot level. . Differences in microbial beta diversity were estimated via PERMANOVA of using index adonis2 function from package Vegan v2.6.2 over the dissimilarity matrix with Bray-Curtis distance (Oksanen et al. 2022).

Results

Drought and plant diversity effects on soil bacterial diversity

Linear mixed effects model results revealed a significant positive effect of the drought treatment and the sown plant diversity on the inverse Simpson diversity index ($p < 0.001$), while drought treatment and the interaction between drought and sown diversity were significant for the Shannon index ($p < 0.001$ – Table 1 - A). The number of observed ASVs (observed richness) was not significantly affected by the drought treatment. The difference between drought and control for Shannon and inverse Simpson can be observed in Figure 1A and 1B, where drought-treated plots showed higher diversity (blue dots) when compared to the control treatment (gray dots), while no clear separation could be observed in the number of observed ASVs (Figure 1C). We did not observe any significant relationship between plant richness and the Shannon and observed ASVs, i.e. bacterial richness indexes (Table 1A, Figure 1A and 1C). However, plant richness presented a significant negative effect on inverse Simpson diversity ($p < 0.001$, Table 1, Figure 1B).

To investigate the effects of the plant richness on the drought effects, we performed a post hoc test, contrasting the means of control plots against drought plots using Shannon and Inverse Simpson diversity indexes as response variables. First, we can observe that the difference between control and drought treated plots is significant at all plant richness levels ($p < 0.001$, Table 1B), in alignment with the trend observed in the correlation plots in Figure 1 and highlights the consistent effect of the

drought treatment on the soil bacterial community. Secondly, the negative values of estimated marginal means while contrasting control against drought samples presents a consistent negative result, showing that average Shannon and Inverse Simpson diversity values are consistently higher in drought plots (Table 1B, Figure 1).

*Table 1. A) ANOVA table displaying the numerator and denominator degrees of freedom, alongside the F and p values for the linear mixed effects models (lme). We used Shannon, inverse Simpson, and richness (number of observed ASVs)of soil bacterial communities as response variables. The fitting order was AlphaDiversity~block+log(PlantRichness)*treatment, random=~1|plot. B) Estimated marginal means (EMMs) generated using the emmeans package in R. The statistical model employed was as previously described. Post hoc comparisons were conducted using Tukey's method, using pairwise comparison between control and drought across the plant diversity levels. We used the Shannon and Simpson as microbial diversity metrics, since they presented significant values in the linear models.*

A

Linear mixed effect models

	Shannon				**Inverse Simpson**				**Richness**			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
(Intercept)	1	76	10397176	**<.0001**	1	76	128388.44	**<.0001**	1	76	30.007.124	<.0001
Block	3	75	82	**<.0001**	3	75	20.28	**<.0001**	3	75	0.7395	0.5680
Plant Richness	1	75	1	0.2631	1	75	23.07	**<.0001**	1	75	0.4644	0.4977
Drought Treatment	1	76	28790	**<.0001**	1	76	28349.18	**<.0001**	1	76	0.1488	0.7008
Plant Richness and Drought	1	76	35	**<.0001**	1	76	0.02	0.8895	1	76	29.417	0.0904

B

Post hoc analysis

	Shannon						**Inverse Simpson**					
Contrast	Plant species richness	estimate	SE	df	t.ratio	p.value	Plant species richness	estimate	SE	df	t.ratio	p-value
control - drought	1	-0.00936	1.17e-04	76	99.994	**<.0001**	1	-3.02	0.0257	76	-117.604	**<.0001**
control - drought	2	-0.00920	7.25e-05	76	136.111	**<.0001**	2	-2.97	0.0188	76	-157.432	**<.0001**
control - drought	4	-0.00904	4.30e-05	76	167.521	**<.0001**	4	-2.91	0.0153	76	-190.494	**<.0001**
control - drought	8	-0.00888	5.94e-05	76	148.959	**<.0001**	8	-2.86	0.0172	76	-166.478	**<.0001**
control - drought	16	-0.00872	1.01e-04	76	110.186	**<.0001**	16	-2.81	0.0232	76	-120.992	**<.0001**
control - drought	60	-0.00841	1.93e-04	76	65.791	**<.0001**	60	-2.71	0.0389	76	-69.790	**<.0001**

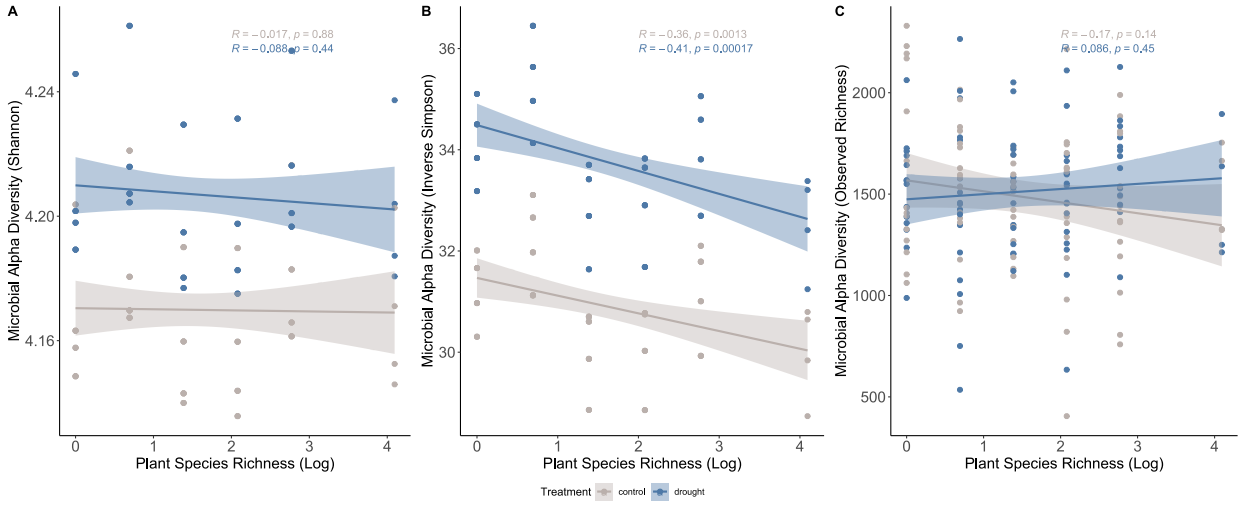


Figure 2: Pearson correlation plots between alpha diversity measurements (Shannon, Inverse Simpson and Observed richness) against plant species richness (natural log). Drought plots are represented in blue color, while gray dots and lines represent the control plots. Drought plots present higher diversity for both Shannon (A) and Inverse Simpson (B), while there is no clear differentiation between the treatments in the number of observed ASVs (C).

To investigate the effects of the experimental design on soil bacterial composition, we used Reduction-Diffusion Analysis (RDA) over Bray–Curtis dissimilarity distance (Figure 2) using soil moisture, soil pH, and number of functional groups as explanatory variables. We observed that soil bacterial community composition follows the sown diversity gradient, and PERMANOVA results indicate a significant effect of both plant species richness and drought treatments on the bacterial community composition (p = 0.001 in both cases – Table 2). Although drought exerted a significant effect on soil bacterial composition, we can observe plant species richness to be the main driver of those bacterial communities (Figure 2). This data also complements the results observed in the linear models and correlation plots for alpha diversity (Figure 1 and table 1), indicating that the impact of plant richness resides on the community composition, not on the overall diversity. Regarding the covariates, we observed a positive correlation between the number of functional groups and plant diversity, which is expected due to the increase in functional groups alongside the diversity gradient in the Jena Experiment design. Soil moisture content displayed a slight negative correlation with the plant diversity gradient, while pH did not seem to be influenced by the same gradient (Figure 2).

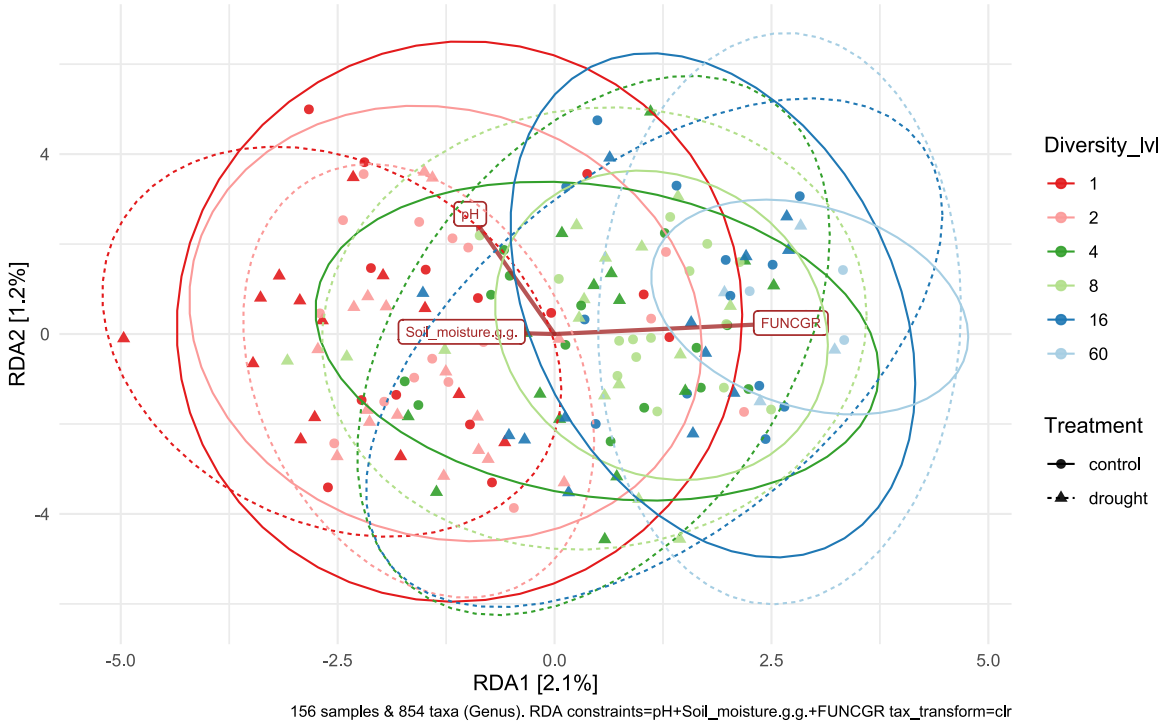


Figure 3: Redundancy analyses (RDA) plot displaying the relationships between pH, soil moisture, and number of plant functional groups as covariates. In the plot, the environmental variables (pH, soil moisture, and number of functional groups) are represented by arrows, indicating their direction and strength of influence. The length of the arrows represents the magnitude of the effect each variable has on the biological communities. The ellipses are colored according to the diversity level of each plot, and the line is dashed according to treatment (straight line for control and dashed line for drought-treated plots).

Table 2: PERMANOVAs over Bray–Curtis dissimilarity distance displaying the effect size and the significance of each tested variable. Plant diversity and drought treatment both presented significant effects on bacterial community composition, while the interaction between both factors was not significant.

	Permanova (Bray Curtis distance)				
	Df	SumOfSqs	R^2	F	Pr(>F)
Plant Species Richness	5	0.8097	0.05448	17.605	0.001***
Drought Treatment	1	0.2312	0.01556	25.135	0.005**
Plant Richness and Drought Treatment	5	0.3903	0.02626	0.8486	0.836
Residual	146	134.292	0.90370		
Total	157	148.603	100.000		

Taxonomical responses to Drought and Plant Species Richness

We also investigated the effects of both plant species richness and summer drought on the taxonomical composition of soil bacterial communities. The taxonomical annotation of sequencing reads indicates that the overall bacterial community composition in our experiment was dominated by the same taxa, regardless of the drought treatment or plant diversity level. Gemmytimonadota, Verrucomicrobiota, Patescibacteria, Myxococcota, Bacteroidota, Chloroflexi, Acidobacteriota, Actinobacteriota, Proteobacteria, and Planctomycetota were the most predominant phyla, being present in all treatments and all plant diversity levels (Supplementary Figure 1). To distinguish responsive taxa across the treatments, we applied a differential compositional analysis using the ANCOMBC package. We filtered significantly increased/decreased ASVs ($p < 0.05$), contrasting their abundances between control and drought-exposed plots across the plant diversity gradient (Figure 3).

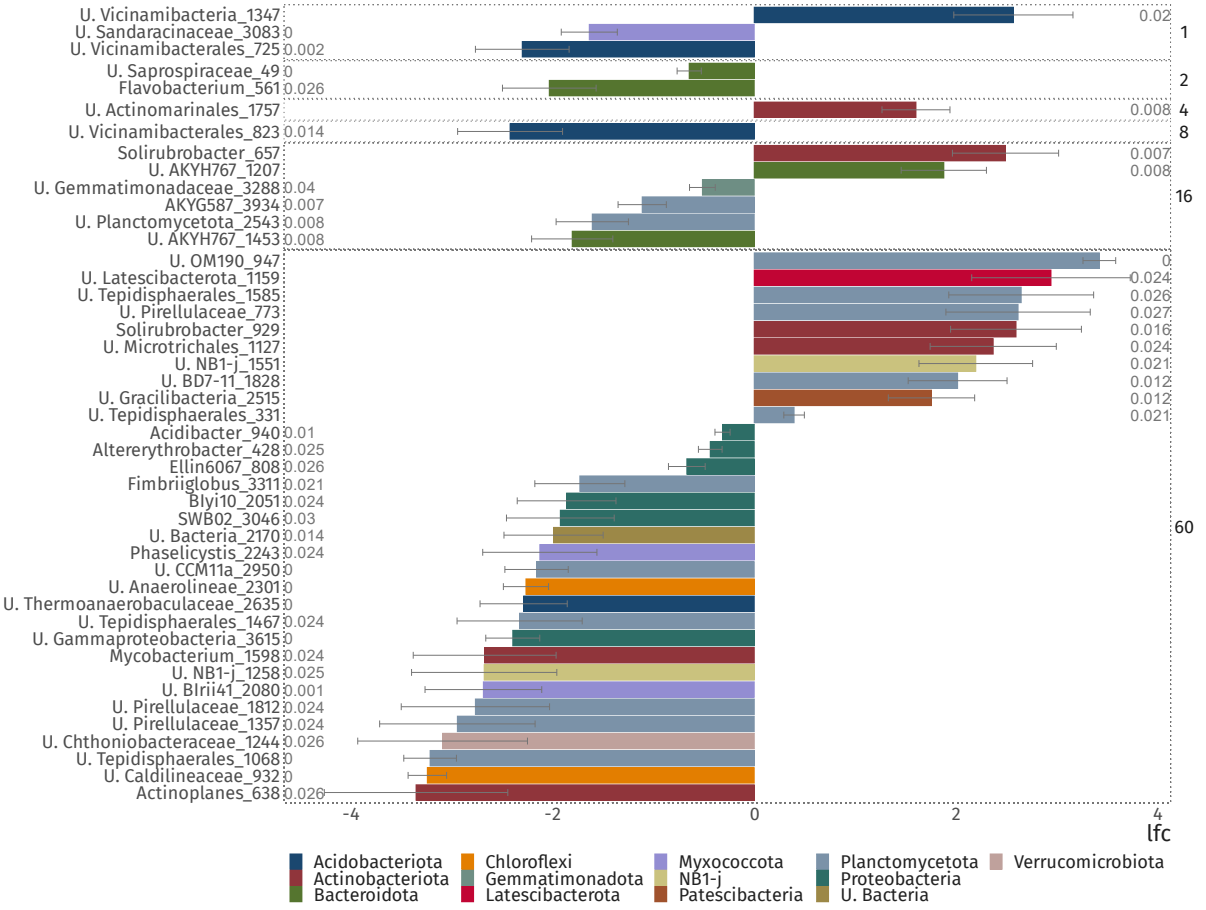


Figure 3: Differential analysis performed with ANCOMB package. The analysis estimated significant differences in ASV abundances between drought and control samples across the plant diversity gradient, with positively (right) and negatively (left) impacted taxa. Taxonomy on the left-hand side indicates the last possible level where that given ASV could be assigned, while bars are colored according to phylum (legend in the bottom). Plant diversity levels are indicated by the numbers on the right.

1-species diversity had both increasing and decreasing ASVs belonging to Acidobacteriota phylum, both assigned to the Vicinamibacteria class, while 1 ASVs assigned to the Myxococcota phylum (Figure 3). Diversity levels 2 and 8 both had only significantly decreased ASVs, assigned to Bacteroidota and Acidobacteriota phyla, respectively. In the other hand, 4-species diversity level only presented a single ASV with significantly response to the drought treatment, increasing in abundance. This ASV was assigned to the Actiboacteriota phylum. In the 16-species plots, we observed an increase in Actinobacteriota and a decrease in Gemmatimonadota and Plantomycetota, and Bacteroidota had both increasing and decreasing ASVs, belonging to the uncultivated order AKYH767 (Mauch, Serra Moncadas, and Andrei 2022). The 60-species diversity level was the one with higher variance in terms of significantly increased/decreased ASVs, with 32 taxa (compared to 3 from levels 1, 2 and 4 and 10 from levels 8 and 6 on level 16), which however might be an artifact caused by a lower number of plots at this plant diversity level (4 plots, with a total of 8 samples). The increasing ASVs were assigned to the Planctomycetota, Latescibacterota, Actinobacteriota and Patescibacteria phyla, while ASVs belonging to Proteobacteriota, Planctomycetota and Chloroflexi were severely reduced. Our analyzes did not associate any specific taxa/ASV with the drought treatment. In this case, the affected bacterial taxa were particular to each of the analyzed diversity levels, without a consistent bacterial taxon being affected by the drought treatment consistently.

Discussion

Our study investigated the effects of increasing plant diversity on the response of the soil bacterial community to extended periods of summer drought. We demonstrated that drought consistently increased bacterial diversity in comparison to ambient precipitation plots. This increase was consistent along the plant richness gradient, which also significantly impacted the composition of soil bacterial community.

Bacterial diversity increases with long-term summer drought.

The effects of drought events have already been largely studied in the soil sciences (Geng et al. 2015; Lipiec et al. 2013; J. P. Schimel 2018). Drought events have a drastic impact on plant and soi bacterial community abundance and composition. The lack of water increases temperature oscillations, reduces nutrient availability, and causes changes in the overall soil structure, making soils more compact and less porous (Yuxin Chen et al. 2022; Geng et al. 2015; Lipiec et al. 2013; Zeppel, Wilks, and Lewis 2014).

Those effects can impair plant growth and development and are largely detrimental for ecosystem functioning. Drought effects have also been largely demonstrated in soil bacterial communities (Preece et al. 2019; Sardans and Pen 2008; J. Schimel, Balser, and Wallenstein 2007), with the microbial response to drought representing a major disturbing factor for soil ecosystem functioning. The lack of water impacts soil microbes in several ways, limiting nutrient availability (Carson et al. 2010; Lawrence, Neff, and Schimel 2009), reducing community connectivity through dissolved molecules (Carson et al. 2010; Manzoni et al. 2016; Manzoni and Katul 2014), and, obviously, diminishing the availability of water as a resource itself, which is essential to the basic functioning and maintenance of cellular processes (Potts 2001). Taking this into consideration, a drop in soil bacterial diversity shortly after a drought event is expected. On the one hand, drought events can quickly change soil properties and nutrient availability, a situation where copiotrophic bacterial taxa do not have enough time to react to drought exposure or simply do not possess the necessary machinery to cope with the changes imposed by drought.

In our study, however, we tested the consequences of long-term, repeated summer drought, which might have an important effect on the adaptation of soil bacterial communities to drought events. In this case, chronic drought imposes a different challenge to soil bacterial communities. The lack of water diminishes soil homogenization, isolating the bacterial community in smaller compartments, which promotes niche formation (Carson et al. 2010), species differentiation (Dumbrell et al. 2010), and an increase in bacterial metabolic dependency (Morris, Lenski, and Zinser 2012). The absence of water also leaves space for more air and therefore more oxygen (Preece, Farré-Armengol, and Peñuelas 2020), increasing the access of the soil bacterial community to other gaseous and volatile substrates (Insam and Seewald 2010), such as methane. The extra oxygen and new substrate availability can then be used as high energy sources for soil bacteria to explore less available and more diverse substrates (Fest et al. 2017; Hartmann, Buchmann, and Niklaus 2011).

Our data indicate that the diversity of soil bacterial communities increases with long-term drought. This trend has already been observed in soils from natural holm oak forest in exposed to chronic drought (Preece et al. 2019); however, it has – to our knowledge – never been reported in grasslands. We observed significant effects of drought treatment on both the Shannon and inverse Simpson diversity indexes ($p < 0.001$), while richness (the number of observed ASVs) did not show any significant responses to the drought treatment ($p = 0.7$). This indicates that the number of taxa did not differ between the treatments; however, their relative abundance did. This trend indicates long-lasting shifts in dominant species in each treatment, as observed in the taxonomic composition analysis. Drought is also shown to significantly affect the composition of soil bacterial community, as observed on the PERMANOVA calculations ($p = 0.005$).

A possible explanation for the increasing bacterial diversity might be the isolation of bacterial communities in the soil, alongside the promotion of less competitive bacteria in this less connected environment. Treves et al. (2002) demonstrated that less dominant taxa have a better chance of establishing as soil moisture decreases (Treves et al. 2003), with the competition between highly abundant taxa and less abundant taxa being more even under this condition. In this scenario, drought can reduce the nutrient availability to fast-growing taxa, allowing the growth of fastidious, less abundant taxa. Similarly, Carson et al. (2010) demonstrated that bacterial diversity increased in soils with low water content in comparison with the same soils with higher moisture (Carson et al. 2010). The changes in this case could be attributed to low pore connectivity due to the lack of water, increasing spatial isolation and reducing soil homogenization. In this sense, our experiment indicates a persistent difference existing in bacterial communities after 9 years of repeated drought, even 1 year after the last treatment period, which indicates the potential long-term effects of moderate droughts on soil bacterial communities. Since plots have only been covered from environmental precipitation during summer months, the breaks in between could represent a sufficiently long recovering time.

Early publications on the same experiment observed contrasting effects, in comparison with our data. Albracht et al. (Albracht et al. 2023) working on the same experiment design, investigated the effects of both plant richness increase and drought treatment on the diversity and composition of arbuscular mycorrhiza (AMF) and total fungal community. They reported a significant impact of plant richness gradient on the diversity and composition of AMF and total fungal community but did not observe any significant effect of drought treatment on the same variables. Wagg et al. (Wagg et al. 2017) observed a reduction on plant biomass on drought treated plots in comparison to control plots, however reported a less pronounced biomass loss as the plant richness increases. In contrast with our findings, however, Vogel et al. (Vogel, Eisenhauer, et al. 2013) observed a significant effect of drought treatment on the litter decomposition, however irrespective of plant diversity. Translating to your context, we observed a highly significant effect of drought treatment on the soil bacterial diversity, however this effect doesn't seem to be correlated with the plant diversity gradient.

Plant richness gradient changes soil microbial composition.

Biodiversity is crucial for ecosystem resilience, which refers to the ability of an ecosystem to withstand and recover from disturbances (Cardinale et al. 2012). A diverse range of species provides functional redundancy, ensuring that ecosystem processes and services are maintained even if some species are lost (Yachi and Loreau 1999). Biodiversity also enhances ecosystem resistance by reducing competition through niche complementarity and increasing adaptability through a broader genetic pool. In this

sense, plant and soil bacterial communities are intimately linked, with plant communities directly impacting and modulating soil bacterial communities (L. Liu et al. 2020). This modulation takes place through diversification of plant exudates in soil (Eisenhauer et al. 2017; El Moujahid et al. 2017), which can be used as a substrate for bacterial growth, as well as the recruitment of specific bacterial taxa to complement plant growth needs, such as phosphorus and nitrogen supply (Berihu et al. 2023). In drought-exposed soils, increasing plant diversity reportedly improves ecosystem resistance and resilience (Wagg et al. 2017), mitigating the effects of drought on biomass loss with compensatory growth after rewetting (Wagg et al. 2017). However, the interaction between drought, the soil bacterial community and plant diversity is still poorly explored.

The effect of increasing plant richness varied according to the diversity metric analyzed. We couldn´t observed a direct effect of plant richness on the Shannon and richness diversity metrics for soil bacterial diversity, with no significant effect on the lme analyzes (Table 1) and no significant linear correlation between plant riches and those metrics (Figure 1). We did, however, observed significant effects of plant richness gradient on the Inverse Simpson metrics, as well as slightly negative correlation between this diversity metric and the plant richness gradient, which indicates that diversity calculated by Simpson slightly decreases along the plant richness gradient. This difference might reside on the different calculation for both diversity index, when the Shannon index emphasizes both species richness and evenness, whereas the Inverse Simpson (derived from the Simson) index places more emphasis on the dominance or concentration of individuals in a few species. Therefore, we estimate that decrease in inverse Simson indicated the increase in dominant species along the plant richness gradient. We do, however, have significant interaction between plant richness and drought treatment on the Shannon metrics, which can be mostly attributed to the strong effect of drought treatment. The impact of plant richness gradient on the soil samples can also be observed on the PERMANOVA analysis, which shows significant effects of broth plant richness gradient and drought treatment. Moreover, previous works on the Jena experiment observed that biodiversity effects on belowground environments might not be significant even though aboveground effects can be observed (Bessler et al. 2009), which can indicate that diversity effects on soil bacterial diversity can be confounded by other environmental factors.

The overall dominance of bacterial taxa did not change across the plant diversity levels or between drought and control plots. Soil samples were dominated by Actinobacteria, Acidobacteria, and Proteobacteria phyla, groups commonly abundant in grassland soil samples (Fierer, Bradford, and Jackson 2007; Janssen 2006). The ANCOMB analyses (Figure 3), however, identified ASVs which significantly increased/decreased after the drought treatment along the plant diversity gradient. The selection and increase/decrease in ASV abundance does not seem to be exclusive to a single phylum,

since we could observe different genera inside of the same phylum being depleted, while others are increased. The Actinobacteriota phylum, for example, had phyla with a 2-fold increase in plant diversity levels 4 and 16 (Actinomarinales order and *Solirubrobacter* genus, respectively), while in diversity level 60 two of the present ASVs were depleted (*Mycobacterium* and *Actinoplanes* genera) while two others were increased (*Solirubrobacter* genus and Microtrichales order). Pérez Castro et al. (Pérez Castro et al. 2019) also observed the decrease in Proteobacteria, Verrucomicrobia, and Acidobacteria, while Actinobacteria abundance increased after drought stress. Therefore, despite the observation of a significant effect on the overall bacterial diversity, the drought treatment in our experiment did not select any specific group or taxa.

This preferential accumulation of specific taxa according to plant diversity level indicates that the complementarity between bacterial and plant metabolism in the face of drought follows individual interactions at the species or even strain level, despite the overall consistent positive effect of plant diversity composition under drought. A possible explanation for these patterns might be the variation in the number of plant functional groups implemented in parallel in the Jena Experiment (see Supplementary table 1.) and/or the variation on species on each diversity level, being the changes on soil bacterial composition a specie-specific interaction, instead of an overall response to the increase in surrounding diversity. The drought experiment conducted by Preece et al. (Preece et al. 2019) also observed high variability in fungal community composition as affected by long-term drought, representing the most affected taxa highly dependent according to their experiment design. As previously mentioned, Albracht et al (Albracht et al. 2023) observed the opposite: non- significant effect of drought and significant effect of plant diversity gradient over fungal community. Together, this data indicates that microbial response to drought is dynamic, changes according to the investigated microbial group and according to the experimental design.

In summary, our data indicate that the diversity of the soil bacterial community positively reacts to long-term drought, with a stable response to the plat richness gradient. This response may be explained by the spatial isolation of soil bacterial communities promoted by a reduction in water potential and the diversification of ecological opportunities offered by increasing plant diversity (Roscher et al. 2004; G. Wang et al. 2019; Weisser et al. 2017). These findings indicate that soil bacterial diversity can adapt to long term drought conditions, being affected by the increase in plant richness, which might have important consequences for ecosystem functioning in a changing climate.

Acknowledgments

We would like to thank the Jena Experiment coordination team, Anne Ebeling and the gardeners and technicians with their help during the experiment's setup and maintenance.

Author contribution

AV, CW and NE installed and maintained the experiment. CA performed DNA extraction. Amplicon sequencing, statistics and data visualization and manuscript writing was performed by YP under the supervision of SS and MS. RS performed bioinformatic data processing. YH helped with data analysis. All authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Funding statement

Furthermore, we thank the Deutsche Forschungsgemeinschaft (DFG) for funding The Jena Experiment (FOR 5000). The authors MS, WW and SS received the following grants: SCHL446/45-1, WE3081/39-1 SCHU2907/8-1. NE gratefully acknowledges additional support by the DFG (Ei 862/29-1).

Data availability

Raw sequencing data was deposited on the NCBI database under the BioProject number PRJNA937585 and BioSample SAMN37746197. Data analysis R script is deposited in the GitHub repository https://github.com/rsiani/yuri_et_al_22.

References

Aanderud, Zachary T. et al. 2018. "Stoichiometric Shifts in Soil C:N:P Promote Bacterial Taxa Dominance, Maintain Biodiversity, and Deconstruct Community Assemblages." *Frontiers in Microbiology* 9(JUL).

Abalos, Diego et al. 2021. "Manipulating Plant Community Composition to Steer Efficient N-Cycling in Intensively Managed Grasslands." *Journal of Applied Ecology* 58(1): 167–80.

Abdelfattah, Ahmed et al. 2022. "From Seed to Seed: The Role of Microbial Inheritance in the Assembly of the Plant Microbiome." *Trends in Microbiology* xx(xx): 1–10. https://doi.org/10.1016/j.tim.2022.10.009.

Abdelfattah, Ahmed, Michael Wisniewski, Leonardo Schena, and Ayco J.M. Tack. 2021. "Experimental Evidence of Microbial Inheritance in Plants and Transmission Routes from Seed to Phyllosphere and Root." *Environmental Microbiology* 23(4): 2199–2214.

Abedini, Davar, Sébastien Jaupitre, Harro Bouwmeester, and Lemeng Dong. 2021. "Metabolic Interactions in Beneficial Microbe Recruitment by Plants." *Current Opinion in Biotechnology* 70: 241–47.

Afzal, Imran, Zabta Khan Shinwari, Shomaila Sikandar, and Shaheen Shahzad. 2019. "Plant Beneficial Endophytic Bacteria: Mechanisms, Diversity, Host Range and Genetic Determinants." *Microbiological Research* 221(April 2018): 36–49.

Ahmed, Moddassir, Muhammad Rauf, Zahid Mukhtar, and Nasir Ahmad Saeed. 2017. "Excessive Use of Nitrogenous Fertilizers: An Unawareness Causing Serious Threats to Environment and Human Health." *Environmental Science and Pollution Research* 24(35): 26983–87.

Albracht, Cynthia et al. 2023. "Effects of Recurrent Summer Droughts on Arbuscular Mycorrhizal and Total Fungal Communities in Experimental Grasslands Differing in Plant Diversity and Community Composition."

Alizon, S., A. Hurford, N. Mideo, and M. Van Baalen. 2009. "Virulence Evolution and the Trade-off Hypothesis: History, Current State of Affairs and the Future." *Journal of Evolutionary Biology* 22(2): 245–59.

Apprill, Amy, Sean Mcnally, Rachel Parsons, and Laura Weber. 2015. "Minor Revision to V4 Region SSU RRNA 806R Gene Primer Greatly Increases Detection of SAR11 Bacterioplankton." *Aquatic Microbial Ecology* 75(2): 129–37.

Atuchin, Victor V. et al. 2023. "Microorganisms for Bioremediation of Soils Contaminated with Heavy Metals." *Microorganisms* 11(4): 1–19.

Babu, Anam Giridhar et al. 2015. "Penicillium Menonorum: A Novel Fungus to Promote Growth and Nutrient Management in Cucumber Plants." *Mycobiology* 43(1): 49–56.

Bach, H. J., J. Tomanova, M. Schloter, and J. C. Munch. 2002. "Enumeration of Total Bacteria and Bacteria with Genes for Proteolytic Activity in Pure Cultures and in Environmental Samples by Quantitative PCR Mediated Amplification." *Journal of Microbiological Methods* 49(3): 235–45.

Baedke, Jan, Alejandro Fábregas-Tejeda, and Abigail Nieves Delgado. 2020. "The Holobiont Concept before Margulis." *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 334(3): 149–55.

Baker, Edward. 2015. "The Worldwide Status of Phasmids (Insecta: Phasmida) as Pests of Agriculture and Forestry, with a Generalised Theory of Phasmid Outbreaks." *Agriculture and Food Security* 4(1): 1–19.

Balvanera, Patricia et al. 2014. "Linking Biodiversity and Ecosystem Services: Current Uncertainties and the Necessary next Steps." *BioScience* 64(1): 49–57.

Banerjee, Samiran, Klaus Schlaeppi, and Marcel G.A. van der Heijden. 2018. "Keystone Taxa as Drivers of Microbiome Structure and Functioning." *Nature Reviews Microbiology* 16(9): 567–76. http://dx.doi.org/10.1038/s41579-018-0024-1.

Bar-On, Yinon M., Rob Phillips, and Ron Milo. 2018. "The Biomass Distribution on Earth." *Proceedings of the National Academy of Sciences of the United States of America* 115(25): 6506–11.

Barnes, Andrew D. et al. 2018. "Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning." *Trends in Ecology and Evolution* 33(3): 186–97.

Barnett, David, Ilja Arts, and John Penders. 2021. "MicroViz: An R Package for Microbiome Data Visualization and Statistics." *Journal of Open Source Software* 6(63): 3201.

Bartelt-Ryser, Janine et al. 2005. “Soil Feedbacks of Plant Diversity on Soil Microbial Communities and Subsequent Plant Growth.” *Perspectives in Plant Ecology, Evolution and Systematics* 7(1): 27–49.

Baslam, Marouane, Idoia Garmendia, and Nieves Goicoechea. 2011. “Plant Genotype, Microbial Recruitment and Nutritional Security.” *Journal of Agricultural and Food Chemistry* 59(10): 5504–15.

Berendse, Frank, Jasper van Ruijven, Eelke Jongejans, and Saskia Keesstra. 2015. “Loss of Plant Species Diversity Reduces Soil Erosion Resistance.” *Ecosystems* 18(5): 881–88. http://dx.doi.org/10.1007/s10021-015-9869-6.

Berg, Gabriele, and Jos M. Raaijmakers. 2018. “Saving Seed Microbiomes.” *ISME Journal* 12(5): 1167–70. http://dx.doi.org/10.1038/s41396-017-0028-2.

van Bergeijk, Doris A., Barbara R. Terlouw, Marnix H. Medema, and Gilles P. van Wezel. 2020. “Ecology and Genomics of Actinobacteria: New Concepts for Natural Product Discovery.” *Nature Reviews Microbiology* 18(10): 546–58. http://dx.doi.org/10.1038/s41579-020-0379-y.

Bergna, Alessandro et al. 2018. “Tomato Seeds Preferably Transmit Plant Beneficial Endophytes.” *Phytobiomes Journal* 2(4): 183–93.

Berihu, Maria et al. 2023. “A Framework for the Targeted Recruitment of Crop-Beneficial Soil Taxa Based on Network Analysis of Metagenomics Data.” *Microbiome* 11(1): 1–21. https://doi.org/10.1186/s40168-022-01438-1.

Bessler, Holger et al. 2009. “Aboveground Overyielding in Grassland Mixtures Is Associated with Reduced Biomass Partitioning to Belowground Organs.” *Ecology* 90(6): 1520–30.

Bever, James D., Scott A. Mangan, and Helen M. Alexander. 2015. “Maintenance of Plant Species Diversity by Pathogens.” *Annual Review of Ecology, Evolution, and Systematics* 46(September): 305–25.

Bramble, De Shorn E. et al. 2024. “Formation of Mineral-Associated Organic Matter in Temperate Soils Is Primarily Controlled by Mineral Type and Modified by Land Use and Management Intensity.” *Global Change Biology* 30(1): 1–19.

Branco, Sara, Annie Schauster, Hui Ling Liao, and Joske Ruytinx. 2022. “Mechanisms of Stress Tolerance and Their Effects on the Ecology and Evolution of Mycorrhizal Fungi.” *New Phytologist* 235(6): 2158–75.

Bray, R. J., and J.T. Curtis. 1957. “An Ordination of the Upland Forest Communities of Southern Wisconsin.” *Ecological Society of America* 27(4): 325–49.

Bulgarelli, Davide et al. 2013. “Structure and Functions of the Bacterial Microbiota of Plants.” *Annual Review of Plant Biology* 64: 807–38.

Bundy, J. G., G. I. Paton, and C. D. Campbell. 2002. “Microbial Communities in Different Soil Types Do Not Converge after Diesel Contamination.” *Journal of Applied Microbiology* 92(2): 276–88.

Callahan, Benjamin J, Amy Jo A , Paul J McMurdie, Michael J Rosen, Andrew W Han, and and Susan P Holmes Johnson. 2016. “Dada2: High Resolution Sample Inference from Illumina Amplicon Data.” *Nature Methods* 13(7): 1–7.

Canarini, Alberto et al. 2021. “Ecological Memory of Recurrent Drought Modifies Soil Processes via Changes in Soil Microbial Community.” *Nature Communications* 12(1): 1–14. http://dx.doi.org/10.1038/s41467-021-25675-4.

Cania, Barbara et al. 2019. “A Long-Term Field Experiment Demonstrates the Influence of Tillage on the Bacterial Potential to Produce Soil Structure-Stabilizing Agents Such as Exopolysaccharides and Lipopolysaccharides.” *Environmental Microbiomes* 14(1): 1–14.

Capdevila-Cortada, Marcal. 2019. “Electrifying the Haber–Bosch.” *Nature Catalysis* 2(12): 1055. http://dx.doi.org/10.1038/s41929-019-0414-4.

Cardinale, Bradley J. et al. 2012. “Biodiversity Loss and Its Impact on Humanity.” *Nature* 486(7401): 59–67.

Carini, Paul et al. 2016. “Relic DNA Is Abundant in Soil and Obscures Estimates of Soil Microbial Diversity.” *Nature Microbiology* 2(December 2016).

Carlson, C. A., and J. L. Ingraham. 1983. “Comparison of Denitrification by Pseudomonas Stutzeri, Pseudomonas Aeruginosa, and Paracoccus Denitrificans.” *Applied and Environmental Microbiology* 45(4): 1247–53.

Carson, Jennifer K. et al. 2010. “Low Pore Connectivity Increases Bacterial Diversity in Soil.” *Applied and Environmental Microbiology* 76(12): 3936–42.

Cesarz, Simone et al. 2017. “Plant Species Richness Sustains Higher Trophic Levels of Soil Nematode Communities after Consecutive Environmental Perturbations.” *Oecologia* 184(3): 715–28.

Chaparro, Jacqueline M., Amy M. Sheflin, Daniel K. Manter, and Jorge M. Vivanco. 2012. “Manipulating the Soil Microbiome to Increase Soil Health and Plant Fertility.” *Biology and Fertility of Soils* 48(5): 489–99.

Chase, Jonathan M et al. 2011. “Disentangling the Importance of Ecological Niches from Stochastic Processes across Scales Disentangling the Importance of Ecological Niches from Stochastic Processes across Scales.” : 2351–63.

Chen, Youhua, and Tsung Jen Shen. 2017. “A General Framework for Predicting Delayed Responses of Ecological Communities to Habitat Loss.” *Scientific Reports* 7(1): 1–11. http://dx.doi.org/10.1038/s41598-017-01070-2.

Chen, Yuxin et al. 2022. “Drought-Exposure History Increases Complementarity between Plant Species in Response to a Subsequent Drought.” *Nature Communications* 13(1): 1–11.

Civitello, David J. et al. 2015. “Biodiversity Inhibits Parasites: Broad Evidence for the Dilution Effect.” *Proceedings of the National Academy of Sciences of the United States of America* 112(28): 8667–71.

Cong, Wen Feng et al. 2014. “Plant Species Richness Promotes Soil Carbon and Nitrogen Stocks in Grasslands without Legumes.” *Journal of Ecology* 102(5): 1163–70.

Contreras-Cornejo, Hexon Angel, Lourdes Macías-Rodríguez, Carlos Cortés-Penagos, and José López-Bucio. 2009. “Trichoderma Virens, a Plant Beneficial Fungus, Enhances Biomass Production and Promotes Lateral Root Growth through an Auxin-Dependent Mechanism in Arabidopsis.” *Plant Physiology* 149(3): 1579–92.

Cornwell, William K. et al. 2008. “Plant Species Traits Are the Predominant Control on Litter Decomposition Rates within Biomes Worldwide.” *Ecology Letters* 11(10): 1065–71.

Crawford, K. M., and K. D. Whitney. 2010. “Population Genetic Diversity Influences Colonization Success.” *Molecular Ecology* 19(6): 1253–63.

Crous, Kristine Y. 2019. “Plant Responses to Climate Warming: Physiological Adjustments and Implications for Plant Functioning in a Future, Warmer World.” *American Journal of Botany*

106(8): 1049–51.

Csonka, L N. 1989. “Physiological and Genetic Responses of Bacteria to Osmotic Stress.” *Microbiological Reviews* 53(1): 121–47.

Cuddington, Kim. 2011. “Legacy Effects: The Persistent Impact of Ecological Interactions.” *Biological Theory* 6(3): 203–10.

Dadzie, Frederick A. et al. 2023. “Inoculating Native Microorganisms Improved Soil Function and Altered the Microbial Composition of a Degraded Soil.” *Restoration Ecology* 32(2): 1–11.

Davis, Nicole M. et al. 2018. “Simple Statistical Identification and Removal of Contaminant Sequences in Marker-Gene and Metagenomics Data.” *Microbiome* 6(1): 1–14.

Decaëns, T. et al. 2006. “The Values of Soil Animals for Conservation Biology.” *European Journal of Soil Biology* 42(SUPPL. 1).

Dell’Anno, Antonio et al. 2012. “High Bacterial Biodiversity Increases Degradation Performance of Hydrocarbons during Bioremediation of Contaminated Harbor Marine Sediments.” *Environmental Pollution* 167: 85–92. http://dx.doi.org/10.1016/j.envpol.2012.03.043.

Delmont, Tom O. et al. 2014. “Microbial Community Development and Unseen Diversity Recovery in Inoculated Sterile Soil.” *Biology and Fertility of Soils* 50(7).

DeLong, Edward F., and Norman R. Pace. 2001. “Environmental Diversity of Bacteria and Archaea.” *Systematic Biology* 50(4): 470–78.

Deng, Lei et al. 2021. “Drought Effects on Soil Carbon and Nitrogen Dynamics in Global Natural Ecosystems.” *Earth-Science Reviews* 214(September 2020): 103501. https://doi.org/10.1016/j.earscirev.2020.103501.

Dhankhar, Neha, and Jagdeep Kumar. 2023. “Impact of Increasing Pesticides and Fertilizers on Human Health: A Review.” *Materials Today: Proceedings* (xxxx). https://doi.org/10.1016/j.matpr.2023.03.766.

Dias, Teresa, Angela Dukes, and Pedro M. Antunes. 2014. “Accounting for Soil Biotic Effects on Soil Health and Crop Productivity in the Design of Crop Rotations.” *Journal of the Science of Food and Agriculture* 95(3): 447–54.

Ding, Tao, and Ulrich Melcher. 2016. “Influences of Plant Species, Season and Location on Leaf Endophytic Bacterial Communities of Non-Cultivated Plants.” *PLoS ONE* 11(3): 1–13.

Dogan, Gokhan, and Bilgin Taskin. 2021. “Hydrolytic Enzymes Producing Bacterial Endophytes of Some Poaceae Plants.” *Polish Journal of Microbiology* 70(3): 297–304.

Dorn-In, Samart et al. 2015. “Specific Amplification of Bacterial DNA by Optimized So-Called Universal Bacterial Primers in Samples Rich of Plant DNA.” *Journal of Microbiological Methods* 113: 50–56. http://dx.doi.org/10.1016/j.mimet.2015.04.001.

Downs, Diana M. 2006. “Understanding Microbial Metabolism.” *Annual Review of Microbiology* 60: 533–59.

Dumbrell, Alex J et al. 2010. “Relative Roles of Niche and Neutral Processes in Structuring a Soil Microbial Community.” *The ISME Journal*: 337–45.

Eisenhauer, Nico et al. 2017. “Root Biomass and Exudates Link Plant Diversity with Soil Bacterial and Fungal Biomass.” *Scientific Reports* 7: 1–8.

Elbasiouny, Heba et al. 2022. “Plant Nutrition under Climate Change and Soil Carbon Sequestration.”

Sustainability (Switzerland) 14(2): 1–20.

Van Elsas, Jan Dirk et al. 2012. "Microbial Diversity Determines the Invasion of Soil by a Bacterial Pathogen." *Proceedings of the National Academy of Sciences of the United States of America* 109(4): 1159–64.

Eriyagama, Nishadi, Vladimir Smakhtin, and Nilantha Gamage. 2009. IWMI Research Report *Mapping Drought Patterns and Impacts: A Global Perspective In Ternational Water Management Institute*.

Estendorfer, Jennifer et al. 2017. "The Influence of Land Use Intensity on the Plant-Associated Microbiome of Dactylis Glomerata L." *Frontiers in Plant Science* 8(June): 1–10.

Evans, Sarah E., and Matthew D. Wallenstein. 2012. "Soil Microbial Community Response to Drying and Rewetting Stress: Does Historical Precipitation Regime Matter?" *Biogeochemistry* 109(1–3): 101–16.

Eyre, Alexander W., Mengying Wang, Yeonyee Oh, and Ralph A. Dean. 2019. "Identification and Characterization of the Core Rice Seed Microbiome." *Phytobiomes Journal* 3(2): 148–57.

Fadiji, Ayomide Emmanuel, and Olubukola Oluranti Babalola. 2020. "Exploring the Potentialities of Beneficial Endophytes for Improved Plant Growth." *Saudi Journal of Biological Sciences* 27(12): 3622–33. https://doi.org/10.1016/j.sjbs.2020.08.002.

Fahrig, Lenore. 2003. "Effects of Habitat Fragmentation on Biodiversity." *Annual Review of Ecology, Evolution, and Systematics* 34: 487–515.

Farrelly, Damien J., Colm D. Everard, Colette C. Fagan, and Kevin P. McDonnell. 2013. "Carbon Sequestration and the Role of Biological Carbon Mitigation: A Review." *Renewable and Sustainable Energy Reviews* 21: 712–27. http://dx.doi.org/10.1016/j.rser.2012.12.038.

Fest, Benedikt et al. 2017. "Soil Methane Uptake Increases under Continuous Throughfall Reduction in a Temperate Evergreen, Broadleaved Eucalypt Forest." *Ecosystems* 20(2): 368–79.

Fierer, Noah, Mark A. Bradford, and Robert B. Jackson. 2007. "Toward an Ecological Classification of Soil Bacteria." *Ecology* 88(6): 1354–64.

Fluegge, Keith, and Kyle Fluegge. 2017. "Air Pollution and Risk of Hospitalization for Epilepsy: The Role of Farm Use of Nitrogen Fertilizers and Emissions of the Agricultural Air Pollutant, Nitrous Oxide." *Arquivos de Neuro-Psiquiatria* 75(9): 614–19.

Fowler, A. M., and K. J. Hennessy. 1995. "Potential Impacts of Global Warming on the Frequency and Magnitude of Heavy Precipitation." *Natural Hazards* 11(3): 283–303.

Freschet, Grégoire T. et al. 2021. "Root Traits as Drivers of Plant and Ecosystem Functioning: Current Understanding, Pitfalls and Future Research Needs." *New Phytologist* 232(3): 1123–58.

Galindo-Solís, Juan M., and Francisco J. Fernández. 2022. "Endophytic Fungal Terpenoids: Natural Role and Bioactivities." *Microorganisms* 10(2): 1–22.

Geisen, Stefan, Diana H. Wall, and Wim H. van der Putten. 2019. "Challenges and Opportunities for Soil Biodiversity in the Anthropocene." *Current Biology* 29(19): R1036–44. https://doi.org/10.1016/j.cub.2019.08.007.

Geisseler, Daniel, and Kate M. Scow. 2014. "Long-Term Effects of Mineral Fertilizers on Soil Microorganisms - A Review." *Soil Biology and Biochemistry* 75: 54–63. http://dx.doi.org/10.1016/j.soilbio.2014.03.023.

Geng, S. M. et al. 2015. “Effects of Drought Stress on Agriculture Soil.” *Natural Hazards* 75(2): 1997–2011.

Goldstein, Ellie J.C., Diane M. Citron, Victoria A. Peraino, and Sally A. Cross. 2003. “Desulfovibrio Desulfuricans Bacteremia and Review of Human Desulfovibrio Infections.” *Journal of Clinical Microbiology* 41(6): 2752–54.

Goyal, Nishu, Zhi Zhou, and Iftekhar A. Karimi. 2016. “Metabolic Processes of Methanococcus Maripaludis and Potential Applications.” *Microbial Cell Factories* 15(1): 1–19.

Grant, Kirsten N., Merrin L. Macrae, Fereidoun Rezanezhad, and W. Vito Lam. 2019. “Nutrient Leaching in Soil Affected by Fertilizer Application and Frozen Ground.” *Vadose Zone Journal* 18(1): 1–13.

Gregorich, E. G., C. F. Drury, and J. A. Baldock. 2001. “Changes in Soil Carbon under Long-Term Maize in Monoculture and Legume-Based Rotation.” *Canadian Journal of Soil Science* 81(1): 21–31.

Groussin, Mathieu, Florent Mazel, and Eric J. Alm. 2020. “Co-Evolution and Co-Speciation of Host-Gut Bacteria Systems.” *Cell Host and Microbe* 28(1): 12–22. https://doi.org/10.1016/j.chom.2020.06.013.

van de Guchte, Maarten, Hervé M. Blottière, and Joël Doré. 2018. “Humans as Holobionts: Implications for Prevention and Therapy.” *Microbiome* 6(1): 81.

Guenay, Yasemin et al. 2013. “Transgressive Overyielding of Soil Microbial Biomass in a Grassland Plant Diversity Gradient.” *Soil Biology and Biochemistry* 60: 122–24. http://dx.doi.org/10.1016/j.soilbio.2013.01.015.

Habibullah, Muzafar Shah, Badariah Haji Din, Siow Hooi Tan, and Hasan Zahid. 2022. “Impact of Climate Change on Biodiversity Loss: Global Evidence.” *Environmental Science and Pollution Research* 29(1): 1073–86.

Haines-young, Roy. 2009. “Land Use and Biodiversity Relationships.” *Land Use Policy*: 178–86.

Handa, I. Tanya et al. 2014. “Consequences of Biodiversity Loss for Litter Decomposition across Biomes.” *Nature* 509(7499): 218–21.

Hanson, China A., Jed A. Fuhrman, M. Claire Horner-Devine, and Jennifer B.H. Martiny. 2012. “Beyond Biogeographic Patterns: Processes Shaping the Microbial Landscape.” *Nature Reviews Microbiology* 10(7): 497–506.

Hartman, Kyle, and Susannah G. Tringe. 2019. “Interactions between Plants and Soil Shaping the Root Microbiome under Abiotic Stress.” *Biochemical Journal* 476(19): 2705–24.

Hartmann, Adrian A., Nina Buchmann, and Pascal A. Niklaus. 2011. “A Study of Soil Methane Sink Regulation in Two Grasslands Exposed to Drought and N Fertilization.” *Plant and Soil* 342(1–2): 265–75.

Hawkes, Christine V., James J. Bull, and Jennifer A. Lau. 2020. “Symbiosis and Stress: How Plant Microbiomes Affect Host Evolution.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 375(1808).

Hector, A. et al. 1999. “Plant Diversity and Productivity Experiments in European Grasslands.” *Science* 286(5442): 1123–27.

Helfrich, Eric J.N., Geng Min Lin, Christopher A. Voigt, and Jon Clardy. 2019. “Bacterial Terpene Biosynthesis: Challenges and Opportunities for Pathway Engineering.” *Beilstein Journal of Organic Chemistry* 15: 2889–2906.

Higginbottom, Thomas P, and Elias Symeonakis. 2014. “Assessing Land Degradation and Desertification Using Vegetation Index Data: Current Frameworks and Future Directions.” (I): 9552–75.

Hoffmann, K. et al. 2014. *Klimauntersuchungen in Jena Für Die Anpassung an Den Klimawandel Und Seine Erwarteten Folgen. Offenbach Am Main: Selbstverlag Des Deutschen Wetterdienstes.*

Hoiss, Bernhard et al. 2013. “Combined Effects of Climate and Management on Plant Diversity and Pollination Type in Alpine Grasslands.” *Diversity and Distributions* 19(4): 386–95.

Hu, Ruifa et al. 2015. “Long- and Short-Term Health Effects of Pesticide Exposure: A Cohort Study from China.” *PLoS ONE* 10(6): 1–13.

Huang, Yuanyuan et al. 2023. “Enhanced Stability of Grassland Soil Temperature by Plant Diversity.” *Nature geoscience*.

Hubálek, Z. 2000. “Measures of Species Diversity in Ecology: An Evaluation.” *Folia Zoologica* 49(4): 241–60.

Hughes, A. Randall et al. 2008. “Ecological Consequences of Genetic Diversity.” *Ecology Letters* 11(6): 609–23.

Humphreys, Aelys M. et al. 2019. “Global Dataset Shows Geography and Life Form Predict Modern Plant Extinction and Rediscovery.” *Nature Ecology and Evolution* 3(7): 1043–47.

Huntington, Thomas G. 2006. “Evidence for Intensification of the Global Water Cycle: Review and Synthesis.” *Journal of Hydrology* 319(1–4): 83–95.

Insam, Heribert, and Martin S.A. Seewald. 2010. “Volatile Organic Compounds (VOCs) in Soils.” *Biology and Fertility of Soils* 46(3): 199–213.

Isbell, Forest et al. 2015. “Biodiversity Increases the Resistance of Ecosystem Productivity to Climate Extremes.” *Nature* 526(7574): 574–77.

———. 2017. “Benefits of Increasing Plant Diversity in Sustainable Agroecosystems.” *Journal of Ecology* 105(4): 871–79.

Jaacks, Lindsay M., and Lisa R. Staimez. 2015. “Association of Persistent Organic Pollutants and Non-Persistent Pesticides with Diabetes and Diabetes-Related Health Outcomes in Asia: A Systematic Review.” *Environment International* 76: 57–70. http://dx.doi.org/10.1016/j.envint.2014.12.001.

Jaccard, Paul. 1912. “The Distribution of the Flora in the Alpine Zone.” *New Phytologist* 11(2): 37–50.

Janssen, Peter H. 2006. “Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S RRNA and 16S RRNA Genes.” *Applied and Environmental Microbiology* 72(3): 1719–28.

Jansson, Janet K., and Kirsten S. Hofmockel. 2020. “Soil Microbiomes and Climate Change.” *Nature Reviews Microbiology* 18(1): 35–46. http://dx.doi.org/10.1038/s41579-019-0265-7.

Jha, S, and K S Bawa. 2006. “Population Growth , Human Development , and Deforestation in Biodiversity Hotspots.” 20(3): 906–12.

Johnson, Christopher N. et al. 2017. “Biodiversity Losses and Conservation Responses in the Anthropocene.” *Science* 356(6335): 270–75.

Johnston-Monje, David, Janneth P. Gutiérrez, and Luis Augusto Becerra Lopez-Lavalle. 2021. “Seed-Transmitted Bacteria and Fungi Dominate Juvenile Plant Microbiomes.” *Frontiers in Microbiology* 12(October).

Jones, Stuart E., and Jay T. Lennon. 2010. “Dormancy Contributes to the Maintenance of Microbial Diversity.” *Proceedings of the National Academy of Sciences of the United States of America* 107(13): 5881–86.

Jr, F A, A The Origin, and New Haven. 2000. “Genetic Diversity and Disease Control in Rice.” 406(August): 718–22.

Kaisermann, Aurore, Franciska T. de Vries, Robert I. Griffiths, and Richard D. Bardgett. 2017. “Legacy Effects of Drought on Plant–Soil Feedbacks and Plant–Plant Interactions.” *New Phytologist* 215(4): 1413–24.

Kakouridis, Anne et al. 2022. “Routes to Roots: Direct Evidence of Water Transport by Arbuscular Mycorrhizal Fungi to Host Plants.” *New Phytologist* 236(1): 210–21.

Kembel, Steven W. et al. 2010. “Picante: R Tools for Integrating Phylogenies and Ecology.” *Bioinformatics* 26(11): 1463–64.

Kennedy, A. C., and K. L. Smith. 1995. “Soil Microbial Diversity and the Sustainability of Agricultural Soils.” *Plant and Soil* 170(1): 75–86.

Khare, Ekta, Jitendra Mishra, and Naveen Kumar Arora. 2018. “Multifaceted Interactions between Endophytes and Plant: Developments and Prospects.” *Frontiers in Microbiology* 9(NOV): 1–12.

King, K. C., and C. M. Lively. 2012. “Does Genetic Diversity Limit Disease Spread in Natural Host Populations.” *Heredity* 109(4): 199–203.

Kirkegaard, J. A., and J. R. Hunt. 2010. “Increasing Productivity by Matching Farming System Management and Genotype in Water-Limited Environments.” *Journal of Experimental Botany* 61(15): 4129–43.

Knights, Dan et al. 2011. “Bayesian Community-Wide Culture-Independent Microbial Source Tracking.” *Nature Methods* 8(9): 761–65.

Koops, H. P. et al. 1991. “Classification of Eight New Species of Ammonia-Oxidizing Bacteria: Nitrosomonas Communis Sp. Nov., Nitrosomonas Ureae Sp. Nov., Nitrosomonas Aestuarii Sp. Nov., Nitrosomonas Marina Sp. Nov., Nitrosomonas Nitrosa Sp. Nov., Nitrosomonas Eutropha Sp. Nov., N.” *Journal of General Microbiology* 137(7): 1689–99.

Kopittke, Peter M. et al. 2019. “Soil and the Intensification of Agriculture for Global Food Security.” *Environment International* 132(August): 105078. https://doi.org/10.1016/j.envint.2019.105078.

Kostenko, Olga, and T. Martijn Bezemer. 2020. “Abiotic and Biotic Soil Legacy Effects of Plant Diversity on Plant Performance.” *Frontiers in Ecology and Evolution* 8(April): 1–12.

Kowalchuk, George A. et al. 2002. “Effects of Above-Ground Plant Species Composition and Diversity on the Diversity of Soil-Borne Microorganisms.” *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology* 81(1–4): 509–20.

Kruskal, Joseph B., and Roger N. Shepard. 1974. “A Nonmetric Variety of Linear Factor Analysis.” *Psychometrika* 39(2): 123–57.

Lal, Rattan, and William C. Moldenhauer. 1987. “Effects of Soil Erosion on Crop Productivity.” *Critical Reviews in Plant Sciences* 5(4): 303–67.

Lange, Markus et al. 2014. “Biotic and Abiotic Properties Mediating Plant Diversity Effects on Soil Microbial Communities in an Experimental Grassland.” *PLoS ONE* 9(5).

———. 2015. “Plant Diversity Increases Soil Microbial Activity and Soil Carbon Storage.” *Nature*

Communications 6.

Lata, R., S. Chowdhury, S. K. Gond, and J. F. White. 2018. "Induction of Abiotic Stress Tolerance in Plants by Endophytic Microbes." *Letters in Applied Microbiology* 66(4): 268–76.

Lawrence, Corey R., Jason C. Neff, and Joshua P. Schimel. 2009. "Does Adding Microbial Mechanisms of Decomposition Improve Soil Organic Matter Models? A Comparison of Four Models Using Data from a Pulsed Rewetting Experiment." *Soil Biology and Biochemistry* 41(9): 1923–34. http://dx.doi.org/10.1016/j.soilbio.2009.06.016.

Leek, Jeffrey T. et al. 2012. "The SVA Package for Removing Batch Effects and Other Unwanted Variation in High-Throughput Experiments." *Bioinformatics* 28(6): 882–83.

Lehmann, Anika, Weishuang Zheng, and Matthias C. Rillig. 2017. "Soil Biota Contributions to Soil Aggregation." *Nature Ecology and Evolution* 1(12): 1828–35. http://dx.doi.org/10.1038/s41559-017-0344-y.

Lekli, Istvan, Diptarka Ray, and Dipak K. Das. 2010. "Longevity Nutrients Resveratrol, Wines and Grapes." *Genes and Nutrition* 5(1): 55–60.

Leo, Lahti. "Microbiome R Package." http://microbiome.github.io.

Letourneau, Deborah K., and Sara G. Bothwell. 2008. "Comparison of Organic and Conventional Farms: Challenging Ecologists to Make Biodiversity Functional." *Frontiers in Ecology and the Environment* 6(8): 430–38.

Li, Sheng Jin et al. 2014. "Microbial Communities Evolve Faster in Extreme Environments." *Scientific Reports* 4: 1–9.

Li, Yuanyuan, Hongyu Wen, Longqian Chen, and Tingting Yin. 2014. "Succession of Bacterial Community Structure and Diversity in Soil along a Chronosequence of Reclamation and Re-Vegetation on Coal Mine Spoils in China." *PLoS ONE* 9(12).

de Lima Procópio, Rudi Emerson et al. 2012. "Antibiotics Produced by *Streptomyces*." *Brazilian Journal of Infectious Diseases* 16(5): 466–71. http://dx.doi.org/10.1016/j.bjid.2012.08.014.

Lin, Huang, and Shyamal Das Peddada. 2020. "Analysis of Compositions of Microbiomes with Bias Correction." *Nature Communications* 11(1): 1–11. http://dx.doi.org/10.1038/s41467-020-17041-7.

Lipiec, J., C. Doussan, A. Nosalewicz, and K. Kondracka. 2013. "Effect of Drought and Heat Stresses on Plant Growth and Yield: A Review." *International Agrophysics* 27(4): 463–77.

Liu, Lan, Kai Zhu, Nina Wurzburger, and Jian Zhang. 2020. "Relationships between Plant Diversity and Soil Microbial Diversity Vary across Taxonomic Groups and Spatial Scales." *Ecosphere* 11(1).

Liu, Yonghong, Wenting Liu, and Zongsuo Liang. 2015. "Endophytic Bacteria from Pinellia Ternata, a New Source of Purine Alkaloids and Bacterial Manure." *Pharmaceutical Biology* 53(10): 1545–48.

Liu, Zidi et al. 2023. "Abiotic Factors and Endophytes Co-Regulate Flavone and Terpenoid Glycoside Metabolism in Glycyrrhiza Uralensis." *Applied Microbiology and Biotechnology* 107(7–8): 2671–88. https://doi.org/10.1007/s00253-023-12441-3.

Lively, Curtis M. 2010. "The Effect of Host Genetic Diversity on Disease Spread." *American Naturalist* 175(6): 2–5.

Long, Hoang Hoa, Dominik D. Schmidt, and Ian T. Baldwin. 2008. "Native Bacterial Endophytes

Promote Host Growth in a Species-Specific Manner; Phytohormone Manipulations Do Not Result in Common Growth Responses." *PLoS ONE* 3(7).

Loreau, M. et al. 2001. "Ecology: Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges." *Science* 294(5543): 804–8.

Loreau, Michel, and Claire de Mazancourt. 2013. "Biodiversity and Ecosystem Stability: A Synthesis of Underlying Mechanisms." *Ecology Letters* 16(SUPPL.1): 106–15.

Ludwig-Müller, Jutta. 2011. "Auxin Conjugates: Their Role for Plant Development and in the Evolution of Land Plants." *Journal of Experimental Botany* 62(6): 1757–73.

Ludwig, B. et al. 2011. "Effects of Fertilization and Soil Management on Crop Yields and Carbon Stabilization in Soils. A Review." *Agronomy for Sustainable Development* 31(2): 361–72.

Lueders, Tillmann, Mike Manefield, and Michael W. Friedrich. 2004. "Enhanced Sensitivity of DNA- and RRNA-Based Stable Isotope Probing by Fractionation and Quantitative Analysis of Isopycnic Centrifugation Gradients." *Environmental Microbiology* 6(1): 73–78.

Manzoni, S., and G. Katul. 2014. "Invariant Soil Water Potential at Zero Microbial Respiration Explained by Hydrological Discontinuity in Dry Soils." *Geophysical Research Letters* 41(20): 7151–58.

Manzoni, S., F. Moyano, T. Kätterer, and J. Schimel. 2016. "Modeling Coupled Enzymatic and Solute Transport Controls on Decomposition in Drying Soils." *Soil Biology and Biochemistry* 95(January): 275–87.

Marhuenda-muñoz, Mar et al. 2019. "Microbial Phenolic Metabolites : WWhich Molecules Actually Have an Effect on Human Health?" *Nutrients* 11: 1–15.

Maron, Pierre Alain et al. 2018. "High Microbial Diversity Promotes Soil Ecosystem Functioning." *Applied and Environmental Microbiology* 84(9).

Mauch, Emanuel, Lucas Serra Moncadas, and Adrian-Stefan Andrei. 2022. "Complete Genome Sequence of an Uncultivated Freshwater Bacteroidota Lineage." *Microbiology Resource Announcements* 11(10): 7–8.

McDonald-Madden, Eve, Michael C. Runge, Hugh P. Possingham, and Tara G. Martin. 2011. "Optimal Timing for Managed Relocation of Species Faced with Climate Change." *Nature Climate Change* 1(5): 261–65. http://dx.doi.org/10.1038/nclimate1170.

McMurdie, Paul J., and Susan Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS ONE* 8(4).

Mead, A. 1992. "Review of the Development of Multidimensional Scaling Methods." *The Statistician* 41(1): 27.

Mellado-Vázquez, Perla Griselle et al. 2016. "Plant Diversity Generates Enhanced Soil Microbial Access to Recently Photosynthesized Carbon in the Rhizosphere." *Soil Biology and Biochemistry* 94: 122–32.

Miliute, Inga, Odeta Buzaite, Danas Baniulis, and Vidmantas Stanys. 2015. "Bacterial Endophytes in Agricultural Crops and Their Role in Stress Tolerance: A Review." *Zemdirbyste* 102(4): 465–78.

Mitchell, Ruth J., Martin H.D. Auld, Michael G. Le Duc, and Marrs H. Robert. 2000. "Ecosystem Stability and Resilience: A Review of Their Relevance for the Conservation Management of Lowland Heaths." *Perspectives in Plant Ecology, Evolution and Systematics* 3(2): 142–60.

Moreno-Mateos, David et al. 2017. “Anthropogenic Ecosystem Disturbance and the Recovery Debt.” *Nature Communications* 8: 8–13.

Morris, J. Jeffrey, Richard E. Lenski, and Erik R. Zinser. 2012. “The Black Queen Hypothesis: Evolution of Dependencies through Adaptive Gene Loss.” *mBio* 3(2).

El Moujahid, Lamiae et al. 2017. “Effect of Plant Diversity on the Diversity of Soil Organic Compounds.” *PLoS ONE* 12(2): 1–16.

Mraja, Anne et al. 2011. “Plant Community Diversity Influences Allocation to Direct Chemical Defence in Plantago Lanceolata.” *PLoS ONE* 6(12).

Nakagawa, Tatsunori, and Reiji Takahashi. 2015. “Nitrosomonas Stercoris Sp. Nov., a Chemoautotrophic Ammonia-Oxidizing Bacterium Tolerant of High Ammonium Isolated from Composted Cattle Manure.” *Microbes and Environments* 30(3): 221–27.

Nanda, Sonil, Sivamohan N. Reddy, Sushanta K. Mitra, and Janusz A. Kozinski. 2016. “The Progressive Routes for Carbon Capture and Sequestration.” *Energy Science and Engineering* 4(2): 99–122.

Naylor, Dan, Stephanie Degraaf, Elizabeth Purdom, and Devin Coleman-derr. 2017. “Drought and Host Selection Influence Bacterial Community Dynamics in the Grass Root Microbiome.” 11(12): 2691–2704. http://dx.doi.org/10.1038/ismej.2017.118.

Nelson, Eric B. 2018. “The Seed Microbiome: Origins, Interactions, and Impacts.” *Plant and Soil* 422(1–2): 7–34.

Nemecek, Thomas et al. 2012. “Estimation of the Variability in Global Warming Potential of Worldwide Crop Production Using a Modular Extrapolation Approach.” *Journal of Cleaner Production* 31: 106–17. http://dx.doi.org/10.1016/j.jclepro.2012.03.005.

Nemergut, Diana R. et al. 2013. “Patterns and Processes of Microbial Community Assembly.” *Microbiology and Molecular Biology Reviews* 77(3): 342–56.

Nicol, Graeme W., L. Anne Glover, and James I. Prosser. 2003. “Molecular Analysis of Methanogenic Archaeal Communities in Managed and Natural Upland Pasture Soils.” *Global Change Biology* 9(10): 1451–57.

Nishihara, H., Y. Igarashi, and T. Kodama. 1991. “Hydrogenovibrio Marinus Gen. Nov., Sp. Nov., a Marine Obligately Chemolithoautotrophic Hydrogen-Oxidizing Bacterium.” *International Journal of Systematic Bacteriology* 41(1): 130–33.

Oksanen, r Jari et al. 2022. “Title Community Ecology Package.” *Vegan: Community Ecol Package*.

Oldroyd, Giles E.D. 2013. “Speak, Friend, and Enter: Signalling Systems That Promote Beneficial Symbiotic Associations in Plants.” *Nature Reviews Microbiology* 11(4): 252–63.

Osborne, Tom, Gillian Rose, and Tim Wheeler. 2013. “Variation in the Global-Scale Impacts of Climate Change on Crop Productivity Due to Climate Model Uncertainty and Adaptation.” *Agricultural and Forest Meteorology* 170: 183–94. http://dx.doi.org/10.1016/j.agrformet.2012.07.006.

Ostad-Ali-Askari, Kaveh, Hossein Ghorbanizadeh Kharazi, Mohammad Shayannejad, and Mohammad Javad Zareian. 2020. “Effect of Climate Change on Precipitation Patterns in an Arid Region Using GCM Models: Case Study of Isfahan-Borkhar Plain.” *Natural Hazards Review* 21(2): 04020006.

Ostfeld, Richard S., and Felicia Keesing. 2000. “Biodiversity and Disease Risk: The Case of Lyme Disease.” *Conservation Biology* 14(3): 722–28.

Oteino, Nicholas et al. 2015. “Plant Growth Promotion Induced by Phosphate Solubilizing Endophytic Pseudomonas Isolates.” *Frontiers in Microbiology* 6(JUL): 1–9.

Ouzari, Hadda et al. 2008. “Diversity of Auxin-Producing Bacteria Associated to Pseudomonas Savastanoi-Induced Olive Knots.” *Journal of Basic Microbiology* 48(5): 370–77.

Parada, Alma E., David M. Needham, and Jed A. Fuhrman. 2016. “Every Base Matters: Assessing Small Subunit RRNA Primers for Marine Microbiomes with Mock Communities, Time Series and Global Field Samples.” *Environmental Microbiology* 18(5): 1403–14.

Park, Inmyoung, Young Su Seo, and Mohamed Mannaa. 2023. “Recruitment of the Rhizo-Microbiome Army: Assembly Determinants and Engineering of the Rhizosphere Microbiome as a Key to Unlocking Plant Potential.” *Frontiers in Microbiology* 14.

Pascale, Alberto, Silvia Proietti, Iakovos S. Pantelides, and Ioannis A. Stringlis. 2020. “Modulation of the Root Microbiome by Plant Molecules: The Basis for Targeted Disease Suppression and Plant Growth Promotion.” *Frontiers in Plant Science* 10(January): 1–23.

Pavoine, S., and M. B. Bonsall. 2011. “Measuring Biodiversity to Explain Community Assembly: A Unified Approach.” *Biological Reviews* 86(4): 792–812.

Pérez Castro, Sherlynette et al. 2019. “Soil Microbial Responses to Drought and Exotic Plants Shift Carbon Metabolism.” *ISME Journal* 13(7): 1776–87. http://dx.doi.org/10.1038/s41396-019-0389-9.

Pons, Simon et al. 2020. “Phytohormone Production by the Arbuscular Mycorrhizal Fungus Rhizophagus Irregularis.” *PLoS ONE* 15(10 October): 1–18.

Porter, Stephanie S. et al. 2020. “Beneficial Microbes Ameliorate Abiotic and Biotic Sources of Stress on Plants.” *Functional Ecology* 34(10): 2075–86.

Potts, Malcolm. 2001. “Desiccation Tolerance: A Simple Process?” *Trends in Microbiology* 9(11): 553–59.

Powlson, D. S. 1993. “Understanding the Soil Nitrogen Cycle.” *Soil Use and Management* 9(3): 86–93.

Preece, Catherine et al. 2019. “Effects of Past and Current Drought on the Composition and Diversity of Soil Microbial Communities.” *Soil Biology and Biochemistry* 131: 28–39. https://doi.org/10.1016/j.soilbio.2018.12.022.

Preece, Catherine, Gerard Farré-Armengol, and Josep Peñuelas. 2020. “Drought Is a Stronger Driver of Soil Respiration and Microbial Communities than Nitrogen or Phosphorus Addition in Two Mediterranean Tree Species.” *Science of the Total Environment* 735: 139554. https://doi.org/10.1016/j.scitotenv.2020.139554.

Price, Samantha A., and John L. Gittleman. 2007. “Hunting to Extinction: Biology and Regional Economy Influence Extinction Risk and the Impact of Hunting in Artiodactyls.” *Proceedings of the Royal Society B: Biological Sciences* 274(1620): 1845–51.

Prober, Suzanne M. et al. 2015. “Plant Diversity Predicts Beta but Not Alpha Diversity of Soil Microbes across Grasslands Worldwide.” *Ecology Letters* 18(1): 85–95.

Pugh, Brittany E. et al. 2022. “A Possible Role for River Restoration Enhancing Biodiversity through Interaction with Wildfire.” *Global Ecology and Biogeography* 31(10): 1990–2004.

Quast, Christian et al. 2013. “The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools.” *Nucleic Acids Research* 41(D1): 590–96.

Rashid, Muhammad Imtiaz et al. 2016. “Bacteria and Fungi Can Contribute to Nutrients Bioavailability and Aggregate Formation in Degraded Soils.” *Microbiological Research* 183: 26–41. http://dx.doi.org/10.1016/j.micres.2015.11.007.

Reed, David H., and Richard Frankham. 2003. “Correlation between Fitness and Genetic Diversity.” *Conservation Biology* 17(1): 230–37.

Renard, Delphine, Lucie Mahaut, and Frederik Noack. 2023. “Crop Diversity Buffers the Impact of Droughts and High Temperatures on Food Production.” *Environmental Research Letters* 18(4).

Roscher, Christiane et al. 2004. “The Role of Biodiversity for Element Cycling and Trophic Interactions: An Experimental Approach in a Grassland Community.” *Basic and Applied Ecology* 5(2): 107–21.

———. 2011. “Plant Resource-Use Characteristics as Predictors for Species Contribution to Community Biomass in Experimental Grasslands.” *Perspectives in Plant Ecology, Evolution and Systematics* 13(1): 1–13.

Roscher, Christiane, Bernhard Schmid, and Ernst Detlef Schulze. 2009. “Non-Random Recruitment of Invader Species in Experimental Grasslands.” *Oikos* 118(10): 1524–40.

Saeid, Agnieszka, Ewelina Prochownik, and Justyna Dobrowolska-Iwanek. 2018. “Phosphorus Solubilization by Bacillus Species.” *Molecules* 23(11): 1–18.

Salam, Md Tareq Bin, and Ryota Kataoka. 2023. “Changes in the Endophytic Bacterial Community of Brassica Rapa after Application of Systemic Insecticides.” *International Journal of Molecular Sciences* 24(20).

Santos, Mariana Sanches, Marco Antonio Nogueira, and Mariangela Hungria. 2019. “Microbial Inoculants: Reviewing the Past, Discussing the Present and Previewing an Outstanding Future for the Use of Beneficial Bacteria in Agriculture.” *AMB Express* 9(1). https://doi.org/10.1186/s13568-019-0932-0.

Santoyo, Gustavo. 2022. “How Plants Recruit Their Microbiome? New Insights into Beneficial Interactions.” *Journal of Advanced Research* 40: 45–58. https://doi.org/10.1016/j.jare.2021.11.020.

Sapoukhina, Natalia, Yuri Tyutyunov, Ivan Sache, and Roger Arditi. 2010. “Spatially Mixed Crops to Control the Stratified Dispersal of Airborne Fungal Diseases.” *Ecological Modelling* 221(23): 2793–2800. http://dx.doi.org/10.1016/j.ecolmodel.2010.08.020.

Sardans, Jordi, and Josep Pen. 2008. “Mediterranean Forest.” 54(5): 513–22.

Scherber, Christoph et al. 2010. “Bottom-up Effects of Plant Diversity on Multitrophic Interactions in a Biodiversity Experiment.” *Nature* 468(7323): 553–56.

Schimel, Joshua, Teri Balser, and Matthew Wallenstein. 2007. “Microbial Stress-Response Physiology and Its Implications.” *Ecology* 88(6): 1386–94.

Schimel, Joshua P. 2018. “Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes.” *Annual Review of Ecology, Evolution, and Systematics* 49(August): 409–32.

Schimel, Joshua P., and Sean M. Schaeffer. 2012. “Microbial Control over Carbon Cycling in Soil.” *Frontiers in Microbiology* 3(SEP): 1–11.

Schmid, B. et al. 2004. “The Role of Biodiversity for Element Cycling and Trophic Interactions: An Experimental Approach in a Grassland Community.” *Basic and Applied Ecology* 5(2): 107–21.

Schmid, Marc W. et al. 2021. “Effects of Plant Community History, Soil Legacy and Plant Diversity on Soil Microbial Communities.” *Journal of Ecology* 109(8): 3007–23.

Schöler, Anne et al. 2017. “Analysis of Soil Microbial Communities Based on Amplicon Sequencing of Marker Genes.” *Biology and Fertility of Soils* 53(5): 485–89.

Schubert, Mikkel, Stinus Lindgreen, and Ludovic Orlando. 2016. “AdapterRemoval v2: Rapid Adapter Trimming, Identification, and Read Merging.” *BMC Research Notes* 9(1): 1–7.

Schultz, J. E., and P. F. Weaver. 1982. “Fermentation and Anaerobic Respiration by Rhodospirillum Rubrum and Rhodopseudomonas Capsulata.” *Journal of Bacteriology* 149(1): 181–90.

Schulz, S. et al. 2013. “Diversity Pattern of Nitrogen Fixing Microbes in Nodules of Trifolium Arvense (L.) at Different Initial Stages of Ecosystem Development.” *Biogeosciences* 10(2): 1183–92.

Sohoulande Djebou, Dagbegnon C., and Vijay P. Singh. 2016. “Impact of Climate Change on Precipitation Patterns: A Comparative Approach.” *International Journal of Climatology* 36(10): 3588–3606.

Somers, E. et al. 2005. “Azospirillum Brasilense Produces the Auxin-like Phenylacetic Acid by Using the Key Enzyme for Indole-3-Acetic Acid Biosynthesis.” *Applied and Environmental Microbiology* 71(4): 1803–10.

de Souza, Rocheli, Adriana Ambrosini, and Luciane M.P. Passaglia. 2015. “Plant Growth-Promoting Bacteria as Inoculants in Agricultural Soils.” *Genetics and Molecular Biology* 38(4): 401–19.

Spaepen, Stijn, and Jos Vanderleyden. 2011. “Auxin and Plant-Microbe Interactions.” : 1–14.

Spain, Anne M., Lee R. Krumholz, and Mostafa S. Elshahed. 2009. “Abundance, Composition, Diversity and Novelty of Soil Proteobacteria.” *ISME Journal* 3(8): 992–1000.

Spohn, Marie et al. 2023. “The Positive Effect of Plant Diversity on Soil Carbon Depends on Climate.” *Nature Communications* 14(1): 1–10.

Stegen, James C, Xueju Lin, Allan E Konopka, and James K Fredrickson. 2012. “Stochastic and Deterministic Assembly Processes in Subsurface Microbial Communities.” 6(9): 1653–64. http://dx.doi.org/10.1038/ismej.2012.22.

Steinauer, Katja et al. 2016. “Convergence of Soil Microbial Properties after Plant Colonization of an Experimental Plant Diversity Gradient.” *BMC Ecology* 16(1): 1–9.

Strecker, Tanja, Odette González Macé, Stefan Scheu, and Nico Eisenhauer. 2016. “Functional Composition of Plant Communities Determines the Spatial and Temporal Stability of Soil Microbial Properties in a Long-Term Plant Diversity Experiment.” *Oikos* 125(12): 1743–54.

Taketani, Rodrigo Gouvêa et al. 2017. “Dry Season Constrains Bacterial Phylogenetic Diversity in a Semi-Arid Rhizosphere System.” *Microbial Ecology*: 153–61. http://dx.doi.org/10.1007/s00248-016-0835-4.

Tardy, Vincent et al. 2014. “Stability of Soil Microbial Structure and Activity Depends on Microbial Diversity.” *Environmental Microbiology Reports* 6(2): 173–83.

Thompson, Janelle R., Hanny E. Rivera, Collin J. Closek, and Mónica Medina. 2014. “Microbes in the Coral Holobiont: Partners through Evolution, Development, and Ecological Interactions.” *Frontiers in Cellular and Infection Microbiology* 4(DEC): 1–20.

Tilman, David. 1996. “Biodiversity: Population versus Ecosystem Stability.” *Ecology* 77(2): 350–63.

Tilman, David, and John A. Downing. 1994. “Biodiversity and Stability in Grasslands.” *Nature*

367(6461): 363–65.

Tilman, David, Forest Isbell, and Jane M. Cowles. 2014. “Biodiversity and Ecosystem Functioning.” *Annual Review of Ecology, Evolution, and Systematics* 45: 471–93.

Torsvik, V., J. Goksoyr, and F. L. Daae. 1990. “High Diversity in DNA of Soil Bacteria.” *Applied and Environmental Microbiology* 56(3): 782–87.

Tóth, Z. et al. 2017. “Extreme Effects of Drought on Composition of the Soil Bacterial Community and Decomposition of Plant Tissue.” *European Journal of Soil Science* 68(4): 504–13.

Treves, D. S., B. Xia, J. Zhou, and J. M. Tiedje. 2003. “A Two-Species Test of the Hypothesis That Spatial Isolation Influences Microbial Diversity in Soil.” *Microbial Ecology* 45(1): 20–28.

Trimble, Stanley W. 2020. “Surface Water: Pollution by Nitrogen Fertilizers.” *Encyclopedia of Water Science, Second Edition* (January 2003): 1185–90.

Trivedi, Pankaj, Bruna D. Batista, Kathryn E. Bazany, and Brajesh K. Singh. 2022. “Plant–Microbiome Interactions under a Changing World: Responses, Consequences and Perspectives.” *New Phytologist* 234(6): 1951–59.

Tucker, Caroline M. et al. 2017. “A Guide to Phylogenetic Metrics for Conservation, Community Ecology and Macroecology.” *Biological Reviews* 92(2): 698–715.

Tyc, Olaf et al. 2020. “The Ecological Role of Bacterial Seed Endophytes Associated with Wild Cabbage in the United Kingdom.” *MicrobiologyOpen* 9(1): 1–19.

Ungar Irwin A. 1987. “Population Ecology of Halophyte Seeds.” *The Botanical Review* 53(3): 301–34.

V., Anju M., Rekha R. Warrier, and C. Kunhikannan. 2022. “Significance of Soil Seed Bank in Forest Vegetation—A Review.” *Seeds* 1(3): 181–97.

Valdés, Jorge et al. 2008. “Acidithiobacillus Ferrooxidans Metabolism: From Genome Sequence to Industrial Applications.” *BMC Genomics* 9: 1–24.

Valletta, Alessio, Lorenzo Maria Iozia, and Francesca Leonelli. 2021. “Impact of Environmental Factors on Stilbene Biosynthesis.” *Plants* 10(1): 1–40.

Vives-Peris, Vicente, Carlos de Ollas, Aurelio Gómez-Cadenas, and Rosa María Pérez-Clemente. 2020. “Root Exudates: From Plant to Rhizosphere and Beyond.” *Plant Cell Reports* 39(1): 3–17. https://doi.org/10.1007/s00299-019-02447-5.

Vogel, Anja, Thomas Fester, et al. 2013. “Separating Drought Effects from Roof Artifacts on Ecosystem Processes in a Grassland Drought Experiment.” *PLoS ONE* 8(8).

Vogel, Anja, Nico Eisenhauer, Alexandra Weigelt, and Michael Scherer-Lorenzen. 2013. “Plant Diversity Does Not Buffer Drought Effects on Early-Stage Litter Mass Loss Rates and Microbial Properties.” *Global Change Biology* 19(9): 2795–2803.

Vogel, Anja, Michael Scherer-Lorenzen, and Alexandra Weigelt. 2012. “Grassland Resistance and Resilience after Drought Depends on Management Intensity and Species Richness.” *PLoS ONE* 7(5).

De Vrieze, Jo et al. 2020. “Stochasticity in Microbiology: Managing Unpredictability to Reach the Sustainable Development Goals.” *Microbial Biotechnology* 13(4): 829–43.

Wagg, Cameron et al. 2017. “Plant Diversity Maintains Long-Term Ecosystem Productivity under Frequent Drought by Increasing Short-Term Variation.” *Ecology* 98(11): 2952–61.

———. 2022. “Biodiversity–Stability Relationships Strengthen over Time in a Long-Term Grassland Experiment.” *Nature Communications* 13(1): 1–11.

Wagi, Shabana, and Ambreen Ahmed. 2019. “Bacillus Spp.: Potent Microfactories of Bacterial IAA.” *PeerJ* 2019(7).

Walsh, Corinne M., Isadore Becker-Uncapher, Madeline Carlson, and Noah Fierer. 2021. “Variable Influences of Soil and Seed-Associated Bacterial Communities on the Assembly of Seedling Microbiomes.” *ISME Journal* 15(9): 2748–62. http://dx.doi.org/10.1038/s41396-021-00967-1.

Wan, Qian et al. 2023. “Different and Unified Responses of Soil Bacterial and Fungal Community Composition and Predicted Functional Potential to 3 Years’ Drought Stress in a Semiarid Alpine Grassland.” *Frontiers in Microbiology* 14(March): 1–14.

Wan, Xiaohua, Xinli Chen, Zhiqun Huang, and Han Y.H. Chen. 2021. “Contribution of Root Traits to Variations in Soil Microbial Biomass and Community Composition.” *Plant and Soil* 460(1–2): 483–95.

Wang, Guangzhou et al. 2019. “Soil Microbiome Mediates Positive Plant Diversity-Productivity Relationships in Late Successional Grassland Species.” *Ecology Letters* 22(8): 1221–32.

Wang, Jichen et al. 2021. “Arbuscular Mycorrhizal Fungi and Plant Diversity Drive Restoration of Nitrogen-Cycling Microbial Communities.” *Molecular Ecology* 30(16): 4133–46.

Wang, Xiao-yan et al. 2021. “Evenness Alters the Positive Effect of Species Richness on Community Drought Resistance via Changing Complementarity.” *Ecological Indicators* 133: 108464. https://doi.org/10.1016/j.ecolind.2021.108464.

Wang, Zhipeng et al. 2020. “Sensitivity of Terrestrial Carbon Cycle to Changes in Precipitation Regimes.” *Ecological Indicators* 113(February): 106223. https://doi.org/10.1016/j.ecolind.2020.106223.

Wang, Zhonghua et al. 2022. “Screening of Phosphate-Solubilizing Bacteria and Their Abilities of Phosphorus Solubilization and Wheat Growth Promotion.” *BMC Microbiology* 22(1): 1–15. https://doi.org/10.1186/s12866-022-02715-7.

Wardle, David A. 2006. “The Influence of Biotic Interactions on Soil Biodiversity.” *Ecology Letters* 9(7): 870–86.

Waters, Colin N. et al. 2016. “The Anthropocene Is Functionally and Stratigraphically Distinct from the Holocene.” *Science* 351(6269).

Weisser, Wolfgang W. et al. 2017. “Biodiversity Effects on Ecosystem Functioning in a 15-Year Grassland Experiment: Patterns, Mechanisms, and Open Questions.” *Basic and Applied Ecology* 23: 1–73.

Whittaker, R. H. 1960. “Vegetation of the Siskiyou Mountains , Oregon and California.” *Ecological Monographs* 30(3): 279–338.

Wickham, Hadley et al. 2019. “Welcome to the Tidyverse.” *Journal of Open Source Software* 4(43): 1686.

Willis, Amy D., and Bryan D. Martin. 2022. “Estimating Diversity in Networked Ecological Communities.” *Biostatistics* 23(1): 207–22.

Woodwell, George M, and Robert H Whittaker. 1968. “Primary Production in Terrestrial Ecosystems Used in Maintaining the Plant — Energy " Burned " in Respiration . The Relationship Applies to Any Green Plant or Any Commu- Nity of Green Plants and Can Be Summarized Most Simply by

This Formula : Where NPP Z .” 30: 19–30.

Wright, Alexandra J. et al. 2015. “Flooding Disturbances Increase Resource Availability and Productivity but Reduce Stability in Diverse Plant Communities.” *Nature Communications* 6: 1–6.

Wrighton, Kelly C. et al. 2008. “A Novel Ecological Role of the Firmicutes Identified in Thermophilic Microbial Fuel Cells.” *ISME Journal* 2(11): 1146–56.

Wu, Wei et al. 2021. “Beneficial Relationships Between Endophytic Bacteria and Medicinal Plants.” *Frontiers in Plant Science* 12(April): 1–13.

Yachi, Shigeo, and Michel Loreau. 1999. “Biodiversity and Ecosystem Productivity in a Fluctuating Environment: The Insurance Hypothesis.” *Proceedings of the National Academy of Sciences of the United States of America* 96(4): 1463–68.

———. 2011. “Biodiversity and Ecosystem Prodi Ictivity in a Fluctuating Environment : The Insurance Hypo Thesis.” *National Academy of Sciences* 96(4): 1463–68.

Yang, Gaowen, Julien Roy, Stavros D. Veresoglou, and Matthias C. Rillig. 2021. “Soil Biodiversity Enhances the Persistence of Legumes under Climate Change.” *New Phytologist* 229(5): 2945–56.

Yang, Yi, David Tilman, George Furey, and Clarence Lehman. 2019. “Soil Carbon Sequestration Accelerated by Restoration of Grassland Biodiversity.” *Nature Communications* 10(1): 1–7. http://dx.doi.org/10.1038/s41467-019-08636-w.

Zeigler, Daniel R. 2014. “The *Geobacillus* Paradox: Why Is a Thermophilic Bacterial Genus so Prevalent on a Mesophilic Planet?” *Microbiology (United Kingdom)* 160(PART 1): 1–11.

Zeppel, M. J.B., J. V. Wilks, and J. D. Lewis. 2014. “Impacts of Extreme Precipitation and Seasonal Changes in Precipitation on Plants.” *Biogeosciences* 11(11): 3083–93.

Zhang, Haihan, Ben Ma, Tinglin Huang, and Yinjie Shi. 2021. “Nitrate Reduction by the Aerobic Denitrifying Actinomycete *Streptomyces Sp.* XD-11-6-2: Performance, Metabolic Activity, and Micro-Polluted Water Treatment.” *Bioresource Technology* 326(December 2020): 124779. https://doi.org/10.1016/j.biortech.2021.124779.

Zhu, Feng et al. 2018. “Species-Specific Plant–Soil Feedbacks Alter Herbivore-Induced Gene Expression and Defense Chemistry in Plantago Lanceolata.” *Oecologia* 188(3): 801–11. https://doi.org/10.1007/s00442-018-4245-9.

7.4. Publication II Supplementary Materials

Supplementary Material

The effect of plant species richness on the recovery of bacterial diversity after successive summer drought periods

Yuri Pinheiro [1,2]; Roberto Siani [1,2]; Cynthia Albracht [3,4], Yuanyuan Huang [5,6], Nico Eisenhauer [5,6], Anja Vogel [5,6], Cameron Wagg [7,8]; Michael Schloter [1,2]; Stefanie Schulz [1]

[1] Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany

[2] Technische Universität München, TUM School of Life Science, Chair of Environmental Microbiology, Neuherberg, Germany

[3]Swammerdam Institute of Life Sciences at University of Amsterdam, Amsterdam, the Netherlands

[4]Department Soil Ecology, Helmholtz Centre for Environmental Research – UFZ, Halle (Saale), Germany

[5] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[6] Institute of Biology, Leipzig University, Leipzig, Germany

[7] Department of Geography, Remote Sensing Laboratories, University of Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland

[8] Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 95 Innovation Road, Post Office Box 20280, Fredericton, E3B 4Z7, NB, Canada

Supplementary table S1: Detailed information about the experiment desing. The desing consists of an split plot desing where drought treates samples and control samples were both nested inside the samme plot. Each plot varies in plant species richness (monoculture, 2, 4, 8, 16 and 60 species) and the composition of functional groups (1, 2, 3 and 4 groups divide among grasses, small herbs, tall herbs and legumes).

Sample	Block	Plot	Treatment	Plant Species Richness	N of functional groups	Grasses	Small Herbs	Tall Herbs	Legumes
1	B4	22	Drought	4	1	0	0	4	0
2	B3	9	Control	16	1	16	0	0	0
3	B3	2	Drought	2	2	1	0	1	0
4	B3	2	Control	2	2	1	0	1	0
5	B3	17	Drought	1	1	0	1	0	0
6	B3	3	Control	4	3	1	1	0	2
7	B3	6	Control	1	1	1	0	0	0
8	B2	17	Control	8	2	0	4	0	4
9	B3	8	Control	2	1	2	0	0	0
10	B2	20	Drought	2	2	0	1	0	1
11	B2	21	Control	8	3	0	2	3	3
12	B3	13	Drought	4	1	4	0	0	0
13	B3	4	Control	8	1	8	0	0	0
14	B4	8	Control	8	2	4	4	0	0
15	B4	10	Control	8	3	2	3	3	0
16	B4	10	Drought	8	3	2	3	3	0
17	B3	11	Control	4	2	2	2	0	0
18	B1	4	Drought	4	4	1	1	1	1
19	B4	12	Drought	1	1	1	0	0	0
20	B3	12	Drought	1	1	0	0	0	1
21	B4	15	Control	2	2	0	1	0	1
22	B3	1	Drought	1	1	0	0	1	0
23	B3	5	Control	8	3	3	0	2	3
24	B3	8	Drought	2	1	2	0	0	0
25	B3	14	Drought	60	4	16	12	20	12
26	B3	23	Drought	4	4	1	1	1	1
27	B3	16	Control	16	2	0	8	0	8
28	B3	6	Drought	1	1	1	0	0	0
29	B4	8	Drought	8	2	4	4	0	0
30	B4	11	Control	4	3	1	0	2	1
31	B1	12	Drought	8	1	0	0	0	8
32	B1	5	Control	2	1	0	0	0	2
33	B2	10	Control	16	2	8	8	0	0
34	B4	17	Control	2	1	0	0	2	0
35	B4	1	Control	60	4	16	12	20	12
36	B2	3	Drought	60	4	16	12	20	12
37	B1	2	Drought	8	2	4	0	4	0
38	B4	13	Control	1	1	0	0	1	0
39	B1	8	Control	1	1	0	0	0	1

40	B4	7	Control	4	2	0	0	2	2
41	B4	2	Drought	16	3	5	5	6	0
42	B1	12	Control	8	1	0	0	0	8
43	B1	6	Control	16	2	8	0	8	0
44	B1	11	Drought	16	1	0	0	16	0
45	B2	9	Control	4	1	0	4	0	0
46	B3	19	Drought	2	2	1	1	0	0
47	B2	21	Drought	8	3	0	2	3	3
48	B1	15	Control	1	1	0	0	1	0
49	B2	16	Drought	4	3	0	2	1	1
50	B3	23	Control	4	4	1	1	1	1
51	B4	4	Control	4	4	1	1	1	1
52	B4	9	Control	1	1	0	0	0	1
53	B1	16	Drought	2	2	1	1	0	0
54	B4	14	Control	2	1	0	2	0	0
55	B4	6	Control	8	1	0	8	0	0
56	B1	21	Control	4	2	2	0	2	0
57	B1	16	Control	2	2	1	1	0	0
58	B3	20	Control	8	2	0	0	4	4
59	B1	13	Drought	4	1	0	0	0	4
60	B3	21	Control	2	1	0	0	0	2
61	B3	9	Drought	16	1	16	0	0	0
62	B1	18	Control	1	1	0	1	0	0
63	B4	16	Drought	8	4	2	2	2	2
64	B3	24	Control	16	3	6	5	0	5
65	B3	21	Drought	2	1	0	0	0	2
66	B3	22	Control	16	4	4	4	4	4
67	B2	17	Drought	8	2	0	4	0	4
68	B1	19	Drought	2	3	2	1	1	0
69	B4	16	Control	8	4	2	2	2	2
70	B1	7	Control	2	1	0	0	2	0
71	B1	13	Control	4	1	0	0	0	4
72	B4	18	Control	16	4	4	4	4	4
73	B2	1	Control	4	4	1	1	1	1
74	B4	2	Control	16	3	5	5	6	0
75	B2	5	Drought	1	1	1	0	0	0
76	B1	5	Drought	2	1	0	0	0	2
77	B1	22	Control	60	4	16	12	20	12
78	B3	20	Drought	8	2	0	0	4	4
79	B4	6	Drought	8	1	0	8	0	0
80	B4	1	Drought	60	4	16	12	20	12
81	B2	4	Drought	1	1	0	0	1	0
82	B4	7	Drought	4	2	0	0	2	2
83	B4	13	Drought	1	1	0	0	1	0

84	B2	1	Drought	4	4	1	1	1	1
85	B1	19	Control	2	3	2	1	1	0
86	B3	7	Drought	8	4	2	2	2	2
87	B4	15	Drought	2	2	0	1	0	1
88	B2	13	Drought	1	1	0	1	0	0
89	B4	21	Drought	2	2	0	0	1	1
90	B4	21	Control	2	2	0	0	1	1
91	B2	6	Control	4	2	0	2	0	2
92	B1	8	Drought	1	1	0	0	0	1
93	B3	7	Control	8	4	2	2	2	2
94	B2	6	Drought	4	2	0	2	0	2
95	B1	15	Drought	1	1	0	0	1	0
96	B2	15	Drought	1	1	0	0	0	1
97	B1	11	Control	16	1	0	0	16	0
98	B1	6	Drought	16	2	8	0	8	0
99	B1	17	Drought	2	2	1	0	1	0
100	B1	1	Control	16	4	4	4	4	4
101	B1	17	Control	2	2	1	0	1	0
102	B1	14	Control	8	4	2	2	2	2
103	B2	3	Control	60	4	16	12	20	12
104	B1	7	Drought	2	1	0	0	2	0
105	B2	2	Drought	2	1	2	0	0	0
106	B4	11	Drought	4	3	1	0	2	1
107	B1	1	Drought	16	4	4	4	4	4
108	B2	2	Control	2	1	2	0	0	0
109	B4	4	Drought	4	4	1	1	1	1
110	B2	16	Control	4	3	0	2	1	1
111	B3	14	Control	60	4	16	12	20	12
112	B4	18	Drought	16	4	4	4	4	4
113	B3	11	Drought	4	2	2	2	0	0
114	B2	19	Control	2	1	0	2	0	0
115	B2	9	Drought	4	1	0	4	0	0
116	B2	5	Control	1	1	1	0	0	0
117	B1	4	Control	4	4	1	1	1	1
118	B1	22	Drought	60	4	16	12	20	12
119	B4	20	Control	16	2	0	0	8	8
120	B3	3	Drought	4	3	1	1	0	2
121	B2	22	Drought	16	3	5	0	5	6
122	B3	1	Control	1	1	0	0	1	0
124	B3	5	Drought	8	3	3	0	2	3
125	B3	13	Control	4	1	4	0	0	0
126	B4	14	Drought	2	1	0	2	0	0
127	B2	4	Control	1	1	0	0	1	0
128	B2	18	Control	16	4	4	4	4	4

129	B1	3	Drought	8	3	3	3	0	2
130	B3	12	Control	1	1	0	0	0	1
131	B4	17	Drought	2	1	0	0	2	0
132	B3	17	Control	1	1	0	1	0	0
133	B4	9	Drought	1	1	0	0	0	1
134	B1	2	Control	8	2	4	0	4	0
135	B2	18	Drought	16	4	4	4	4	4
136	B2	10	Drought	16	2	8	8	0	0
137	B3	22	Drought	16	4	4	4	4	4
138	B2	12	Drought	8	1	0	0	8	0
139	B3	16	Drought	16	2	0	8	0	8
140	B3	4	Drought	8	1	8	0	0	0
141	B2	20	Control	2	2	0	1	0	1
142	B3	19	Control	2	2	1	1	0	0
143	B2	19	Drought	2	1	0	2	0	0
144	B2	14	Control	8	4	2	2	2	2
145	B4	20	Drought	16	2	0	0	8	8
146	B2	15	Control	1	1	0	0	0	1
147	B2	8	Drought	2	2	0	0	1	1
148	b2	13	Control	1	1	0	1	0	0
149	B2	22	Control	16	3	5	0	5	6
150	B1	20	Drought	16	3	0	6	5	5
151	B4	22	Control	4	1	0	0	4	0
152	B1	14	Drought	8	4	2	2	2	2
153	B2	12	Control	8	1	0	0	8	0
154	B4	12	Control	1	1	1	0	0	0
155	B1	21	Drought	4	2	2	0	2	0
156	B1	20	Control	16	3	0	6	5	5
157	B1	18	Drought	1	1	0	1	0	0
158	B2	14	Drought	8	4	2	2	2	2
159	B2	8	Control	2	2	0	0	1	1

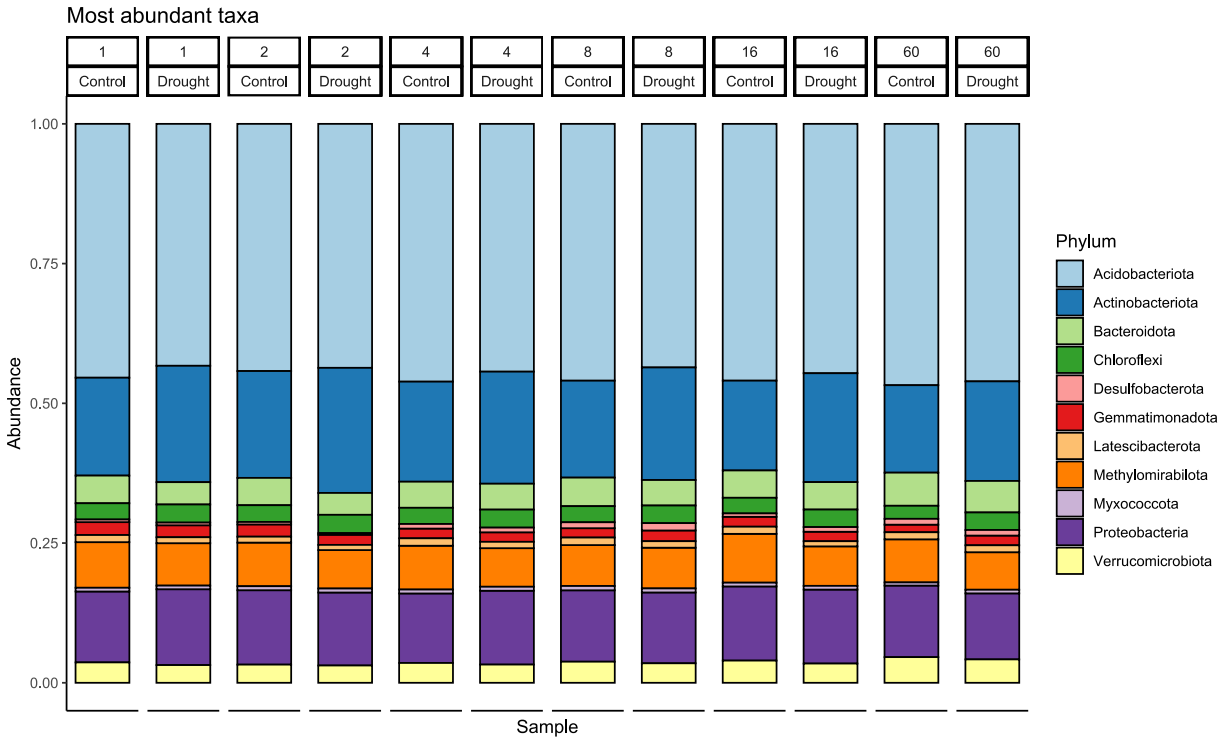
Supplementary table S2: Sumary of the sample destribution along the plant richess gradient. On A the number of functional groups destribute along the plant richness gradient and on B the number of replicates on each treatment along the gradient.

A

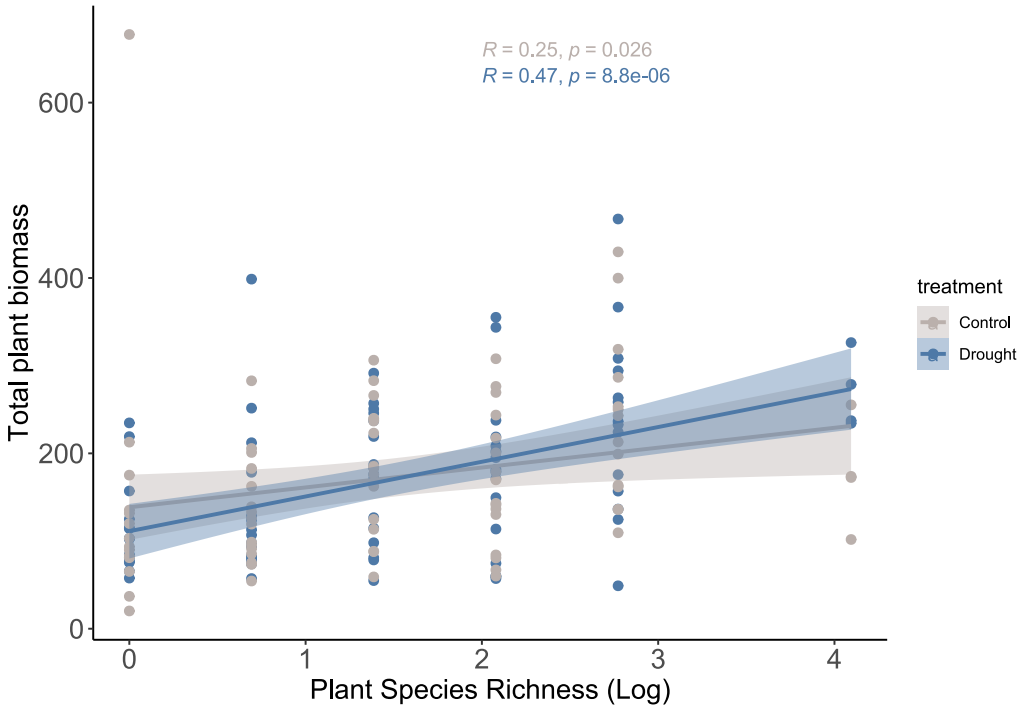
	Number of functional groups			
Plant species richness	1	2	3	4
1	28	0	0	0
2	16	16	0	0
4	8	8	8	8
8	8	8	7	8
16	4	8	7	8
60	0	0	0	8

B

Plant species richness	Drought	Control
1	14	14
2	16	16
4	16	16
8	16	15
16	13	14
60	4	4



Supplementary Figure S1: Bar plot displaying the relative abundance of top 12 taxa at phylum level. Samples were merged at diversity and treatment level. Overall microbial diversity is stable along the diversity levels and treatment, being dominated by Acidobacteria (especially Vicinamibacteraceae family) Actinobacteria, Proteobacteria and Bacteroidota. The taxa with significantly differs between the control and drought plots are displayed on the main text, figure 3.



Supplementary Figure S2: Correlation plot displaying the relationship between plant scpecies richness and total plant biomass on the time of sampling from soil samples (August 2017). We observed a slightly positve corelation between the plant species richness gradient and the plant biomass production, however drought treatment did not impact biomass estimation after 1 year of termination of the drought treatment.

Supplementary table S3: table displaying the result of linear mixed effect models analyzes over total biomass data. We observed highy significant effect of plant richness on the over total biomass productivity while the drought treatment did not show significant effects. The fitting order of the terms in the model was TotalBiomass~Block+DroughtTreatment+log(PlantRichness)

	numDF	denDF	F-value	p-value
(Intercept)	1	131	5.732.677	<.0001
block	3	131	0.3629	0.7799
treatment	1	131	0.0000	10.000
Plant richness	1	131	2.638.730	<.0001

7.5. Publication III

Souza de *et al. Environmental Microbiome* (2024) 19:11
https://doi.org/10.1186/s40793-024-00552-x

BRIEF REPORT Open Access

The seeds of *Plantago lanceolata* comprise a stable core microbiome along a plant richness gradient

Yuri Pinheiro Alves de Souza[1,2], Michael Schloter[1,2*], Wolfgang Weisser[3], Yuanyuan Huang[4,5] and Stefanie Schulz[2]

Abstract

Background Seed endophytic bacteria are beneficial to plants. They improve seedling growth by enhancing plant nutrient uptake, modulating stress-related phytohormone production, and targeting pests and pathogens with antibiotics. Seed endophyte composition can be influenced by pollination, plant cultivar, and soil physicochemical conditions. However, the effects of plant community richness on seed endophytes are unknown. To investigate the effects of increasing plant species richness on the diversity and composition of the seed microbiome, we made use of a well-established long-term biodiversity experiment in Germany (The Jena Experiment). We sampled seeds from different *Plantago lanceolata* blossoms in a plant diversity gradient ranging from monoculture to 16 species mixtures. The seeds were surface sterilized to remove seed surface-associated bacteria and subjected to a metabarcoding approach to assess bacterial community structure.

Results Our data indicate a very stable core microbiome, which accounted for more than 90% of the reads and was present in all seeds independent of the plant richness level the seeds originated from. It consisted mainly of reads linked to *Pseudomonas rhizosphaerae, Sphingomonas faeni* and *Pirellulla spp*. 9% of the obtained reads were not part of the core microbiome and were only present in plots of specific diversity levels. The number of unique ASVs was positively correlated with plant richness. Interestingly, most reads described as non-core members belonged to the same genera described as the core microbiome, indicating the presence of different strains or species with possibly different functional properties important for seed performance.

Conclusion Our data indicate that *Plantago lanceolata* maintains a large seeds core microbiome across the plant richness gradient. However, the number of unique ASVs increases alongside the plant community richness, indicating that ecosystem biodiversity also mitigates diversity loss in seed endophytes.

Keywords *Plantago lanceolata*, Seed microbiome, Plant diversity gradient, Core microbiome

*Correspondence:
Michael Schloter
schloter@tum.de
[1]TUM School of Life Science, Chair of Environmental Microbiology, Technische Universität München, Neuherberg, Germany
[2]Research Unit Comparative Microbiome Analysis, Helmholtz Zentrum München, Neuherberg, Germany
[3]TUM School of Life Science, Chair of Terrestrial Ecology, Technische Universität München, Freising, Germany
[4]German Centre of Integrative Bioaffiliationersity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
[5]Institute of Biology, Experimental Interaction Ecology, Leipzig University, Leipzig, Germany

Background

Seed endophytic microbial communities play an essential role in seed protection and germination [1, 2]. Moreover, the seed microbiome contributes to important responses during seedling development and in early plant life stages, acting as the initial inoculum for the microbiome of the next plant generation and representing a heritage link between the parental plant and its offspring [3]. Changes in the structure and function of the endophytic seed microbiome can critically affect the development of the seedling by modulating growth as well as stress responses of the plant at early stages of life [3]. Especially for domesticated plants a reduction in seed microbiome diversity was observed with negative consequences for plant resistance against pathogen invasion, for example [4].

A study by Bergna et al. [5] indicates that the seed microbiome composition is determined by parental filtering, which selects microbes from the air, water and soil. On the one hand, this filtering results in plant species specific and even genotype specific seed microbiomes as it was shown for rice [6], *Cucurbita pepo* [7] or oilseed rape [8]. On the other hand, environmental conditions modulate the composition of the seed microbiome as well as it was shown for the type and presence of pollinators [9] or differences in soil properties [10]. However, the effect of increasing plant diversity has not been explored so far. Although many studies proved the positive effect of diverse plant communities on several ecosystem functions [11–13]. A similar positive link between plant diversity and seed endophytic microbial diversity can indicate that ecosystem biodiversity can impact not only the endophytic microbiome of the current plant generation but also their offspring, representing an important legacy effect of biodiversity increase [10].

Here, we hypothesize that plant diversity also influences the structure and diversity of the seed bacteria. Therefore, we used *Plantago lanceolata*, a plant that is widely distributed in European grasslands and is often used as a model in ecological studies [12, 14]. We analyzed the seed microbiome of *P. lanceolata* in frame of the setting provided by "The Jena Experiment" [12, 14], one of the largest plant diversity experiments worldwide, and enabled us to compare changes in the seed microbiome in monocultures, 4-, 8- and 16-species plant communities.

Methods

Experimental design and sampling

This experiment was conducted in the frame of "The Jena Experiment" [6, 7 - http://the-jena-experiment.de/], which is running since 2002. The experiment was established in the city of Jena, Germany, next to the Saale River (50°55'43.61"N, 11°35'23.64"E). A pool of 60 regional grassland species was combined to create a diversity gradient, which resulted in 82 plots (20 m × 20 m; 400 m^2). The gradient spans from monoculture plots to 60-species mixtures. For our experiment, we sampled only plots that had *Plantago lanceolata* in their mixture. Thus, seeds from all available monoculture plots (3) and from 4-, 8- and 16-species mixtures were sampled, which were 4, 2 and 3 plots, respectively. Detailed information about plant taxonomical composition of each sampled plot as well as the identity of the direct neighbor of the sampled *P. lanceolata* individuals can be found in Supplementary Table S1. The *P. lanceolata* coverage in the 60-species plots was too low for additional sampling. This summed up to 12 plots, which were sampled in September 2021. The sampling was performed in a nested design, which is depicted in Supplementary Figure S1. Three individual plants were sampled per plot, and three blossoms were sampled per individual. Blossoms were collected using gloves and surface-disinfected scissors. Intact blossoms were stored in sterile 15 ml falcon tubes and transported to the laboratory at room temperature where the seeds were removed from the blossoms. The resulting seeds were surface sterilized as follows: 1 min incubation in 1% Tween; 2 min incubation in 70% ethanol; 3 washes in sterile water; 5 min incubation in 5% sodium hypochlorite solution; and 3 washes in sterile water [15]. The surface-sterilized seeds were stored at -20 °C until further processing.

DNA extraction

Surface-sterilized seeds were ground using sterilized mortars and liquid nitrogen. Per DNA extraction, 50 seeds were used, equaling 97.5 mg of seeds. The number of seeds was estimated based on the weight of 1000 *P. lanceolata* seeds, which is 1.95 g for plants from "The Jena Experiment" field site [16]. For blossoms with a lower amount of seeds, all material was used. Seed DNA was extracted following a phenol/chloroform/isoamyl alcohol-based method [17]. Sample lysis was performed using Lysing Matrix E tubes (MP Biomedicals™, Germany). The beat beating was done using a TissueLyser II bead beater (QIAGEN®, Germany) at a frequency of 15 Hz for 2 min. The resulting DNA was quantified by Qubit fluorometric system (Thermo Fisher Scientific, Germany) using the broad range assay kit. The quality of the DNA was checked using the Nanodrop photometric system (Thermo Fisher Scientific, Germany) and by agarose gel electrophoresis. To exclude contamination during DNA extraction, a blank control without seed material was processed.

Amplicon library preparation and sequencing

We performed metabarcoding targeting the V3 and V4 regions of the 16S rRNA gene using

chloroplast exclusion primers S-D-Bact-0335-a-S-17 (338f -TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCADACTCCTACGGGAGGC) and S-D-Bact-0769-a-A-19 (789r-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCCTGTTTGMTMCCCVCRC) [18] with overhang sequences at the 5′ end compatible with the Nextera® XT Index Kit. The primers used reduce the overamplification of plant derived 16 S rRNA genes including chloroplast and mitochondria sequences, while targeting the same region of the 16 S rRNA gene as the frequently used earth microbiome primers [19, 20] which improves comparability of data sets across different habitats. PCR amplification was performed using 20 ng of template DNA, and negative controls without DNA template were processed alongside. Each PCR consisted of 25 µL containing 12.5 µL NEB Next High-Fidelity Master Mix (Thermo Fisher Scientific, Germany), 0.5 µL of each primer at 10 pmol/µl, 2.5 µL of 3% BSA, 1 µl of 5 ng/µL diluted DNA, and 8 µL of DEPC-treated water. The thermal profile was as follows: 98 °C for 1 min, followed by 35 cycles of 98 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s, and a final extension at 72 °C for 5 min, which was repeated 35 times. Samples were indexed using the Nextera® XT Index Kit v2 (Illumina, USA) and purified with MagSi-NGSprep Plus Beads (ratio 0.8 beads:1 sample) according to the manufacturer's protocol, and quality assessment was performed via the Fragment Analyzer System 5300 (Agilent, Germany). High-quality DNA was diluted to 4 nM and sequenced on an Illumina MiSeq using a MiSeq Reagent v3 (600 Cycle) kit. PhiX (5 pM, 20%) was loaded alongside the samples. Raw sequencing files were uploaded to the NCBI SRA database under the bio project number PRJNA937585 and bio sample SAMN33409010.

Sequence processing

After sequencing, samples were uploaded to the European Galaxy server (https://usegalaxy.eu). The Cutadpat [21] tool was used to remove adapters, and the quality of reads was assessed via FastQC [22]. Forward reads with quality scores below 30 and reverse reads with quality scores below 20 were removed. For further analysis, dada2 version 1.16 [23] was used. The plotQualityProfile option was used to determine the trimming parameters, which were set to 280 bp for the forward reads and 220 bp for the reverse reads. The next steps included the calculation of error rates and sample inference followed by merging reads and removal of chimeric sequences (https://benjjneb.github.io/dada2/tutorial.html). The loss of reads during that process is summarized in Table S1. Taxonomy was assigned using assignTaxonomy and addSpecies function, aligning the amplicon sequence variants (ASVs) against the Silva database [24] version 138.

Statistical analyses

Plots and statistical analysis were conducted in R version 4.2.2 using the packages phyloseq version 1.42.0 [25] and vegan [26] version 4.0.5. Before analysis, all ASVs detected in both extraction and PCR negativecontrols were removed from the dataset. This resulted in 8.772 reads distributed among 129 ASVs. Additionally, reads taxonomically assigned to chloroplast or mitochondria were removed. The sample specific read loss during sample processing is summarized in Table S2. In total, 4007 different ASVs were detected in the full dataset. To estimate whether the sequencing depth of the remaining reads was enough to reach sufficient coverage, rarefaction curves were drawn using the *rarecurve* command in the package Vegan v 2.6.4. Nine samples with fewer than 1000 reads were excluded from further downstream analysis. Afterwards, the blossoms per individual were treated as ps pseudo-replicates per plant individual level. Thus, only consensus sequences detected on all three blossoms per individual were further considered. For the statistical analyses, plant species richness (SR) was treated as continuous variable, and it was log2-transformed to reach linearity. To estimate alpha diversity, the number of observed ASVs was used as a richness estimate and calculated using the *estimate_richness* command in Phyloseq. We fitted the transformed data to a linear mixed-effects model using the lme function of package *lme4* [27] to investigate the effects of plant species richness on the number of observed ASVs (AD). We applied random term corrections to the plot (PL) and individual (IN) levels. The fitting order was m1<-lme(AD ~ log2(SR), random=~1|PL/IN). Beta diversity was calculated by using the Bray–Curtis dissimilarity matrix as input for a PCoA using the *ordinate* command from phyloseq [25]. Significant differences in community composition were tested using PERMANOVA ($p<0.05$). To account for the dependency of samples coming from the same plot we used the *strata* command in the adonis2 package [28] to restrict permutations within the plot (999 permutations). In addition to the sown plant richness gradient, we calculated plant realized diversity by using species-level aboveground plant biomass data from August 2021 [29, 30] to calculate both the Shannon and Simpson diversity indices. Biomass data was obtained by harvesting all aboveground plant materials using a 0.1 m^2 (20×50 cm) frame randomly positioned in each plot. The Shannon and Simpson indices were calculated using the "diversity" function from the vegan package (version 2.6-4). We used the calculated plant diversity to calculate the correlation between the number of observed bacterial ASVs per plot to the actual realized plant diversity in each plot. The core microbiome was calculated using the *trans_venn* function of the package MicroEco [31] v 0.14.2. Venn diagrams were drawn taking into consideration 100%

prevalence, meaning that only ASVs present in all individuals of a given plant richness level were considered.

Results

On average, we obtained 19,625 high-quality reads per sample (Table S2) and reached sufficient coverage of the bacterial richness in the seeds of *P. lanceolata* for all samples, as depicted in Supplementary Figure S2 (Additional file 1).

In line with no significant effects of plant richness on overall seed microbiome diversity (lme; F: 3.13379, p: 0.1071 Fig. 1A), our analysis identified a stable core microbiome of *P. lanceolata* seeds across the plant diversity gradient. The core microbiome contained 91% of all detected reads, explaining the poor separation of samples when ß-diversity was analyzed (PCoA on Bray–Curtis dissimilarity; PERMANOVA: $p=0.401$– Fig. 1C). We observed, however, a slight positive correlation between the number of observed ASVs and the plant richness gradient ($R=0.22$, $p=0.046$ - Fig. 1B), however no correlation was observed when the number of observed ASVs was correlated with realized Shannon and Simpson plant diversity on each plot ($R=0.085$, $p=0.44$ for Shannon realized diversity and $R=0.14$, $p=0.21$ - Supplementary Figure S3, Additional file 1). The core microbiome consisted of 81 ASVs assigned to the genera *Paracoccus*, *Alteribacillus*, *Sphingomonas*, *Pseudomonas*, *Massilia* and *Pirellula* (Fig. 2A). Among those, *Pseudomonas rhizosphaerae* followed by *Sphingomonas faeni* and *Pirellulla spp.* were the most abundant species of the core microbiome (Fig. 2B).

In contrast to the core microbiome, the number of unique ASVs at each plant richness level was positively correlated with plant richness ($R=0.9$, $p=0.00006$, Fig. 2C and D). The identity of unique ASVs of the *P. lanceolata* seed microbiome differed at the different plant richness levels and mostly consisted of rare ASVs. Interestingly, no unique ASVs were found in the seeds of the monoculture plots, while 12 unique ASVs could be detected in seeds from 4 species plots, which further increased to 41 and 55 unique ASVs in samples from 8 to 16 plant species plots, respectively (Fig. 2C). Interestingly, most of the ASVs detected in the core microbiome and among the unique ASVs belonged to the same genera.

Discussion

In the present study, we demonstrate for the first time that the microbiome of *Plantago lanceolata* seeds is highly conserved across the tested plant richness gradient. However, we also observed that the stable core microbiome is extended by additional unique ASVs, whose number was positively correlated with plant richness.

Previous studies reported that seed endophytic bacteria are present in extremely low counts, especially in surface-sterilized seeds [4, 32]. We acknowledged these low biomass properties during our data analysis in two ways. First, we processed blank extraction as well as PCR negative controls and removed all ASVs found in those controls from the samples. Second, to account for the high oscillation in low biomass samples [33, 34], we worked with the consensus community per plant individual, which was achieved by conglomerating the sequencing results of three individual blossoms (biological replicates) per plant as explained in the methods section. This procedure allowed the removal of 90% of the ASVs, which were not consistent within the same individual. Thus, we are confident that the obtained results are robust and do not originate from contamination or random fluctuation.

As result of our study, we identified a large, conserved bacterial core microbiome of *P. lanceolata* seeds across the plant richness gradient. This indicates that *P. lanceolata* is capable of recruiting a very specific set of bacteria from the environmental pool, even though the surrounding plant richness and community composition was not the same. On the one hand, this finding is in line with other studies, which identified large seed core microbiomes of similar plant species. For example, Eyre et al. (2019) [6] described a consistent core microbiome for rice coming from different geographical locations and genotypes. However, rice and other crops are mostly cultivated in monocultures and based on breeding efforts which drive comparable traits, namely productivity. On the other hand, previous studies demonstrated that increasing plant richness significantly changed ecosystem functions and thus the properties of the surrounding habitat. For example, the number and diversity of pollinators increases with plant richness and the soil microbial community composition changes [12]. Both directly change the sources from which the seed microbiome is selected from. However, it might be possible that these effects are bigger for the microbiome colonizing the seed surface [6], which was excluded in our analysis. Most of the detected bacterial taxa have already been described as part of the seed microbiome of other plant species. For example, *Pseudomonas* and *Alternaria* were identified as consistent seed endophytes in a metastudy, which compared 50 different plant species [2]. *Pseudomonas rhizosphaerae* has been previously isolated from vegetal tissue, demonstrating important plant growth-promoting traits such as phosphate solubilization [35], while *Sphingomonas faeni*, originally isolated from indoor dust, belongs to a genus widely known for its phytohormone production [36]. To date, *Pirelulla* has been mostly found in marine samples and has not yet been described in plant tissues [37].

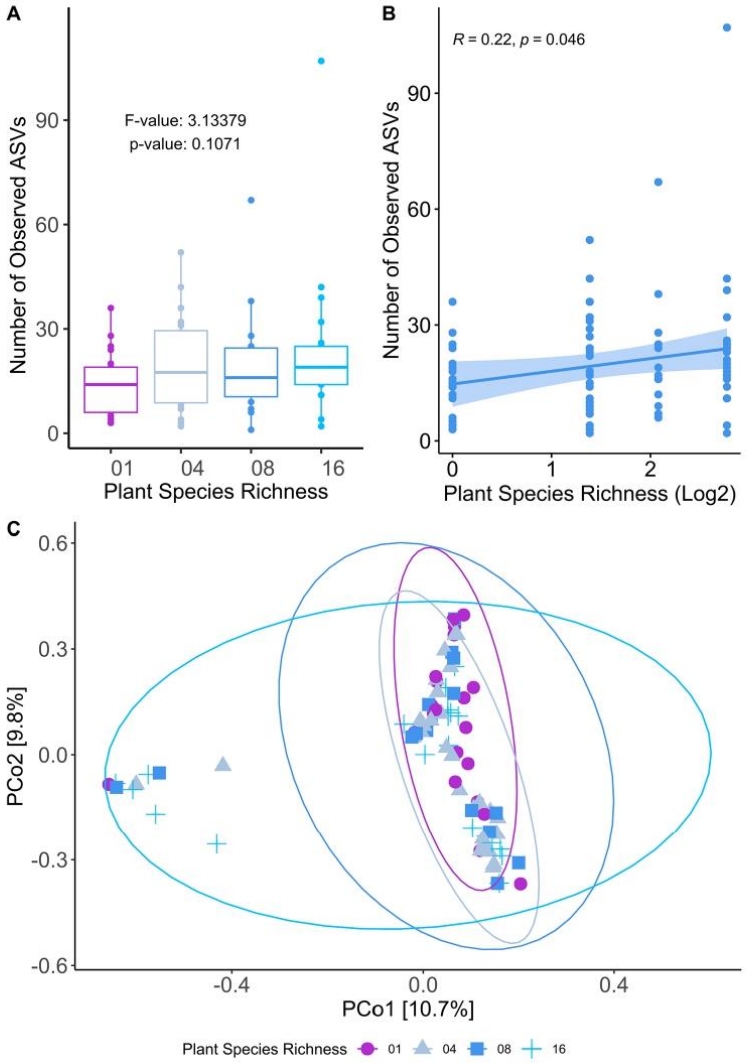


Fig. 1 **(A)** Number of observed ASVs per plant richness level and linear mixed effects models used to investigate the relationship between plant richness and the number of bacterial observed ASVs. A nested design was applied where Plot and Individual were used as random term in the model, correcting for grouping effects. **(B)** Spearman correlation plot between the number of observed ASVs and plant species richness (log2 scale) **(C)** PcoA analyzes built over Bray–Curtis dissimilarity distance

In addition to the core microbiome, the number of unique ASVs of the *P. lanceolata* seed microbiome increased with plant richness, but their identity differed at the different plant richness levels. We attribute the increasing number of additional ASVs to changes in parental filtering processes [10]. Current literature suggests that microbial recruitment of seed endophytes depends on plant topology and phytochemistry [38]. Studies on the plant properties along the investigated plant richness gradient revealed that plant phenotypes, genotypes and chemical defense mechanisms [12, 39, 40] significantly differed in *P. lanceolata* monocultures

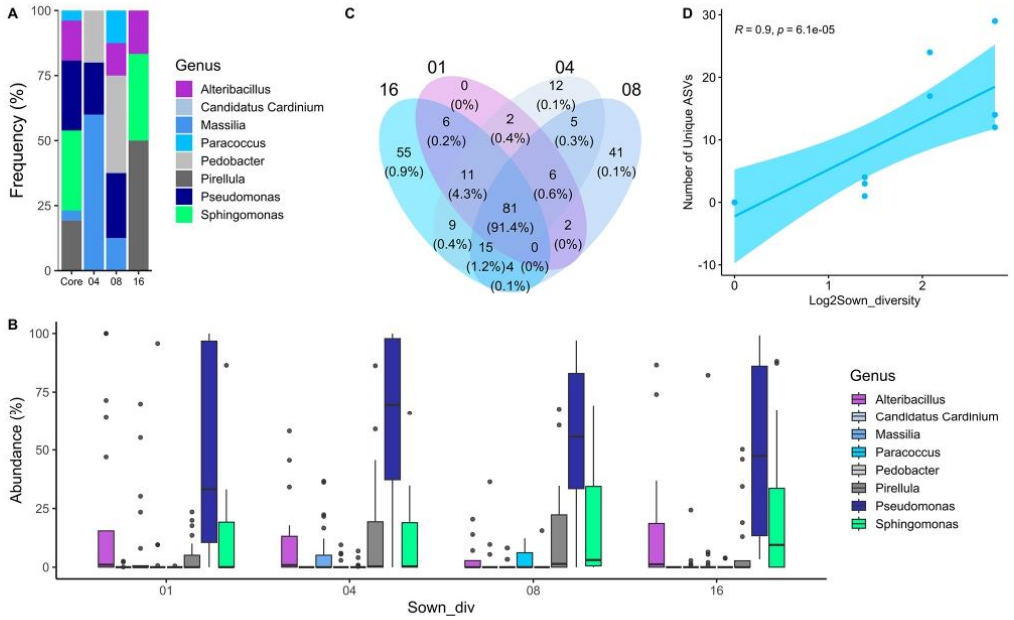


Fig. 2 The seed microbiome of *P. lanceolata* across the different plant diversity levels. **(A)** Frequency and phylogenetic assignment of ASVs belonging to the core microbiome (Core) or the unique taxa of 4-, 8- and 16-species plots. Monoculture plots did not harbor any unique ASV. **(B)** Relative abundance of ASVs being part of the core microbiome in seeds obtained from plants of plots from the different diversity levels (Sown_div). **(C)** Venn diagram displaying the number of shared ASVs among the diversity levels. Numbers outside brackets indicate the absolute number of ASVs shared on that given combination, while numbers inside brackets represent the percentage of reads assigned to those ASVs. Relative abundances below 0.1% are displayed as 0%. **(D)** Spearman correlation plot between the number of unique ASVs per diversity level and plant species diversity displayed as log2sown diversity. In monocultures, the number of unique ASVs is zero

compared to plots with higher plant richness. Thus, the recruitment of additional taxa might be related to changes in plant performance along the plant richness gradient. These differences seemed to induce a stricter selection in plots with low plant richness, which are characterized by a higher pathogen load for example [12]. Similar trends were observed for crop monocultures with long term domestication history [4].The increasing number of unique ASVs at plant species rich plots provides additional complexity of the endophytic seed bacteria along the plant richness gradient. The beneficial effects of increasing diversity on ecosystem stability have been vastly investigated [12, 41, 42]. Biodiversity enhances the stability and resilience of ecosystems by improving the complementary use of resources and functional redundancy [43], which prevents ecosystems to collapse during disturbances [16, 44]. Although the positive feedback of plant richness on the diversity of soil [45] and rhizosphere microbiomes has already been observed [45, 46], this interaction has not been observed for the bacterial seed endophytes. The data indicates that the beneficial effects of mixed plantations can be carried on to the next generation to a certain extent, possibly improving seed defense, seedling germination and overall plant robustness [1, 47].

Interestingly, most of the ASVs detected in the core microbiome and among the unique ASVs belonged to the same genera, indicating a differentiation between core and unique taxa below the genus level. Differences in the manifestation of plant growth promotion traits have been reported at the species or strain level [48], indicating the importance of the non-core ASVs for the plant performance of the next generation. Only ASVs linked to the genus *Pedobacter* were exclusively found in plots with 4 and 8 plant species, apparently being sorted out by parental filtering on the 16 plant species level plot. *Pedobacter* has been described as widely ubiquitous in soil and water [49] and was recently described as a plant endophyte of *Carex pumilia* [50]. Interestingly, *Carex* and *Plantago* are only poorly phylogenetically related but have been described as functionally similar in grasslands [51], which might also be linked to the previously undescribed functional profiles of *Pedobacter*. The strong influence of functional traits on the selection of seed endophytes was

also observed for hyperaccumulating plants, which conserved specific plant growth promoting bacteria across different plant families [52].

Conclusion

Our study identified a stable seed core microbiome of *P. lanceolata* along a plant richness gradient. The core taxa include bacteria frequently identified as plant growth promoting bacteria underlining the important role of the transgenerational transfer of endophytic bacteria to ensure the provision of a consistent starter community for the next plant generation. In addition to the maintenance of a specific core microbiome, our data suggests that this can be supplemented by additional taxa if *P. lanceolata* is grown in plant species rich grasslands. The number of these additional taxa was positively correlated with the number of plant species on the plot. This indicates that the beneficial effects of mixed plantations can be carried on to the next generation, possibly improving seed defense, seedling germination and overall plant robustness [1, 47]. Future studies should investigate whether a similar pattern can be found for other plant species and other microbial groups like fungi or protists to figure out if this might be a future strategy to mitigate consequences of domestication. Moreover, the investigation of consequences for the performance of the next plant generation are open questions resulting from our study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-024-00552-x.

Supplementary Material 1

Acknowledgements
We would like to thank the Jena Experiment coordination team, Nico Eisenhauer, Anne Ebeling and the field coordinator, Annette Jesch, for the support on organizing the seed sampling and the access to the field site and the gardeners and technicians with the help during the sampling campaing.

Author contributions
All authors contributed to the study conception and design. Sampling was performed by YP. Sample processing, sequencing and data analyses were performed by YP. Statistical analyses were performed by YP, YH and SS. The first draft of the manuscript was written by YP, SS and MS, and all authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Funding
Furthermore, we thank the Deutsche Forschungsgemeinschaft (DFG) for funding The Jena Experiment (FOR 5000). The authors MS, WW and SS received the following grants: SCHL446/45 – 1, WE3081/39 – 1 SCHU2907/8 – 1.
Open Access funding enabled and organized by Projekt DEAL.

Data availability
The dataset supporting the conclusions of this article is included within the article (and its Additional file 1). The datasets generated and/or analyzed during the current study are available in the NCBI SRA database repository under project number PRJNA937585, biosample SAMN33409010. R script and metadata files can be found at https://github.com/Streptomyces1/plantago_seed_microbiome.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 5 September 2023 / Accepted: 19 January 2024
Published online: 02 February 2024

References

1. Nelson EB. The seed microbiome: origins, interactions, and impacts. Plant Soil. 2018;422:7–34.
2. Simonin M, Briand M, Chesneau G, Rochefort A, Marais C, Sarniguet A, et al. Seed microbiota revealed by large-scale meta-analysis including 50 plant species. New Phytol. 2022;234:1448–63.
3. Miliute I, Buzaite O, Baniulis D, Stanys V. Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. Zemdirbyste. 2015;102:465–78.
4. Berg G, Raaijmakers JM. Saving seed microbiomes. ISME J. 2018;12:1167–70.
5. Bergna A, Cernava T, Rändler M, Grosch R, Zachow C, Berg G. Tomato seeds preferably transmit plant beneficial endophytes. Phytobiomes J. 2018;2:183–93.
6. Eyre AW, Wang M, Oh Y, Dean RA. Identification and characterization of the core rice seed microbiome. Phytobiomes J. 2019;3:148–57.
7. Adam E, Bernhart M, Müller H, Winkler J, Berg G. The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. Plant Soil. 2018;422:35–49.
8. Rybakova D, Mancinelli R, Wikström M, Birch-Jensen AS, Postma J, Ehlers RU, et al. The structure of the Brassica napus seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome. 2017;5:104.
9. Prado A, Marolleau B, Vaissière BE, Barret M, Torres-Cortes G. Insect pollination: an ecological process involved in the assembly of the seed microbiota. Sci Rep. 2020;10:1–11.
10. Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, et al. A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. Front Microbiol. 2017;8 JAN:1–10.
11. Albracht C, Eisenhauer N, Vogel A, Wagg C, Buscot F, Heintz-buschart A. Effects of recurrent summer droughts on arbuscular mycorrhizal and total fungal communities in experimental grasslands differing in plant diversity and community composition. 2023.
12. Weisser WW, Roscher C, Meyer ST, Ebeling A, Luo G, Allan E, et al. Biodiversity effects on ecosystem functioning in a 15-year grassland experiment: patterns, mechanisms, and open questions. Basic Appl Ecol. 2017;23:1–73.
13. Schmid MW, van Moorsel SJ, Hahl T, De Luca E, De Deyn GB, Wagg C, et al. Effects of plant community history, soil legacy and plant diversity on soil microbial communities. J Ecol. 2021;109:3007–23.
14. Roscher C, Schumacher J, Baade J, Wilcke W, Gleixner G, Weisser WW, et al. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl Ecol. 2004;5:107–21.
15. Estendorfer J, Stempfhuber B, Haury P, Vestergaard G, Rillig MC, Joshi J, et al. The influence of land use intensity on the plant-associated microbiome of Dactylis glomerata L. Front Plant Sci. 2017;8:1–10.
16. Schmid B, Weisser WW, Schumacher J, Roscher C, Schulze ED, Wilcke W, et al. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl Ecol. 2004;5:107–21.

17. Lueders T, Manefield M, Friedrich MW. Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. Environ Microbiol. 2004;6:73–8.
18. Dorn-In S, Bassitta R, Schwaiger K, Bauer J, Hölzel CS. Specific amplification of bacterial DNA by optimized so-called universal bacterial primers in samples rich of plant DNA. J Microbiol Methods. 2015;113:50–6.
19. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2016;18:1403–14.
20. Apprill A, Mcnally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75:129–37.
21. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. 2011;17:2803–9.
22. Wingett SW, Andrews S. Fastq screen: a tool for multi-genome mapping and quality control [version 1; referees: 3 approved, 1 approved with reservations]. F1000Research. 2018;7:1–13.
23. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA. Dada2: high resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:1–7.
24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:590–6.
25. McMurdie PJ, Holmes S. Phyloseq: an R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 2013;8.
26. Oksanen r J, Simpson GL, Blanchet FG, Solymos P, Stevens MHH, Szoecs E et al. Title Community Ecology Package. Vegan Community Ecol Packag. 2022.
27. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw. 2015;67.
28. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P et al. vegan: Community Ecology Package_. R package version 2.6-4. 2022.
29. Jesch A, Huang Y, Kratzch G, Ebeling A, Eisenhauer N. Plant aboveground biomass in the main plots in 2021. Jena Exp Inform Syst. 2021. https://jexis.idiv.de/ddm/data/Showdata/320.
30. Jesch A, Huang Y, Roscher C, Weigelt A, Gleixber G, Ebeling A et al. Biomass of old and new (dBEF) monoculture plots in 2021. Jena Experiment Information System. https://jexis.idiv.de/ddm/data/Showdata/512.
31. Liu C, Cui Y, Li X, Yao M. Microeco: an R package for data mining in microbial community ecology. FEMS Microbiol Ecol. 2021;97:1–9.
32. Chesneau G, Torres-Cortes G, Briand M, Darrasse A, Preveaux A, Marais C, et al. Temporal dynamics of bacterial communities during seed development and maturation. FEMS Microbiol Ecol. 2020;96:1–11.
33. Villette R, Autaa G, Hind S, Holm JB, Moreno-Sabater A, Larsen M. Refinement of 16S rRNA gene analysis for low biomass biospecimens. Sci Rep. 2021;11:1–12.
34. Rajar P, Dhariwal A, Salvadori G, Junges R, Åmdal HA, Berild D et al. Microbial DNA extraction of high-host content and low biomass samples: optimized protocol for nasopharynx metagenomic studies. Front Microbiol. 2022;13 December.
35. Asaf S, Numan M, Khan AL, Al-Harrasi A. Sphingomonas: from diversity and genomics to functional role in environmental remediation and plant growth. Crit Rev Biotechnol. 2020;40:138–52.
36. Peix A, Rivas R, Mateos PF, Martínez-Molina E, Rodríguez-Barrueco C, Velázquez E. Pseudomonas rhizosphaerae sp. nov., a novel species that actively solubilized phosphate in vitro. Int J Syst Evol Microbiol. 2003;53:2067–72.
37. Glöckner FO, Kube M, Bauer M, Teeling H, Lombardot T, Ludwig W, et al. Complete genome sequence of the marine planctomycete Pirellula sp. strain 1. Proc Natl Acad Sci U S A. 2003;100:8298–303.
38. Santoyo G. How plants recruit their microbiome? New insights into beneficial interactions. J Adv Res. 2022;40:45–58.
39. Zuppinger-Dingley D, Schmid B, Petermann JS, Yadav V, De Deyn GB, Flynn DFB. Selection for niche differentiation in plant communities increases biodiversity effects. Nature. 2014;515:108–11.
40. Mraja A, Unsicker SB, Reichelt M, Gershenzon J, Roscher C. Plant community diversity influences allocation to direct chemical defence in Plantago lanceolata. PLoS ONE. 2011;6.
41. Rachid CTCC, Balieiro FC, Peixoto RS, Pinheiro YAS, Piccolo MC, Chaer GM, et al. Mixed plantations can promote microbial integration and soil nitrate increases with changes in the N cycling genes. Soil Biol Biochem. 2013;66:146–53.
42. Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. Nature. 2012;486:59–67.
43. Cardinale BJ, Srivastava DS, Duffy JE, Wright JP, Downing AL, Sankaran M, Jouseau C. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature. 2006;443:989–92.
44. Tilman D. Biodiversity: population versus ecosystem stability. Ecology. 1996;77:350–63.
45. Kowalchuk GA, Buma DS, De Boer W, Klinkhamer PGL, Van Veen JA. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2002;81:509–20.
46. Hacker N, Ebeling A, Gessler A, Gleixner G, González Macé O, de Kroon H, et al. Plant diversity shapes microbe-rhizosphere effects on P mobilisation from organic matter in soil. Ecol Lett. 2015;18:1356–65.
47. Walsh CM, Becker-Uncapher I, Carlson M, Fierer N. Variable influences of soil and seed-associated bacterial communities on the assembly of seedling microbiomes. ISME J. 2021;15:2748–62.
48. Khan MY, Nadeem SM, Sohaib M, Waqas MR, Alotaibi F, Ali L, et al. Potential of plant growth promoting bacterial consortium for improving the growth and yield of wheat under saline conditions. Front Microbiol. 2022;13:1–17.
49. Viana AT, Caetano T, Covas C, Santos T, Mendo S. Environmental superbugs: the case study of Pedobacter spp. Environ Pollut. 2018;241:1048–55.
50. Peng Y, Jiang L, Seo J, Li Z, Choe H, Jeong JC, et al. Pedobacter endophyticus sp. nov., an endophytic bacterium isolated from Carex pumila. Int J Syst Evol Microbiol. 2021;71:1–8.
51. Pipenbaher N, Skornik S, de Carvalho GH, Batalha MA. Phylogenetic and functional relationships in pastures and meadows from the North Adriatic Karst. Plant Ecol. 2017;214:501–19.
52. Ancousture J, Durand A, Blaudez D, Benizri E. A reduced but stable core microbiome found in seeds of hyperaccumulators. Sci Total Environ. 2023;887.

Publisher's Note

Supplementary Material

The seeds of *Plantago lanceolata* comprise a stable core microbiome along a plant richness gradient

Yuri Pinheiro [1, 2]; Michael Schloter [1, 2]; Wolfgang Weisser [3]; Yuanyuan Huang[4,5], Stefanie Schulz [1*]

[1]Technische Universität München, TUM School of Life Science, Chair of Environmental Microbiology, Freising, Germany

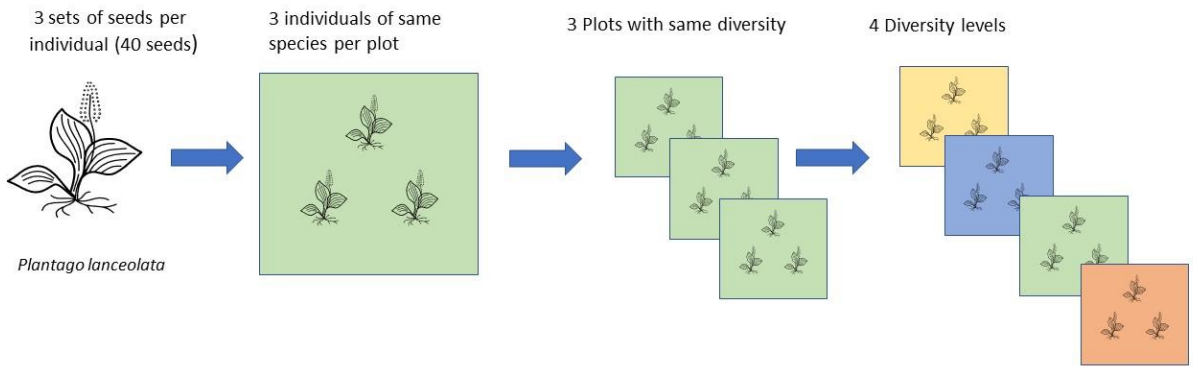
[2]Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Neuherberg, Germany

[3] Technische Universität München, TUM School of Life Science, Chair of Terrestrial Ecology, Freising, Germany

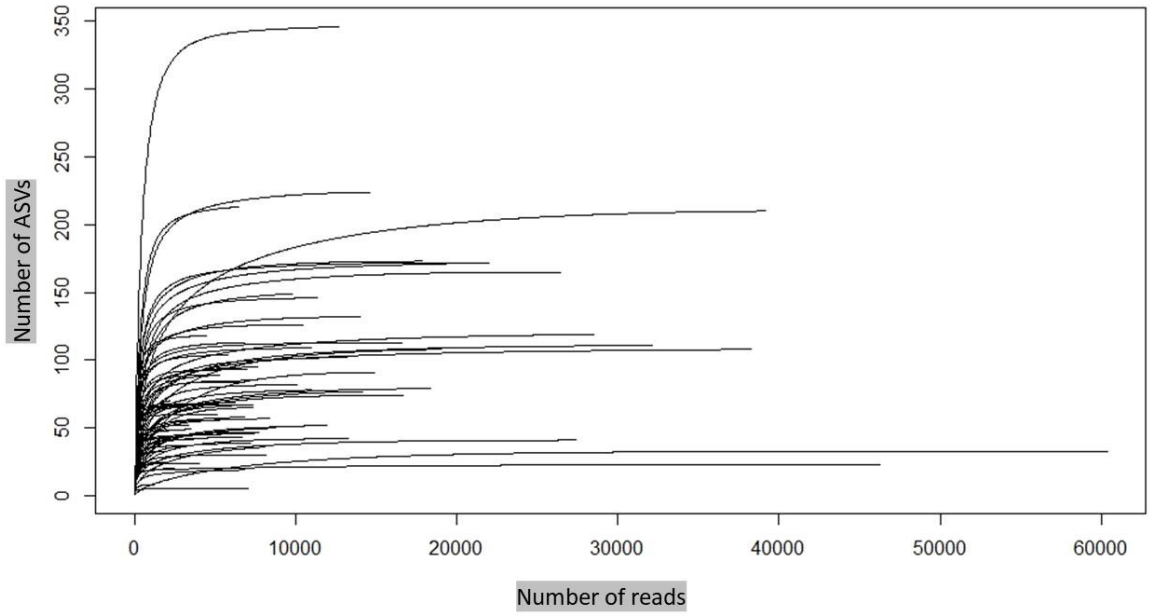
[4]German Centre of Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany

[5]Institute of Biology, Experimental Interaction Ecology, Leipzig University, Germany

*corresponding author: schloter@tum.de



Supplementary Figure S1: Seed sampling scheme. Blossoms were samples on each diversity level containing Plantago lanceolata *in the Jena experiment. In each of the 12 sampled plots, 3 individuals were sampled, each individual is composed by 3 blossoms.*



Supplementary Figure S2: Rarefaction curve drawn using the ASV table prevenient from metabarcoding sequencing.

Table S1: Metadata information with block, plot, indivudal, replicate code, neighbor information of each sampled individual and taxonomical compostion of each sampled plot.

Sample	Code	Block	Plot	Replicate	Plant Species Richness	Individual	Neighbor_1	Neighbor_2	Neighbor_3	Plot_Species
1_S72	B1A01-1-1	B1	B1A01	B1A01-1-1	16	A	*Knautia arvensis*	*Plantago lanceolata*	*Galium mollugo*	*Anthoxanthum odoratum; Avenula pubescens; Bromus erectus; Poa pratensis; Ajuga reptans; Plantago lanceolata; Ranunculus repens; Taraxacum officinale; Anthriscus sylvestris; Carum carvi; Geranium pratense; Tragopogon pratensis; Lathyrus pratensis; Lotus corniculatus; Trifolium campestre; Vicia cracca*
2_S73	B1A01-1-2	B1	B1A01	B1A01-1-2	16	A	*Knautia arvensis*	*Plantago lanceolata*	*Galium mollugo*	
3_S74	B1A01-1-3	B1	B1A01	B1A01-1-3	16	A	*Knautia arvensis*	*Plantago lanceolata*	*Galium mollugo*	
4_S75	B1A01-2-1	B1	B1A01	B1A01-2-1	16	B	*Ranunculus repens*	*Geranium pratense*		
5_S76	B1A01-2-2	B1	B1A01	B1A01-2-2	16	B	*Ranunculus repens*	*Geranium pratense*		
6_S77	B1A01-2-3	B1	B1A01	B1A01-2-3	16	B	*Ranunculus repens*	*Geranium pratense*		
7_S78	B1A01-3-1	B1	B1A01	B1A01-3-1	16	C	*Geranium pratense*	*Plantago lanceolata*	*Galium mollugo*	
8_S79	B1A01-3-2	B1	B1A01	B1A01-3-2	16	C	*Geranium pratense*	*Plantago lanceolata*	*Galium mollugo*	
9_S80	B1A01-3-3	B1	B1A01	B1A01-3-3	16	C	*Geranium pratense*	*Plantago lanceolata*	*Galium mollugo*	
13_S81	B1A04-1-1	B1	B1A04	B1A04-1-1	4	A	*Arenaterum elatius*	*Picris hieracioides*		*Festuca pratensis; Plantago lanceolata; Campanula patula; Onobrychnis viciifolia*
14_S82	B1A04-1-2	B1	B1A04	B1A04-1-2	4	A	*Arenaterum elatius*	*Picris hieracioides*		
15_S83	B1A04-1-3	B1	B1A04	B1A04-1-3	4	A	*Arenaterum elatius*	*Picris hieracioides*		
16_S84	B1A04-2-1	B1	B1A04	B1A04-2-1	4	B	*Arenaterum elatius*	*Picris hieracioides*		
17_S85	B1A04-2-2	B1	B1A04	B1A04-2-2	4	B	*Arenaterum elatius*	*Picris hieracioides*		
18_S86	B1A04-2-3	B1	B1A04	B1A04-2-3	4	B	*Arenaterum elatius*	*Picris hieracioides*		
19_S87	B1A04-3-1	B1	B1A04	B1A04-3-1	4	C	*Arenaterum elatius*	*Picris hieracioides*		
20_S88	B1A04-3-2	B1	B1A04	B1A04-3-2	4	C	*Arenaterum elatius*	*Picris hieracioides*		
21_S89	B1A04-3-3	B1	B1A04	B1A04-3-3	4	C	*Arenaterum elatius*	*Picris hieracioides*		
25_S90	B1A14-1-1	B1	B1A14	B1A14-1-1	8	A	*Knautia arvensis*	*Plantago lanceolata*	*unidentified grass*	*Luzula campestris; Trisetum flavescens; Leontodon hispidus; Plantago lanceolata; Anthriscus sylvestris; Daucus carota; Trifolium campestre; Trifolium fragiferum*
26_S91	B1A14-1-2	B1	B1A14	B1A14-1-2	8	A	*Knautia arvensis*	*Plantago lanceolata*	*unidentified grass*	
27_S92	B1A14-1-3	B1	B1A14	B1A14-1-3	8	A	*Knautia arvensis*	*Plantago lanceolata*	*unidentified grass*	
28_S93	B1A14-2-1	B1	B1A14	B1A14-2-1	8	B	*unidentified grass*	*Knautia arvensis*		
29_S94	B1A14-2-2	B1	B1A14	B1A14-2-2	8	B	*unidentified grass*	*Knautia arvensis*		
30_S95	B1A14-2-3	B1	B1A14	B1A14-2-3	8	B	*unidentified grass*	*Knautia arvensis*		
31_S96	B1A14-3-1	B1	B1A14	B1A14-3-1	8	C	*Trifolium pratense*	*Plantago lanceolata*	*Knautia arvensis*	
32_S97	B1A14-3-2	B1	B1A14	B1A14-3-2	8	C	*Trifolium pratense*	*Plantago lanceolata*	*Knautia arvensis*	
33_S98	B1A14-3-3	B1	B1A14	B1A14-3-3	8	C	*Trifolium pratense*	*Plantago lanceolata*	*Knautia arvensis*	
37_S99	B1A20-1-1	B1	B1A20	B1A20-1-1	16	A	*Knautia arvensis*	*Medicago varia*		

38_S100	B1A20-1-2	B1	B1A20	B1A20-1-2	16	A	*Knautia arvensis*	*Medicago varia*		*Ajuga reptans; Bellis perennis; Leontodon autumnalis; Leontodon hispidus; Plantago lanceolata; Veronica chamaedrys; Achillea millefolium; Geranium pratense; Knautia arvensis; Ranunculus acris; Sanguisorba officinalis; Lotus corniculatus; Medicago varia; Onobrychnis viciifolia; Trifolium hybridum; Trifolium repens*
39_S101	B1A20-1-3	B1	B1A20	B1A20-1-3	16	A	*Knautia arvensis*	*Medicago varia*		
40_S102	B1A20-2-1	B1	B1A20	B1A20-2-1	16	B	*Trifolium pratense*	*Knautia arvensis*	*Medicago varia*	
41_S103	B1A20-2-2	B1	B1A20	B1A20-2-2	16	B	*Trifolium pratense*	*Knautia arvensis*	*Medicago varia*	
42_S104	B1A20-2-3	B1	B1A20	B1A20-2-3	16	B	*Trifolium pratense*	*Knautia arvensis*	*Medicago varia*	
43_S105	B1A20-3-1	B1	B1A20	B1A20-3-1	16	C	*Geranium pratense*	*Medicago varia*	*Knautia arvensis*	
44_S106	B1A20-3-2	B1	B1A20	B1A20-3-2	16	C	*Geranium pratense*	*Medicago varia*	*Knautia arvensis*	
45_S107	B1A20-3-3	B1	B1A20	B1A20-3-3	16	C	*Geranium pratense*	*Medicago varia*	*Knautia arvensis*	
49_S108	B1B045-1-1	B1	B1B045	B1B045-1-1	1	A	*Plantago lanceolata*			*Plantago lanceolata (monoculture)*
50_S109	B1B045-1-2	B1	B1B045	B1B045-1-2	1	A	*Plantago lanceolata*			
51_S110	B1B045-1-3	B1	B1B045	B1B045-1-3	1	A	*Plantago lanceolata*			
52_S111	B1B045-2-1	B1	B1B045	B1B045-2-1	1	B	*Plantago lanceolata*			
53_S1	B1B045-2-2	B1	B1B045	B1B045-2-2	1	B	*Plantago lanceolata*			
54_S2	B1B045-2-3	B1	B1B045	B1B045-2-3	1	B	*Plantago lanceolata*			
58_S3	B1B045-4-1	B1	B1B045	B1B045-4-1	1	C	*Plantago lanceolata*			
59_S4	B1B045-4-2	B1	B1B045	B1B045-4-2	1	C	*Plantago lanceolata*			
60_S5	B1B045-4-3	B1	B1B045	B1B045-4-3	1	C	*Plantago lanceolata*			
61_S6	B1C076-1-1	B1	B1C076	B1C076-1-1	1	A	*Plantago lanceolata*			*Plantago lanceolata (monoculture)*
62_S7	B1C076-1-2	B1	B1C076	B1C076-1-2	1	A	*Plantago lanceolata*			
63_S8	B1C076-1-3	B1	B1C076	B1C076-1-3	1	A	*Plantago lanceolata*			
67_S9	B1C076-3-1	B1	B1C076	B1C076-3-1	1	B	*Plantago lanceolata*			
68_S10	B1C076-3-2	B1	B1C076	B1C076-3-2	1	B	*Plantago lanceolata*			
69_S11	B1C076-3-3	B1	B1C076	B1C076-3-3	1	B	*Plantago lanceolata*			
70_S12	B1C076-4-1	B1	B1C076	B1C076-4-1	1	C	*Plantago lanceolata*			
71_S13	B1C076-4-2	B1	B1C076	B1C076-4-2	1	C	*Plantago lanceolata*			
72_S14	B1C076-4-3	B1	B1C076	B1C076-4-3	1	C	*Plantago lanceolata*			
76_S15	B2A06-2-1	B2	B2A06	B2A06-2-1	4	A	*Cirsium arvense*			*Plantago lanceolata; Taraxacum officinale; Lotus corniculatus; Medicago lupulina*
77_S16	B2A06-2-2	B2	B2A06	B2A06-2-2	4	A	*Cirsium arvense*			
78_S17	B2A06-2-3	B2	B2A06	B2A06-2-3	4	A	*Cirsium arvense*			
79_S18	B2A06-3-1	B2	B2A06	B2A06-3-1	4	B	*Cirsium arvense*			

80_S19	B2A06-3-2	B2	B2A06	B2A06-3-2	4	B	*Cirsium arvense*			
81_S20	B2A06-3-3	B2	B2A06	B2A06-3-3	4	B	*Cirsium arvense*			
83_S22	B2A06-4-2	B2	B2A06	B2A06-4-2	4	C	*Bromus erectus*			
84_S23	B2A06-4-3	B2	B2A06	B2A06-4-3	4	C	*Bromus erectus*			
85_S24	B2A09-1-1	B2	B2A09	B2A09-1-1	4	A	*Trifolium pratense*	*Prunella vulgaris*	*Knautia arvensis*	*Ajuga reptans; Plantago lanceolata; Primula veris; Prunella vulgaris*
86_S25	B2A09-1-2	B2	B2A09	B2A09-1-2	4	A	*Trifolium pratense*	*Prunella vulgaris*	*Knautia arvensis*	
87_S26	B2A09-1-3	B2	B2A09	B2A09-1-3	4	A	*Trifolium pratense*	*Prunella vulgaris*	*Knautia arvensis*	
91_S27	B2A09-3-1	B2	B2A09	B2A09-3-1	4	B	*Prunella vulgaris*	*Primula veris*		
92_S28	B2A09-3-2	B2	B2A09	B2A09-3-2	4	B	*Prunella vulgaris*	*Primula veris*		
93_S70	B2A09-3-3	B2	B2A09	B2A09-3-3	4	B	*Prunella vulgaris*	*Primula veris*		
94_S29	B2A09-4-1	B2	B2A09	B2A09-4-1	4	C	*Primula veris*	*Prunella vulgaris*		
95_S30	B2A09-4-2	B2	B2A09	B2A09-4-2	4	C	*Primula veris*	*Prunella vulgaris*		
96_S31	B2A09-4-3	B2	B2A09	B2A09-4-3	4	C	*Primula veris*	*Prunella vulgaris*		
98_S32	B2A10-1-2	B2	B2A10	B2A10-1-2	16	A	*unidentified grass*	*Geranium pratense*	*Primula veris*	*Alopecurus pratensis; Anthoxanthum odoratum; Arrhenaterum elatius; Bromus erectus; Festuca pratensis; Holcus lanatus; Phleum pratense; Poa pratensis; Bellis perennis; Leontodon autumnalis; Leontodon hispidus; Plantago lanceolata; Primula veris; Prunella vulgaris; Ranunculus repens; Veronica chamaedrys*
99_S33	B2A10-1-3	B2	B2A10	B2A10-1-3	16	A	*unidentified grass*	*Geranium pratense*	*Primula veris*	
100_S34	B2A10-2-1	B2	B2A10	B2A10-2-1	16	B	*unidentified grass*	*Geranium pratense*	*Primula veris*	
101_S35	B2A10-2-2	B2	B2A10	B2A10-2-2	16	B	*unidentified grass*	*Primula veris*		
102_S36	B2A10-2-3	B2	B2A10	B2A10-2-3	16	B	*unidentified grass*	*Primula veris*		
103_S37	B2A10-3-1	B2	B2A10	B2A10-3-1	16	C	*unidentified grass*	*Primula veris*		
104_S38	B2A10-3-2	B2	B2A10	B2A10-3-2	16	C	*unidentified grass*			
105_S39	B2A10-3-3	B2	B2A10	B2A10-3-3	16	C	*unidentified grass*			
109_S40	B2A13-1-1	B2	B2A13	B2A13-1-1	1	A	*Plantago lanceolata*			*Plantago lanceolata (monoculture)*
110_S41	B2A13-1-2	B2	B2A13	B2A13-1-2	1	A	*Plantago lanceolata*			
111_S42	B2A13-1-3	B2	B2A13	B2A13-1-3	1	A	*Plantago lanceolata*			
115_S43	B2A13-3-1	B2	B2A13	B2A13-3-1	1	B	*Equisetum arvense*	*Plantago lanceolata*		
116_S44	B2A13-3-2	B2	B2A13	B2A13-3-2	1	B	*Equisetum arvense*	*Plantago lanceolata*		
117_S45	B2A13-3-3	B2	B2A13	B2A13-3-3	1	B	*Equisetum arvense*	*Plantago lanceolata*		
118_S46	B2A13-4-1	B2	B2A13	B2A13-4-1	1	C	*Campanula barbara*	*Plantago lanceolata*		
119_S47	B2A13-4-2	B2	B2A13	B2A13-4-2	1	C	*Campanula barbara*	*Plantago lanceolata*		
120_S48	B2A13-4-3	B2	B2A13	B2A13-4-3	1	C	*Campanula barbara*	*Plantago lanceolata*		

124_S49	B4A08-2-1	B4	B4A08	B4A08-2-1	8	A	*unidentified grass*	*Crepis vesicaria*		*Avenula pubescens; Bromus hordeaceus; Festuca rubra; Ajuga reptans; Plantago lanceolata; Taraxacum officinale; Veronica chamaedrys; Anthoxanthum odoratum*
125_S50	B4A08-2-2	B4	B4A08	B4A08-2-2	8	A	*unidentified grass*	*Crepis vesicaria*		
126_S51	B4A08-2-3	B4	B4A08	B4A08-2-3	8	A	*unidentified grass*	*Crepis vesicaria*		
127_S52	B4A08-3-1	B4	B4A08	B4A08-3-1	8	B	*Picris hieracioides*	*Plantago lanceolata*	*unidentified grass*	
128_S53	B4A08-3-2	B4	B4A08	B4A08-3-2	8	B	*Picris hieracioides*	*Plantago lanceolata*	*unidentified grass*	
129_S54	B4A08-3-3	B4	B4A08	B4A08-3-3	8	B	*Picris hieracioides*	*Plantago lanceolata*	*unidentified grass*	
130_S55	B4A08-4-1	B4	B4A08	B4A08-4-1	8	C	*Picris hieracioides*			
131_S56	B4A08-4-2	B4	B4A08	B4A08-4-2	8	C	*Picris hieracioides*			
136_S58	B4A04-2-1	B4	B4A04	B4A04-2-1	4	A	*Picris hieracioides*			*Arrhenaterum elatius; Plantago lanceolata; Anthriscus sylvestris; Trifolium dubium*
137_S59	B4A04-2-2	B4	B4A04	B4A04-2-2	4	A	*Arenaterum elatius*	*Picris hieracioides*		
138_S60	B4A04-2-3	B4	B4A04	B4A04-2-3	4	A	*Arenaterum elatius*	*Picris hieracioides*		
139_S61	B4A04-3-1	B4	B4A04	B4A04-3-1	4	B	*Arenaterum elatius*	*Picris hieracioides*		
140_S62	B4A04-3-2	B4	B4A04	B4A04-3-2	4	B	*Arenaterum elatius*	*Picris hieracioides*		
141_S63	B4A04-3-3	B4	B4A04	B4A04-3-3	4	B	*Arenaterum elatius*	*Picris hieracioides*		
142_S64	B4A04-4-1	B4	B4A04	B4A04-4-1	4	C	*Arenaterum elatius*	*Picris hieracioides*		
143_S65	B4A04-4-2	B4	B4A04	B4A04-4-2	4	C	*Arenaterum elatius*	*Picris hieracioides*		
144_S66	B4A04-4-3	B4	B4A04	B4A04-4-3	4	C	*Arenaterum elatius*	*Picris hieracioides*		

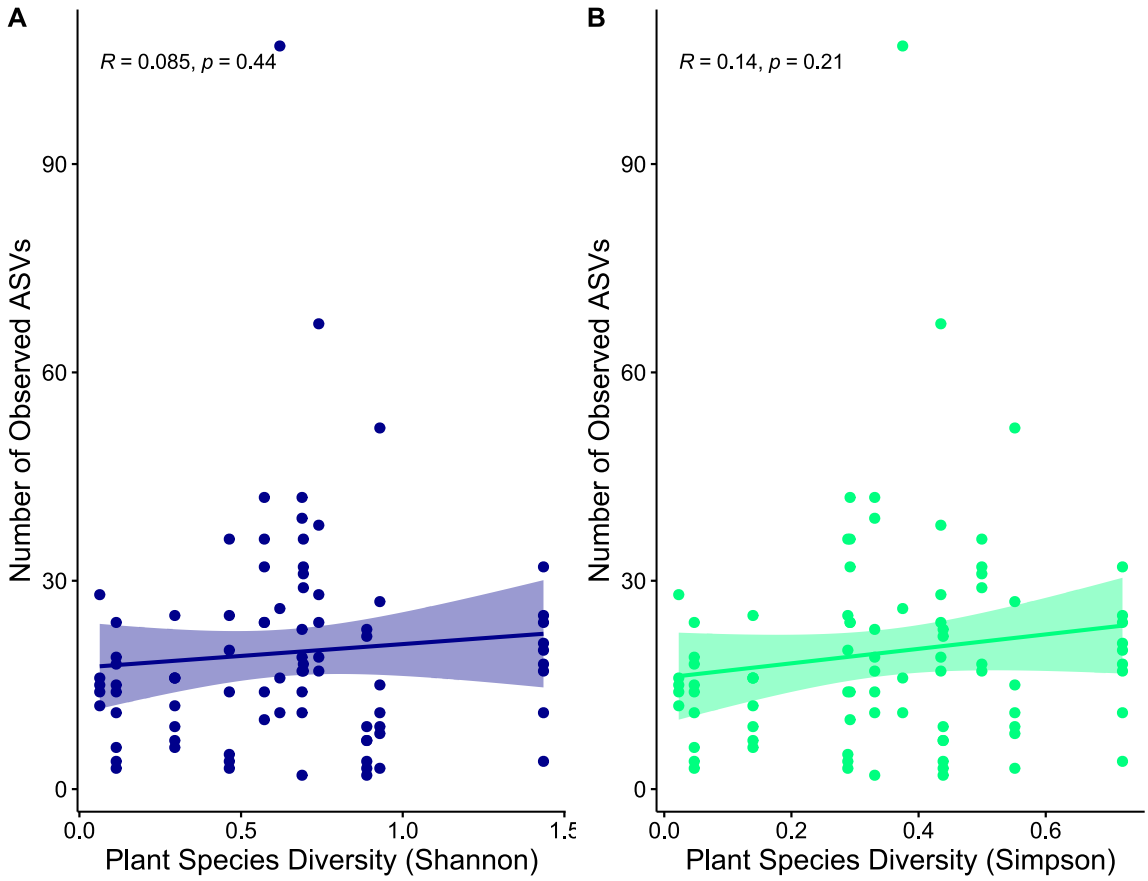
Table S2: Table displaying the read loss per processing step during the bioinformatic pipeline. Samples with blank in their name display extraction controls and with NTC PCR controls.

Sample	Individual	filter+trim reads.in	filter+trim reads.out	dadaF	dadaR	merged	seqtable	removeBimera	Removal chloroplast and mitochondria
1_S72	B1A01-1	13050	10625	10574	10453	10320	10320	10262	3774
100_S34	B1A01-1	15141	10594	10415	10413	9968	9968	9706	1846
101_S35	B1A01-1	53412	46343	45770	45874	44007	44007	35860	14083
102_S36	B1A01-2	15071	12098	11921	11940	11103	11103	10444	3288
103_S37	B1A01-2	63345	55796	55126	55170	50905	50905	38832	32087
104_S38	B1A01-2	19135	16770	16504	16525	15556	15556	14454	7114
105_S39	B1A01-3	73279	61862	61443	61355	59331	59331	48192	18910
109_S40	B1A01-3	40468	34990	34122	34419	30368	30368	24282	9687
110_S41	B1A01-3	9802	7033	6878	6821	6278	6278	6038	1302
111_S42	B1A04-1	28654	23512	23240	23273	22239	22239	21268	4762
115_S43	B1A04-1	16089	13732	13557	13551	12971	12971	11539	254
116_S44	B1A04-1	421	367	248	245	214	214	201	3
117_S45	B1A04-2	84751	74984	74609	74839	73491	73491	47928	46257
118_S46	B1A04-2	964	801	750	735	690	690	666	38
119_S47	B1A04-2	70831	60878	60304	60411	57154	57154	52909	13644
120_S48	B1A04-3	36985	30613	30198	30247	28888	28888	23080	8670
124_S49	B1A04-3	2206	1602	1540	1521	1395	1395	1343	162
125_S50	B1A04-3	6253	5485	5408	5346	5155	5155	4360	4032
126_S51	B1A14-1	9550	7993	7794	7820	7235	7235	5670	2410
127_S52	B1A14-1	62391	54566	54102	54076	52250	52250	42405	14838
128_S53	B1A14-1	9626	8423	8360	8353	8261	8261	8033	7043
129_S54	B1A14-2	73495	63980	63811	63773	63382	63382	62095	60218
13_S81	B1A14-2	85	50	38	32	23	23	23	15
130_S55	B1A14-2	43352	36707	36174	36268	34424	34424	29940	2019
131_S56	B1A14-3	50521	43405	42993	43132	41177	41177	36606	6663

136_S58	B1A14-3	149	88	38	22	14	14	14	14
137_S59	B1A14-3	20697	13339	12882	12783	11332	11332	10940	5624
138_S60	B1A20-1	7725	4672	4556	4538	4275	4275	4002	275
139_S61	B1A20-1	58086	48468	48167	48118	46615	46615	39183	27248
14_S82	B1A20-1	14884	12516	12207	12288	10961	10961	10123	5672
140_S62	B1A20-2	26139	20316	19904	20033	18465	18465	16765	9699
141_S63	B1A20-2	28476	24190	23854	23816	22550	22550	19143	7311
142_S64	B1A20-2	17698	13128	12909	12893	11970	11970	11021	2518
143_S65	B1A20-3	20115	16772	16553	16480	15420	15420	13927	8159
144_S66	B1A20-3	15106	13137	13037	13038	12855	12855	12525	6687
15_S83	B1A20-3	28237	23981	23433	23504	20567	20567	18530	12295
16_S84	B1B045-1	300214	260942	260129	260271	253730	253730	223332	113403
17_S85	B1B045-1	22570	17541	17193	17218	15871	15871	15534	7049
18_S86	B1B045-1	24417	20148	19740	19733	17479	17479	15409	11310
19_S87	B1B045-2	56	34	16	15	9	9	9	6
2_S73	B1B045-2	38404	32779	32558	32550	31915	31915	28940	11605
20_S88	B1B045-2	15704	14085	13920	13897	12721	12721	9561	8103
21_S89	B1B045-4	17316	15293	15105	15159	14382	14382	13523	7079
25_S90	B1B045-4	5850	4476	4354	4283	3692	3692	3587	1012
26_S91	B1B045-4	2173	225	184	178	173	173	173	8
27_S92	B1C076-1	106	60	38	13	13	13	5	5
28_S93	B1C076-1	22302	20108	19723	19643	17264	17264	11542	6013
29_S94	B1C076-1	52147	45783	45380	45406	42647	42647	36651	26267
3_S74	B1C076-3	17363	14435	14299	14158	13683	13683	13395	2878
30_S95	B1C076-3	26	14	1	1	0	0	0	0
31_S96	B1C076-3	18360	13291	12794	12865	11267	11267	10595	4810
32_S97	B1C076-4	24622	21588	21406	21372	20757	20757	19610	1565
33_S98	B1C076-4	21478	18837	18695	18681	18112	18112	17786	3322
37_S99	B1C076-4	18665	16450	16179	16122	14255	14255	13676	4448

38_S100	B2A06-2	33706	30263	29843	29872	27681	27681	19495	16690
39_S101	B2A06-2	27172	24139	23903	23835	23054	23054	18940	7687
4_S75	B2A06-2	16413	12130	11806	11630	10487	10487	9876	6154
40_S102	B2A06-3	30941	22359	22018	22040	20172	20172	19815	8850
41_S103	B2A06-3	46933	41687	41416	41374	39533	39533	37714	32323
42_S104	B2A06-3	8403	7389	7284	7302	6722	6722	5251	1916
43_S105	B2A06-4	25424	22105	21918	21898	21572	21572	21018	19902
44_S106	B2A06-4	33681	29605	29191	29396	27315	27315	24526	9194
45_S107	B2A09-1	17333	15752	15669	15687	15432	15432	14123	14026
49_S108	B2A09-1	7155	3939	3830	3771	3435	3435	3162	976
5_S76	B2A09-1	27080	22372	22071	22052	20652	20652	20066	11701
50_S109	B2A09-3	72233	62713	62317	62375	59935	59935	53240	8880
51_S110	B2A09-3	32309	28033	27642	27717	25379	25379	22146	16244
52_S111	B2A09-3	44476	39182	38877	38961	36890	36890	32047	18807
53_S1	B2A09-4	14678	12349	12085	12101	11002	11002	9486	4945
54_S2	B2A09-4	22610	18518	18274	18356	17582	17582	16731	3265
58_S3	B2A09-4	56298	50723	50384	50383	48182	48182	38624	18354
59_S4	B2A10-1	26448	22669	22431	22471	21147	21147	19480	2360
6_S77	B2A10-1	42606	34739	34130	34183	30922	30922	27912	13783
60_S5	B2A10-2	39415	35086	34877	34925	33630	33630	31536	2663
61_S6	B2A10-2	10404	8885	8780	8725	8374	8374	8176	3067
62_S7	B2A10-2	16998	14896	14697	14673	14064	14064	10327	8082
63_S8	B2A10-3	24583	21341	21137	21076	20124	20124	18561	5115
67_S9	B2A10-3	5771	4988	4838	4845	4457	4457	4154	575
68_S10	B2A10-3	10735	9395	9297	9298	9026	9026	6900	6786
69_S11	B2A13-1	28326	24604	24422	24421	23560	23560	22348	2029
7_S78	B2A13-1	35915	30009	29588	29450	26702	26702	25865	15974
70_S12	B2A13-1	37854	33197	32957	32918	31221	31221	28357	6415
71_S13	B2A13-3	29156	25600	25406	25320	24382	24382	23070	3867

72_S14	B2A13-3	4960	4366	4296	4298	4148	4148	4117	1784
76_S15	B2A13-3	40193	34862	34209	34289	29875	29875	27462	22007
77_S16	B2A13-4	47823	41859	41373	41297	37230	37230	31584	25377
78_S17	B2A13-4	7714	6565	6403	6412	5878	5878	5511	650
79_S18	B2A13-4	21431	18924	18644	18699	17263	17263	14623	6250
8_S79	B4A08-2	12564	10658	10549	10526	10033	10033	9835	4602
80_S19	B4A08-2	32980	29650	29306	29344	27359	27359	23016	13194
81_S20	B4A08-2	12916	11353	11245	11151	10598	10598	9849	4113
82_S21	B4A08-3	11814	9977	9825	9759	9402	9402	8461	0
83_S22	B4A08-3	2973	2622	2519	2472	2244	2244	2168	1872
86_S25	B4A08-3	173822	150022	148170	148895	138419	138419	89751	38793
87_S26	B4A08-4	5489	4723	4588	4627	4339	4339	4226	516
9_S80	B4A08-4	33497	29229	28739	28768	25939	25939	17582	10942
91_S27	B4A04-2	13839	11500	11398	11385	10915	10915	10428	1222
92_S28	B4A04-2	2917	2434	2372	2321	2167	2167	2109	1179
93_S70	B4A04-2	22846	18102	17288	17380	14087	14087	13404	12635
94_S29	B4A04-3	814	670	638	629	586	586	578	152
95_S30	B4A04-3	35151	29760	29370	29493	27615	27615	25326	4370
96_S31	B4A04-3	2040	1691	1540	1529	1259	1259	1212	292
98_S32	B4A04-4	92431	81438	80701	80936	76760	76760	67332	37709
99_S33	B4A04-4	28795	25023	24730	24714	23493	23493	13507	13248
Blank_S67	B4A04-4	12305	9223	9051	9087	8644	8644	8063	REMOVED
NTC-1_S68	NTC	1192	485	435	411	336	336	329	REMOVED
NTC-2_S69	NTC	941	680	631	607	535	535	514	REMOVED
NTC-3_S71	NTC	47	26	11	12	8	8	6	REMOVED



Supplementary figure S3: Correlation plots (Spearman correlation index) between seed endophytic microbiome (number of observed ASVS) and Shanon and Simpson realized plant species diversity on each plot. Plant diversity was calculated based on species biomass harvest on August 2021.