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Master thesis

Bacterial life history strategies in gamma-irradiated arable soil receiving different microbial inocula

Životní strategie bakteriálních společenstev v gamma ozářené půdě po zavedení různých mikrobiálních inokulí

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Declaration

I declare that I am the author of this graduation thesis and that I used only sources and literature displayed in the list of references in its preparation.

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Signature

Abstrakt

Půda obsahuje mimořádnou úroveň mikrobiální biodiverzity, která podporuje klíčové funkce ekosystému. Pochopení toho, jak se půdní mikrobiální společenstva v zemědělských ekosystémech utvářejí a jaké vlastnosti vykazují (tj. jaké funkce a služby zprostředkovávají), je nezbytné pro lepší předvídání důsledků ztráty biologické rozmanitosti. Tato práce analyzuje složení, diverzitu a životní strategie půdních bakteriálních společenstev po zavedení mikrobiálních společenstev z různých zdrojů (tj. půdy a kravských exkrementů) do sterilní půdy (ozářené γ-zářením). Výsledky ukazují, že bakterie z půdy a exkrementů se v γ-ozářené půdě úspěšně etablovaly a že složení a diverzita bakteriálních společenstev silně závisí na zdroji inokulace. Analýzy vybraných funkčních znaků prokázaly, že taxony časných kolonizátorů se vyznačovaly vyšším počtem kopií genu 16S rRNA, menšími genomy a zvýšenou pohyblivostí a schopností sporulace než původní půdní bakteriální společenstva. Kromě toho, přestože půdy vykazovaly podobné počáteční fyzikálně-chemické a biochemické vlastnosti, různá mikrobiální společenstva vytvářela podstatné rozdíly ve vlastnostech půdy a mikrobiálně zprostředkovaných funkcích. Tato práce přispívá k lepšímu pochopení toho, jak je složení bakteriálního společenstva důležité pro mikrobiální biodiverzitu v půdě a jak naopak může biodiverzita přispívat ke změnám půdní úrodnosti a aktivity bakterií prostřednictvím různých životních strategií a vlastností.

Klíčová slova: půda, bakteriální komunita, funkční znaky, životní strategie, zemědělství

Abstract

Soil contains an extraordinary level of microbial biodiversity that supports key ecosystem functions. Understanding how soil microbial communities assemble in agricultural ecosystems, as well as what traits they exhibit (i.e., what functions and services they mediate), is essential to better predict the consequences of biodiversity loss. This thesis analyzes the composition, diversity, and life history strategies of soil bacterial communities after the introduction of microbial communities from different sources (i.e., soil and cow excreta) into sterile soil (γ-irradiated). The results show that bacteria from soil and excreta successfully established in the γ-irradiated soil and that the bacterial composition and diversity strongly depends on the inoculation source. Analyses of selected functional traits proved that early colonizer taxa were characterized by having higher 16S rRNA copy numbers, smaller genomes and elevated motility and sporulation abilities than the original soil bacterial community. In addition, despite the soils showing similar initial physicochemical and biochemical properties, the different bacterial assemblages generated strong differences in soil properties and microbially-mediated functions. This thesis contributes to a better understanding on how the composition of microbial community is important for the microbial biodiversity in soil and in turn, how biodiversity can contribute to changes in soil fertility and bacterial activity through different life history strategies and traits.

Keywords: soil, bacterial community, functional traits, life history strategies, agriculture

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Content

Introduction

Soils have a high microbial diversity which provides important soil functions and ecosystem services. However, intensive agricultural practices that threaten soil organisms, including microorganisms, contribute to soil degradation and potentially affect soil fertility and ecosystem sustainability. Soil degradation and, on the other hand, soil fertility and sustainability are very important issues for agriculture and humankind in general. The application of organic fertilizers, such as manure or compost, increases the organic matter content of the soil and improves its properties. It also makes it possible to recycle the tons of organic waste that are produced each year. At the same time, however, organic materials introduce exogenous microbes, whose contribution to soil biodiversity in agricultural ecosystems is unclear. Investigating the composition of soil bacterial communities, and how these microorganisms modulate soil functions and attributes, such as soil fertility, is important for understanding the consequences of biodiversity loss. While the addition of large amounts of predominantly inorganic fertilisers to agricultural soils simultaneously favours bacteria with rapid growth strategies, it may selectively exclude those that take advantage of more complex resources and thus perform irreplaceable functions in soil. It is important to bring the disturbed microbial status of agricultural soils back into balance through appropriate practices. This thesis, being a part of a larger inoculation experiment, provides a detailed experimental view of the introduction of different communities into disturbed soils and the succession of bacterial communities and their mediated functions by analysing the composition, diversity, and life history strategies of soil bacteria during colonization of sterile soil as well as their impact on soil properties and microbial activity.

1 Literature review

1.1 Agroecology

Agriculture is one of the most influential anthropogenic activities that affect soil properties, their physical, chemical, and biological characteristics and consequently their functioning (Navarro-Noya et al., 2013). However, maintaining soil quality and productivity is essential to meet the increasing demand for food (Vasu et al., 2020). Among other aspects, soil productivity is largely influenced by the activity of soil organism communities (Wang et al., 2018). They mediate vital ecosystem processes such as primary production, decomposition of organic matter, nutrient cycling, climate regulation, biocontrol, and pollutant transformation (Ducklow, 2008). To achieve better sustainability, the science of agroecology has been established (Hatt et al., 2016). Moudrý et al. (2018) define agroecology as the science of the interrelationships between economically important organisms (i.e., plants, animals, microorganisms), their environment and the surrounding landscape.

1.2 Soil

Soil is an integral part of most terrestrial ecosystems and is the basis for food production, and therefore nutrition, for a growing world population (Vasu et al., 2020). Soil is the top layer of the regolith and consists of solid, liquid and gas phases. Its physical and chemical properties determine the water regime of terrestrial plants and allow them to root. Soil is also a source of mineral nutrition and a habitat for various organisms. The solid part of the soil consists mainly of mineral particles, such as rock and mineral fragments and their grains or crystals, but also organic matter, which is a very important component of the soil. Soil organic matter (SOM) is the dead and altered remains of organisms, whether they occur naturally in the soil or are incorporated into the soil, for example in the form of manure.

The lithosphere, atmosphere, hydrosphere and biosphere influence soil formation, and soil in turn alters these spheres (Martin and Johnson., 2012). The lithosphere determines the basic composition of the mineral content of the soil, and soil weathering processes change the nature of the uppermost part of the lithosphere. The hydrosphere determines the presence of water in the soil, and soil influences the movement and

dissolution of substances. The atmosphere determines the climatic conditions under which soil is formed and finally the biosphere determines which species can live in the soil, with soil properties allowing many different types of organisms to thrive and evolve. The interaction of soil-forming factors has resulted in a large number of different soil types. Among other things, they differ in soil organic matter content; while organic soils are rich in organic matter, mineral soils contain only a few weight percent (Burges, 1967). Each soil performs different functions depending on its composition, environmental conditions, and properties. Soil properties are usually divided into physical, chemical, and biological ones. More complex properties or characteristics that cannot be clearly measured and quantified are referred to as attributes, and include for example soil fertility, quality, and productivity.

Fertility of the soil is an important attribute, particularly in agricultural terms. Stockdale et al. (2002) defined soil fertility as: "the ability of a soil to provide the conditions required for plant growth. It is a result of the physical, chemical and biological processes that act together to provide nutrients, water, aeration and stability to the plant, as well as freedom from any substances that may inhibit growth". However, for example erosion, compaction, contamination by pollutants and pharmaceutical residues, acidification, and salinization or alkalinization owing to poor soil and water management can all have a negative impact on soil fertility (Šimek et al., 2019b). Another important attribute of soil is soil quality, which can be defined as: "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). Assessing and monitoring soil quality is essential to sustain agricultural production and overcome the impact of climate change on soil functions (Vasu et al., 2020), as soil plays a key role in ecosystem functioning (Adhikari and Hartemink, 2016). For example, agricultural intensification and poor management leads to the breakdown of soil aggregates and crusting of the soil surface and compaction, which in turn causes reduced water infiltration and consequently increased surface runoff, soil erosion and loss of soil organic matter, which affects most soil functions that are mediated by soil microorganisms and reduces soil fertility (Vasu et al., 2020).

1.3 Life in soil and the role of the soil microbiome

The living component of the soil is the soil biota. Plants are not usually considered part of the soil biota, although their life is closely linked to the soil (Šimek et al., 2019a). Plant root systems affect the soil mechanically by growing through it and chemically by secreting organic acids and many other substances at different depths and distances (Richter et al., 2007).

Soil life is substantially affected by water regime, aeration, nutrient availability, and lack of light (Šimek et al., 2019a). The distribution of organisms in soil is neither uniform nor random and is determined and controlled primarily by the distribution of soil organic matter (Poll et al., 2003). Also, by the ability of organisms to move through the soil environment by either active or passive processes, or a combination of these two factors (Yang and van Elsas, 2018). The source of mineral nutrients for organisms are inorganic components (rocks and minerals) and their weathering (Ehrlich, 1998). The size of mineral particles then determines the spatial arrangement of the soil. Finer-grained (heavier) soils are less aerated, bind water and nutrients more tightly, and create a more stable environment for organisms, whereas sandy soils dry out quickly, are poorer in nutrients, and have a more variable environment, especially for microorganisms (Šimek et al., 2019a). Both inorganic and organic soil components contribute together with organisms to the formation of stable aggregates, thereby improving soil structure (Ehrlich, 1998).

Soil organisms include a wide range of forms and levels of organisation, from viruses and bacteria, fungi, archaea and algae, to protozoa and lower (invertebrate) animals, and to small vertebrates. These interact with plants, leading to a constant flow of substances and energy in the soil (Šimek et al., 2019a). These interactions range from symbiosis and mutualism to parasitism causing serious illness and death of the host (Lee and Pankhurst, 1992). Microorganisms are the smallest living component of soil, but ultimately represent the largest biomass. The ecological community of microorganisms within a particular environment is called microbiome and was defined by Berg et al. (2020) as: “a characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to the microorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and

scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health". Microorganisms are able to divide rapidly and therefore respond quickly to environmental changes; bacteria in particular play a key role in the decomposition and mineralization of organic matter and thus in the fluxes of biogenic elements in trophic networks, making them indispensable for the final phase of organic matter degradation (Condron et al., 2010). As the functional diversity of bacteria is a crucial link between biodiversity patterns and ecosystem functioning (Escalas et al., 2019), this study will focus specifically and mostly on the bacterial community.

1.4 Bacteria

Bacteria are unicellular microscopic organisms with an average size of up to a few µm that have a prokaryotic cell organization. The DNA is arranged in a single circular or linear chromosome and, together with supporting proteins, forms a nucleoid that is not bounded by a nuclear membrane. There are no mitochondria or plastids in the cell. Bacteria often contain smaller circular DNA molecules, that are not part of the chromosome, called plasmids (Tran and Boedicker, 2019). Some bacteria form extremely resistant dormant forms that allow long-term survival under unfavourable conditions (Roszak and Colwell, 1987). These forms are called spores and exhibit minimal metabolism, respiration, and reduced enzyme production (Morrison and Rettger, 1930), with subsequent transformation into the native form if conditions are favourable. For example, some Gram positive bacteria produce intracellular spores, called endospores, to survive when unfavourable conditions occur. Endospores are highly resistant and thick-walled structures formed inside bacterial cells, which under the improved environmental conditions germinate back into active bacterial cells (Piggot and Coote, 1976). Bacteria often form colonies, groups of bacteria derived from the same mother cell, but each bacterium is itself a biotic autonomous system with its own internal cellular capabilities such as storing, processing, and evaluating information (Ben-Jacob, 2008). Bacteria are largely asexual, most often they reproduce by binary fission, although levels of homologous recombination vary and may be high enough to confer evolutionary advantages of sex in many groups (Birky and Barraclough, 2009). However, a bacterial cell can also acquire foreign DNA by i) conjugation – transfer of plasmids between bacteria, ii) transduction – viral infection,

or iii) transformation – taking extracellular DNA from the environment and incorporating it into its genome by recombination (Crits-Christoph et al., 2020) to acquire, for example, resistance to antibiotics. These processes are called horizontal gene transfer, and the ability is encoded in the bacterial genome, which then itself influences these cell-to-cell exchanges (Tran and Boedicker, 2019). Genes encoded on mobile elements are generally not essential for bacterial life and may be lost over time if the stimulant is not present in the environment. Gene loss and convergent evolution can lead to polyphyletic expansion of certain traits, and this can then complicate the link between phylogeny and the presence of certain functional traits in bacteria (Martiny et al., 2013).

1.4.1 Identification

Before the advent of sequencing technologies, bacteria were classified mainly on the basis of morphological and physiological features, using cultivation under different conditions and from biochemical tests. Nowadays, sequencing is used for taxonomical classification almost exclusively. The 16S ribosomal RNA (rRNA) gene was chosen as the most appropriate for phylogenetic and taxonomic classification of bacteria (Armougom and Raoult, 2009). The main reason is its essential function in the living cell, as it is a structural component of the small subunit of the 30S ribosome and thus highly conserved throughout the bacterial domain (Jonasson et al., 2002). This gene is 1 550 bp long and contains 8 conserved (U 1–8) and 9 hypervariable (V 1–9) regions (Armougom and Raoult, 2009). While the conserved regions can be used to reveal old relationships, the variable regions show a spectrum of different nucleotide substitution rates that can be used to study evolutionary relationships in the bacterial domain (van de Peer et al., 1996). The number of rRNA gene copies depends on the species and normally varies between 1 and 15 copies (Jonasson et al., 2002) per cell.

The clonal Sanger sequencing technique is often used to identify individual bacterial species by sequencing an isolated bacterial strain (i.e., a colony cultured from a single cell) with the full-length 16S rRNA gene (Armougom and Raoult, 2009). Other sequencing technologies, such as shotgun metagenomic sequencing, are used to examine whole bacterial genomes (Loman and Pallen, 2015). The isolation of individual strains and their detailed phylogenetic and physiological analysis are of great importance in terms of bacterial ecology. It is a basis for analysing their

properties and, for example, prediction of their ability to adapt to different physical conditions, such as the production of bioactive substances (Vartoukian et al., 2010).

Metagenomic sequencing of 16S rRNA gene amplicons is now often performed as a culture-independent technique to investigate microbial communities in environmental samples (Vilo and Dong, 2012). The technique is based on the isolation of DNA from the environment (Konopka, 2009) and the subsequent amplification and sequencing of a selected hypervariable region of the 16S rRNA gene, using next-generation sequencing platforms, such as Illumina (Jovel et al., 2016). The length of the fragments can vary on the chosen pair of primers and platform, from 100 to 400 pb (Vilo and Dong, 2012). The set of reading fragments, or reads, is called 16S rRNA amplicon library. The reads are usually clustered based on similarity into Operational Taxonomic Units (OTUs) according to a fixed threshold of sequence dissimilarity of 3 % (Westcott and Schloss, 2015). Recently, amplicon sequence variants (ASVs) have been presented as a replacement for OTUs, when ASVs are used without the need for arbitrary dissimilarity standards (Callahan et al., 2017). Nucleotide sequences of 16S rRNA reads can be compared against databases such as SILVA (Quast et al., 2013) to identify the microorganisms in environmental samples, which can be further used to infer microbial traits using novel trait databases, such as BactoTraits (Cébron et al., 2021). However, traits encoded by mobile elements, such as most antibiotic resistances, cannot be monitored through 16S amplicon libraries (Rankin et al., 2011).

Whole-metagenome shotgun culture-independent sequencing is another way to explore bacterial communities in their natural environments (Chen and Pachter, 2005). It is performed by unrestricted genome sequencing of all bacteria present in the sample (Jovel et al., 2016). As with the 16S libraries, short reads are obtained from the platform, but these can be assembled into longer sequences (i.e., “contigs”) by using reference bacteria or by overlapping sequences *de novo* (Jovel et al., 2016). The metagenomic approach allows to compare bacterial composition without the amplification bias, which may be an advantage over 16S amplicon sequencing (Bahram et al., 2021). The technique offers a way to study microbial community structure, composition, species diversity, metabolic capacity, and functional diversity (Shah et al., 2011). However, metagenomic sequencing is usually more expensive than 16S rRNA amplicon sequencing, although the price depends on the depth of sequencing (Jovel et al., 2016). Indeed, the sequencing depth may not be sufficient to characterize the full diversity of soil microorganisms and is then less powerful than

16S libraries for diversity studies. In addition, the interpretation of this type of data also presents several difficult bioinformatic challenges (Chen and Pachter, 2005).

1.4.2 Phylogeny

Phylogeny reflects functional similarity among taxa, as most relevant bacterial traits are phylogenetically conserved (Goberna and Verdú, 2016). Phylogenetically related species tend to share a common evolutionary history; thus, phylogenetically related species in an ecosystem tend to perform comparable roles (Pérez-Valera et al., 2015). Studying the functioning of individual bacteria in environmental samples (e.g., whether they sporulate or are motile) is challenging, and therefore, the phylogeny can be used to predict traits based on their similarity to well-characterized bacteria. Indeed, the more phylogenetically conserved the traits observed and the larger the proportion of species with known values for a given trait, the more accurate the results generated from phylogenetic observations are (Goberna and Verdú, 2016). Trait inference using phylogenetic relatedness can assist in the clarification of concerns about biodiversity, genetics, evolution, and ecology among groups of organisms (Gittleman, 2016). The phylogenetic structure can be helpful in assessing the functional features of bacterial groups and can contribute to a better understanding on how bacterial community composition influences essential ecological activities (Morrissey et al., 2016).

1.4.3 Biodiversity

Diversity is a frequently used term in a wide range of scientific disciplines. In biology, we talk about biodiversity and Díaz et al. (2015) define it as: "the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are a part. This includes variation in genetic, phenotypic, phylogenetic, and functional attributes, as well as changes in abundance and distribution over time and space within and among species, biological communities, and ecosystems". Microbial communities have their own specificities and Dunlop (2001) defined microbial diversity as: "the range of different kinds of unicellular organisms, bacteria, archaea, protists, and fungi. Various microorganisms thrive throughout the biosphere, defining the limits of life and creating conditions conducive for the survival and evolution of other living beings. The

different kinds of microorganisms are distinguished by their differing characteristics of cellular metabolism, physiology, and morphology, by their various ecological distributions and activities, and by their distinct genomic structure, expression, and evolution". Whittaker (1972) defined three concepts for describing biodiversity on a large scale. The first is alpha diversity, which is determined by counting the number of taxa in an ecosystem and refers to diversity within a region, community, or ecosystem. The second is beta diversity, which compares the number of taxa that are unique to each environment and measures species diversity between ecosystems. Gamma diversity, on the other hand, is a measure of total variety for distinct ecosystems within a region. The alpha diversity of a specific area or community can be quantified either by counting the number of species present at that location (species richness) or by using one of the diversity indices. These indices, i.e., Shannon index, also attempt to consider other diversity attributes such as the balance of abundances of individual species (species evenness). Beta diversity can be calculated by Bray-Curtis's dissimilarity, a statistical method used to quantify compositional differences between two different sites based on the numbers of taxa at each site.

1.4.4 Functional traits

Functional traits are morphophysiological characteristics that indirectly affect fitness through their impact on growth, reproduction, and survival of the organism, while they are not species specific (Violle et al., 2007). They can also be used to describe the processes and characteristics of ecosystems, as larger biological organizations grow, reproduce, and survive in the same way as individual organisms (Violle et al., 2007). Trait expression in bacteria is often closely linked to the environment in which they are found, and their expression in an ecosystem lasts as long as the organism is alive (Escalas et al., 2019). The link between genotype and phenotype is tighter in microorganisms than in macroorganisms (Dutilh et al., 2013), and therefore many bacterial traits are genetically regulated, such as metabolism, biofilm formation, or virulence (Escalas et al., 2019). Their induction depends on population size, cell activity and environmental conditions (Escalas et al., 2019).

As mentioned above, evaluation of traits is one of the fundamental tools for studying biodiversity (Findley et al., 2013), and in molecular ecology, functional diversity provides a link between ecosystem functioning and biodiversity patterns

(Escalas et al., 2019). Exploring different traits is important for functional diversity, but caution is required when inferring traits values from phylogenetically close taxa of reference, as it is necessary to select traits that are phylogenetically conserved (Goberna and Verdú, 2016). Functional traits based on complex genetic systems that are integral to the organism evolve slowly and tend to be more phylogenetically conserved, whereas simple traits that include few functional genes tend to occur at low depth in phylogenetic trees and are often not shared by all members of a given taxon (Martiny et al., 2015). Simple traits are more likely to be transmitted by horizontal gene transfer (Escalas et al., 2019). According to Romillac and Santorufo (2021), functional traits should not be identified based on their relationship to the ecosystem function of interest, but rather on their relationship to bacterial fitness, which consists of three components: growth rate, survival, and dispersal. They also suggested observing the variation in trait values aggregated within a community along environmental gradients and tracking relationships between relative abundance and trait values within communities to validate identified functional traits. Assessing microbial communities using a trait-based framework highlights important relationships between microorganisms and their environment. Genomic characteristics such as genome size, GC content and 16S rRNA gene copy number have been shown to be indicators of bacterial life history strategies. Thus, the relationships between genomic traits and environmental factors show the potential usefulness of genomic traits for assessing the relationships between bacteria and their environment (Chuckran et al., 2022).

To study environmental patterns at the bacterial community level, we can use a trait-based approach in combination with a 16S rRNA amplicon library and then analyse selected traits based on taxonomic classification and comparison with related taxa. A "community weighted mean" (CWM) can be used to predict and quantify functional traits in complex microbial communities, where the average trait values for all taxa in a community are weighted by their relative abundance to show the variability of functional traits occurring in a given community (Fierer et al., 2014). Functional traits can be separated to quantitative genotypic and phenotypic traits. Quantitative genotypic traits include for example number of 16S rRNA gene copies, genome size, and guanin and cytosin (GC) base content (Escalas et al., 2019). Phenotypic traits characterize the expression of the genotype, such as shape, Gram stain-type of cell wall, motility, or spore production, can be analysed, as well as traits

related to environmental preferences, which include oxygen requirements, optimal pH, growth temperature or salinity tolerance (Escalas et al. 2019; Madin et al., 2020; Cébron et al., 2021).

Copy number of 16S rRNA gene – The number of ribosomal RNA operons is considered an indicator of bacterial growth rate (Li et al., 2019; Weissman et al., 2021). The copy number of ribosomal genes is related to the rate of resource use. Fast-growing bacteria that use nutrients in a burst mode have generally more ribosomal gene copies than slow-growing microorganisms that have adapted to a steady supply of low nutrient levels (Schmidt et al., 2018). Because ribosomes are the site of protein synthesis in the cell, the more copies of ribosome production genes accumulate in the genome, the faster ribosome biogenesis and protein synthesis can keep pace with opportunities in a changing environment if sufficient nutrients are available (Woolford and Baserga, 2013).

Genome size – The size of genome can be thought of as capturing ecological strategy along the dimension of versatility because differences in genome size usually reflect the number of distinct coding genes (Guieysse and Wuertz, 2012). The size of the genome depends on the type of ecosystem. Specialized organisms that depend on a limited number of C resources, whether fast or slow growing, have smaller genomes than generalists that can use a variety of resources (Schmidt et al., 2018; Chuckran et al., 2022) and respond flexibly to different circumstances (Westboy et al., 2021).

GC content – The G and C content of the bacterial genome is the molar ratio of guanine (G) and cytosine (C) bases in all bases. Values range from less than 20 % to more than 70 % in bacteria, and this variability is generally attributed to differences in mutation patterns among bacteria, with many species undergoing selection for increased GC (Hildebrand et al., 2010). GC content is reported to be in general higher in aerobic bacteria than in anaerobic bacteria (Naya et al., 2002). Also, it is proposed that genomic base composition might be related to abiotic stress tolerance due to better repair of damaged DNA (Romillac and Santorufo, 2021).

Motility – A number of bacterial species are able to move both in liquids or on moist surfaces by swimming, swarming, gliding, oscillating or floating. The mechanisms of movement are diverse. Movement can be provided by surface appendages such as flagella that rotate, pili that drag, or mycoplasma outcrop that walk. Some types of motilities involve internal structures such as the cytoskeleton and gas vesicles. Regardless of the type of motility mechanism that is used, most motile

microorganisms use complex sensory systems to control their movements in response to stimuli, allowing them to migrate to other environments (Jarrell and McBride, 2008). Higher proportion of motility-enabling traits is detected in copiotrophs (Chen et al., 2021).

Cell wall type – Bacteria are classified using Gram staining based on the structural characteristics of their cell walls. Peptidoglycans in the cell wall of Gram positive and Gram negative bacteria give the cell its form and provide mechanical protection. Peptidoglycans are made up of a muramic acid and glucosamine glycan backbone and peptide chains that are extensively cross-linked by bridges in Gram positive bacteria and partially cross-linked in Gram negative bacteria (Milton and Kwang-Shin, 1996). Gram positive bacteria more easily adapt their metabolism to nutrient-poor conditions and are thus usually classified as oligotrophic bacteria. Whereas Gram negative bacteria thrive better in environments with readily available resources and are mostly considered copiotrophs (Fanin et al., 2014; Orwin et al., 2018).

Sporulation – Spore formation is a successful strategy to allow a cell or population to survive life-threatening conditions. The ability to form endospores is mostly observed within Firmicutes phylum, with spore-forming species represented in most classes, including Bacilli and Clostridia. Endospores are most found in rod-shaped and filamentous bacteria, and many endospores have only been observed in samples from the nature (Hutchison et al., 2014). Many actinobacteria also produce spores, but these are more like fungal spores. Depending on the species, spore production can take place directly on the substrate mycelium or from aerial mycelium. Actinobacterial spores take different forms and are characterised by different properties, e.g., the Actinoplanes and Actinosynnema groups are characterised by motile spores, while Thermoactinomyces form unique heat-resistant endospores. Some other genera of Actinobacteria have sclerotia, synnema or vesicles that contain spores (Barka et al., 2016).

1.4.5 Life history strategies

Life history strategies are a collection of traits that tend to correlate as a result of physiological or evolutionary trade-offs, with different strategies favouring different environmental conditions (Malik et al., 2020).

The life histories of plants and animals are often defined by a continuum of r-to-K selection (Fierer et al., 2007). When resources are abundant, r-strategists are adapted to optimize their own growth rate, whereas K-strategists are adapted to compete and survive when populations approach carrying capacity and resources are limited (Lauro et al., 2009). Although the specific ecological criteria used to define r- and K-strategies may differ, the idea of r-/K-strategy should apply to both multicellular and unicellular taxa, providing a valuable basis for comparing ecological traits between taxa (Fierer et al., 2007). In microbiology, the terms copiotroph and oligotroph are frequently used to describe microorganisms that have similar ecological traits to r- and K-strategists (Fierer et al., 2007). Copiotrophs prefer to consume labile pools of organic carbon (e.g., in the soil), have high nutrient requirements, and can grow rapidly when resource conditions are favourable; in contrast, oligotrophs are slower growing and may compete with copiotrophs in low-nutrient environments because of their greater affinity for the substrate (Klappenbach et al., 2000). As a result, copiotrophs should predominate in environments with high amounts of accessible organic carbon, whereas oligotrophs should predominate in environments with low quality and/or quantity of organic carbon (Fierer et al., 2007). Traits such as the operon copy number, genome size or GC base content can be observed at the community level. Based on these observations, it can be said, for example, that organisms with low rRNA operon copy number dominate in later successional communities due to a selection advantage (Ortiz-Álvarez et al., 2018; Nemergut et al., 2016). On the other hand, r-strategists tend to encode higher rRNA operon copy number in the genome (Reznick et al., 2002), giving them an advantage in conditions where nutrient supply increases. (Westboy et al., 2021).

Other alternative frameworks, such as the Grime triangle (C-S-R) distinguishing competitive, stress-tolerant, and ruderal strategies in plants, have recently been adapted for bacteria (Krause et al., 2014; Fierer, 2017; Malik et al., 2020). In his work, Fierer (2017) described competitive bacteria (C) as those with large genomes and high catabolic diversity, stress-tolerators (S) as having low 16S rDNA copy number and high affinity for substrates, and ruderals (R) as having many copies of 16S rRNA, growing rapidly and often sporulate (Fierer, 2017).

1.5 Bacteria in soil

Bacteria are the smallest and most abundant free-living microorganisms in soil, and their spectrum of autotrophic and heterotrophic capabilities is unmatched by any other of the major groups of soil life (Clark, 1967).

Despite the vast number of bacterial species in soil, only about 1 % can be detected by standard techniques, which does not represent the entire taxonomic diversity (Pham and Kim, 2012). As mentioned above, culture-independent techniques based on the analysis of environmental DNA, particularly the 16S rRNA gene, have the potential to overcome these limitations and thus become a powerful tool in modern microbial ecology. According to the databases containing thousands of reference ribosomal sequences, the taxonomic affiliation of uncultured microorganisms can be determined (Kuffner et al., 2004). The development of sequencing methods has provided a closer look at the enormous biodiversity in soil, where each gram of soil contains 10^9–10^{10} prokaryotic cells (Clark, 1967), of which 10^3–10^6 might be unique bacterial species (Fierer et al, 2007). Although abundance, diversity and microbial biomass are generally high in soil, they can be significantly reduced in degraded soils (Šimek et al., 2019b). Microbiome composition and function are also related to stability, dispersal, and microarchitecture within and between soil aggregates (Mikha and Rice, 2004). Soil aggregates occur even in apparently homogeneous soils. They are classified according to size, with large macroaggregates having more than 2 mm in diameter, macroaggregates greater than 250 μm in diameter, and microaggregates less than 250 μm in diameter (Bronick and Lal, 2005). Aggregate structure provides a diverse range of physicochemical niches for microbial habitats, including variations in nutrient quantity and quality, redox conditions, water-filled pore space, and pore size classes (Mummey and Stahl, 2004).

Soil microorganisms are not uniformly spread, occurring perhaps on less than 1 % of available surfaces and forming local aggregations, hotspots of activity, usually associated with nutrient sources, particles of organic matter from dead bodies of plants, animals and microorganisms, organic matter separated in the soil or with exchange surfaces and plant roots (Tecon and Or, 2017). The coexistence of high biodiversity in the soil is enabled by successional specialization in the detrital food web (Bastow, 2012).

1.5.1 The main taxa of soil bacteria

There are about thirty valid phyla described in the Bacteria domain so far. The vast majority of known bacteria occur in the soil environment, but for many of them, knowledge of their physiology, ecological importance and role in the ecosystem is marginal or limited, mostly due to cultivation constraints. Here, six important phyla that are abundant in soil, will be briefly mentioned.

Proteobacteria encompass an enormous level of morphological, physiological, and metabolic diversity (mostly copiotrophs) and are of great importance for the global carbon, nitrogen, and sulphur cycling (Spain et al., 2009). All representatives are Gram negative and include many bacteria responsible for nitrogen fixation, with alpha-, gamma- and deltaproteobacteria being particularly important in the soil environment (Spain et al., 2009).

The members of **Actinobacteria** phylum are Gram positive bacteria, in chromosomal DNA have a high abundance of cytosine and guanine and have a diverse metabolism (Wink et al., 2017). They usually have a large genome containing several genes allowing adaptation to environmental conditions and complex regulatory mechanisms regulating the expression of these genes (Wink et al., 2017). Together with fungi, they play an important role in the decomposition of organic compounds such as cellulose and chitin in the soil, thus contributing to the turnover of organic matter and the carbon cycle, replenishing soil nutrient reserves and being an important part of humus formation (Anandan et al., 2016).

Acidobacteria is one of the most common and diverse bacterial phylum in soils and peatlands (Ivanova et al., 2020). Despite their high abundance and diversity, we still have relatively little information regarding the actual activities and ecology, mainly because of difficulties in cultivating the majority of Acidobacteria (Dedysh and Damsté, 2018). All cultured Acidobacteria species are Gram negative and heterotrophic (Kielak et al., 2016), but generally are classified as oligotrophic (Ramirez et al., 2012). Genomic and metagenomic data predict a number of ecologically relevant capabilities for some Acidobacteria, including the ability to use of nitrite as N source, respond to soil acidity, macro- and micronutrients, express multiple active transporters, and produce exopolysacharide (Kielak et al., 2016).

Bacteria of the phylogenetic phylum **Firmicutes** are Gram positive and have the ability to form endospores. They are important chitinolytic bacteria in soil (Wieczorek

et al., 2019) and some of them have fermentative, iron and sulphate reducing capabilities (Gupta et al., 2018). Within the Bacilli class, there are also antibiotic producers with antagonistic activity against fungal and some bacterial pathogens that allow the bacteria to survive in their natural environment (Sansinenea and Ortiz, 2011).

Members of the **Bacteroidetes** phylum are divided into 4 orders: Bacteroidales, Flavobacteriales, Sphingobacteriales, and Cytophagiales. They are Gram negative non-sporulating rods and have different oxygen requirements, ranging from obligate aerobes to obligate anaerobes. They are specialised in the degradation of complex organic matter, especially in the form of polysaccharides (Wolińska et al., 2017). Bacteroidetes in soil release several carbohydrate-active enzymes that target various glycans in the soil and are often found in association with animals (Larsbrink and McKee, 2020).

The **Verrucomicrobia** phylum includes aerobic or facultatively anaerobic, oligotrophic bacteria (Ramirez et al., 2012), form cytoplasmic flagella and are Gram negative. They are globally distributed, abundant, and active stable component of soil communities (Sangwan et al., 2005). In nutrient-enriched soils, verrucomicrobial community were related to soil factors linked to soil fertility, such as total nitrogen, phosphorous and potassium ammounts (Navarrete et al., 2015).

1.5.2 Fertilization and its impact on the life history strategies and succession of the soil microbiome

Soil productivity is one of the most important soil attributes and is closely related to the structure and activity of microorganisms, as already mentioned. However, soil productivity in agricultural soils is influenced by many factors, whilst the cycling of mineral nutrients such as C, N, and P is critical for determining soil productivity in unfertilized soils (Wang et al., 2018). In unfertilized soils, the main sources of inorganic N are organic N mineralization and microbial N_2 fixation. Most of the inorganic N is released from organic materials in the soil during microbial decomposition, while some of the inorganic N is reabsorbed by microorganisms, with both free-living bacteria and symbiotic bacteria fixing N_2 in the soil (Laungani and Knops, 2012; Sellstedt and Richau, 2013). For example, the family Rhizobiaceae, which has a significant positive association with soil productivity, includes several species with the ability to fix N_2 (Delgado-Baquerizo et al., 2018). On the other hand,

the order Solibacterales (Acidobacteria), which shows a negative correlation with soil productivity, is reported to be a potential indicator of soil degradation (Soman et al., 2017). Soil microbial communities are often sensitive to nutrient supply; nitrogen fertilization typically reduces microbial biomass and respiration rates, as well as recruitment of functionally specific microbial groups, including ammonia-oxidizing bacteria and archaea (Tian et al., 2014). Increased N and P supply causes predictable changes in the taxonomic and functional traits of soil microbial communities, including an increase in the relative abundance of faster-growing copiotrophic bacterial taxa, as suggested by findings of the study by Leff et al. (2015) and these changes are likely to affect belowground ecosystems worldwide. Their findings show that while the composition of microbial communities varied significantly across the grassland sites tested, nutrient availability caused consistent changes in microbial community composition across the sites, thereby, by selecting microbial groups with specific functional characteristics, and highlight that the way soil microbial communities respond to changes in nitrogen availability is of major importance in the context of increasing nitrogen and phosphorus inputs to ecosystems worldwide. For example, nutrient-induced changes in copiotrophic and oligotrophic traits can have a major impact on soil C cycling (Schmidt et al., 2014), similarly, reductions in mycorrhizae and methanogens can have a significant impact on ecosystem processes (Leff et al., 2015). Pérez-Valera et al. (2019a), who investigated how manure affects the soil bacterial community, found that when the soil is healthy, the native bacterial community can control the spread of potentially risky organisms, but also that native soil microorganisms are essential for the fertilizing effect of manure on the soil and therefore soil fertility.

1.5.3 Influence of agricultural practices on the ecology of the soil bacterial community

Erosion, groundwater contamination, soil acidification, soil salinization, and soil biota depletion are typical features of unsustainable farming techniques leading to soil degradation (Nearing et al., 2017; Shi et al., 2009; Zhang et al., 2021). Organic farming, on the other hand, aims to preserve soil quality and fertility while producing healthier food (Stolze et al., 2000; Wood et al., 2006). For sustainable agriculture is important to monitor indicators associated with land degradation (Kuffner et al., 2004).

One of the indicators may be the soil microbiome, that is important for soil fertility because it is engaged in things like nutrient cycling, erosion control and pest and disease regulation (Lori et al., 2017). Anthropogenic interventions, especially agricultural operations, have a significant impact on soil microorganisms, and conventional and organic farming approaches have been discovered to have differing effects on soil microbiome (Kuffner et al., 2004). For assessing soil fertility, microbiological characteristics such as microbial diversity and community structure are particularly important (Lori et al., 2017). Microbial diversity has been found to decrease in response to environmental stressors, compromising the ability of the ecosystem to respond to disturbance and microbial biomass and activity are often higher in organically maintained soils (Bertola et al., 2021). The composition of the microbiome provides details of the condition and history of the soil ecosystem, considering both the immediate condition of the organisms, and the long-term effects on successional processes of the microbial community (Kuffner et al., 2004). Hartmann et al. (2015) compared organic farming and conventionally managed soil fertilized with mineral fertilizers only and showed that organic farming increased the richness, decreased the homogeneity, reduced the dispersion, and changed the organization of the soil microbiome. Moreover Leff et al. (2015) showed that despite significant compositional changes between sites, microbial communities responded to N or P additions in a consistent manner, and the magnitude of these changes was related to the magnitude of plant community responses to N additions, which was characteristic of agricultural soils. Metagenomic data also indicated that nutrient addition reduced the average genome size of bacterial community members and induced changes in the relative abundance of representative functional genes, suggesting a shift in bacterial life history strategies (Leff et al., 2015). Another indicator may be the enzymatic activity of the soil. In their study, Štursová and Baldrián (2011) focused on soil enzyme activity by comparing the profiles of bacterial communities in a field and a meadow. They found that both soil ecosystems were surprisingly similar, with SOM being the most important factor influencing soil enzyme activity. Also, Wallenius et al. (2011) reported that SOM concentration is the main factor determining the level of soil enzyme activity and biomass. According to them, the within-site variability in soil microbiological characteristics is mainly due to the variability in SOM concentration, and SOM content also predicts well the main differences in the level of enzyme activity and microbial biomass between grassland

and field soils. However, microbial variability is not only dependent on variation in SOM concentration, but also on other characteristics of land use type, probably physical heterogeneity (stones, tree roots) and plant diversity. Wallenius et al. (2011) found that enzymatic activities in a meadow have greater variability and coefficients of variation than in a field, implying that increasing plant diversity and/or tillage reduces the variability of microbial activity in the soil. On the other hand, the meadow appeared more homogeneous than the field based on bacterial community profiles, which was probably due to the fact that the permanent rhizosphere is a more stable bacterial environment, and in the field, the disruption of field bacterial communities caused by tillage and cropping, together with the uneven distribution of manure input, created spatially and temporally localised adaptive pressures, which could explain the lower population diversity found within the meadow samples (Wallenius et al., 2011).

2 Aims and hypotheses

The general aim of this thesis was to investigate the ecological processes that determine the composition, life history strategies, and functionality of soil bacterial communities during microbial colonization and succession in experimental microcosms containing sterile soil inoculated with different microbial communities. Specifically, we:

1. Analysed the role of the bacterial pool (i.e., microbial inoculum) in the composition and diversity of soil bacterial communities during colonization and succession in γ-irradiated soil.

2. Investigated the life history strategies (i.e., copiotrophy versus oligotrophy) and functional traits (e.g., maximum growth rate) that are relevant for bacteria during soil colonization and succession.

3. Identified the relative influence of different bacterial assemblages on soil functions (i.e., bacterial activity and nutrient cycling) and services (i.e., soil fertility) regardless of the soil abiotic environment.

We hypothesized that:

1. The composition, diversity, and life history strategies of bacterial communities in soil microcosms will depend on the inoculation source, with bacterial communities tending to resemble those from the inoculum over time. I.e., soil microcosms inoculated with excrement to be more similar to source excrement, and microcosms inoculated with soil to be more similar to source soil.

2. Early colonizer taxa in our microcosms will show higher 16S rRNA copy numbers, smaller genomes and other traits related to a copiotrophy strategy. Soil microcosms inoculated with excrement will show higher number of copiotroph taxa than microcosms inoculated with soil.

3. Bacterial assemblages from soil and excreta will function differently, with soil microcosms inoculated with soil showing higher microbial activity (e.g., respiration) than those inoculated with excrement.

3 Material and methods

3.1 Experimental design

A large-scale laboratory microcosm inoculation experiment was designed to simulate the colonization of sterile arable soil by microorganisms from a different origin. In this thesis, two experimental treatments (S, soil and E, excreta) were evaluated along with a control (F, filtrated soil solution) consisting of a sterile solution from soil. All experimental conditions were set up in triplicate and destructively sampled after 14- and 56-day incubation (3 treatments × 2 time points × 3 replicates = 18 samples). Additionally, source samples (SI and EI) at the time of starting the experiment were analysed, making a total of 24 samples. Experimental design is shown in Figure 3.1. Although other biological groups, such as fungi, can also be involved in some changes in soil properties and microbial activity (e.g., respiration or enzymatic activity), this thesis focuses specially and mostly on bacterial communities.

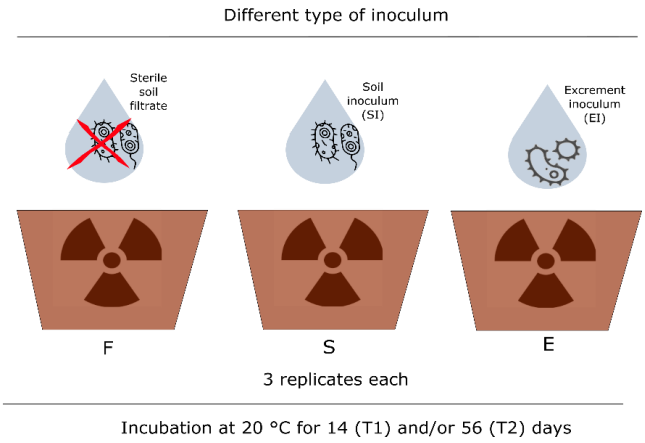

Figure 3.1: Experimental design of γ-irradiated soil microcosmos inoculated with 2 different inocula (SI and EI) and sterile soil solution (F), having three different treatments F, S and E (each of three replicates) sampled after 14- and/or 56-day incubation at 20 °C.

3.2 Soil collecting and processing

The area for soil sampling was in Střížov (GPS 48.881750, 14.525417) and was selected based on preliminary experiments according to soil water holding capacity, basal respiration, conductivity, pH, and field treatment history (data not shown in this thesis). No organic fertilizer was applied on the field for at least 5 previous years. Soil

samples were collected on 13th August 2020, in a winter wheat field shortly after harvest. Three line transects 5 m apart were established. Within the transect line, 5 subsamples (separated 2 m each) were taken, the transects were respected across the whole experiment. Samples were sieved (0.5 cm) and pooled on each transect. A 2 kg aliquot of each transect was separated and stored in a refrigerator at 4 °C and later used for inoculum preparation. Another aliquot of approximately 5 kg was air-dried for 4 days and then placed in plastic bags. The dry soil was weighed to two plastic zip bags, placed to a paper box, and sent for the γ-irradiation to Bioster a.s., Tejny 621, Veverská Bítýška 664 71, Czech Republic. It was irradiated with a total of 50 kGy (2 × 25 kGy with a 3 day pause between radiating cycles). Last irradiation was done on 11th November 2020. The paper box and plastic bags were opened at the time of setting up the experiment.

3.2.1 Inoculum preparation

For the preparation of the fresh soil inoculum (SI), soil stored at 4 ºC was used and pre-incubated for 4 days in the dark at laboratory temperature. For the preparation of the excrement inoculum (EI), intestinal excrements of dairy cows were collected at a dairy farm in the region of South Bohemia. Details about the farm and cattle management can be found in Kyselková et al. (2015). The excrements were sampled on the day of starting the experiment (on 30th November 2020), from the rectum of 7 cows (Red Holstein of age 2–5 years) into plastic examination gloves and homogenized in the laboratory into one sample (analyses were done in triplicate taken from this homogenous sample).

All inoculums were prepared in the same way. A total of 150 g of soil/excrement was weighted, 150 ml of sterile tap water added, stirred with a mixer (Rhonson Hand Blender R-518, 900 W max) at ½ speed for 30 sec and left for 5 min to allow the larger stones to sink. Then the stone-free inoculum was transferred to a graduated cylinder to know the volume and transferred to an Erlenmeyer flask where an equal volume of sterile tap water was added and the content well mixed.

SI (soil inoculum) – prepared from the fresh soil with respect to the transect 150 g of soil + 300 ml of sterile tap water

EI (excrement inoculum) – prepared from a homogeneous mixture of fresh cow excrement 150 g + 300 ml of sterile tap water

F (filtrate) – the remaining SI with respect to transect were filtered by paper filter (Grade: 4b) and then cleared by centrifugation in 50 ml falcons 10 min at 8 000 rpm. Then, 10 ml was collected by syringe and applied through a 0.2 μm filter to the F samples relative to the transects.

3.3 Setting up experimental microcosms

Microcosms were established on 30th November 2020 in plastic containers (300 ml volume, 8 cm base diameter, 9.5 cm top diameter, 6 cm height), closed with perforated lids (4 holes, 2.5 mm diameter) and covered with a ring of filter paper. For sterilisation, the containers, lids, and paper rings were exposed to UV light in sterilisation box (Captair Bio 712, France) for 30 min immediately before use. Microcosms were set up by weighing 80 g of dry γ-irradiated soil with respect to the transect. Using a dispenser, 12 ml of autoclaved tap water was added to each container and then 10 ml of inoculum was pipetted into the samples; treatment F was inoculated by syringe and represents the control. All microcosms were weighed, randomly distributed on trays, and incubated in a Thermostatic Cabinet (Lovibond, Germany) at 20 °C for 14 and/or 56 days. The final inoculation volume was 22 ml (12 ml sterile water + 10 ml of each inoculum) to saturate the soil to 55 % (used soil has a water holding capacity of 50 %, which means that 100 g of dry soil admits 50 ml of water to saturate to 100 %). Moisture in the microcosm was controlled by weighing (weekly) and re-watering (2 ml of sterile tap water was disturbed randomly on the surface of to each microcosm every 2 weeks). The position of trays in the thermostat was changed weekly to minimize the effect of position on the microcosms during incubation.

3.4 Sampling of microcosms

Destructive sampling was performed in two time points T1 – 14 days and T2 – 56 days of incubation. Microcosms were weighted and homogenized, and several aliquots for physicochemical, biochemical, and bacterial analyses were taken (see below). For some analyses, such as DNA isolation and enzymatic activities, the aliquots were kept at −20 °C until analysed. The sample set for further analyses was: 6 source samples (SI and EI, three replicates each), 9 samples for T1 (FT1, ST1 and ET1, three replicates each) and 9 samples for T2 (FT2, ST2 and ET2, three replicates each).

3.5 Isolation of DNA

The DNA was isolated from soil samples with DNeasy® PowerLyzer® PowerSoil® Kit according to protocol as follows. Up to 0.25 g of soil/excrement sample was weighted to the Power-Bead Tube. Added 750 µl of PowerBead Solution to the PowerBead Tube. Added 60 µl of Solution C1 and vortexed briefly. Placed the PowerBead Tubes into the tube holder in the homogenizer (FastPrep-24 5G M.P. Biomedicals) and run the samples 6.5 m/sec for 45 sec. The tubes were centrifugated at 10 000 × g for 2 min and supernatant transferred to a clean 2 ml Collection Tube. Then 250 µl of Solution C2 was added and vortexed for 5 sec and incubated at 4 °C for 5 min. The tubes were centrifugated for 2 min at 10 000 × g and 600 µl of the supernatant was transferred to a clean 2 ml Collection Tube. Then 200 µl of Solution C3 was added to the tube, vortexed briefly and incubated at 4 °C for 5 min. After the tubes were centrifuged for 2 min at 10 000 × g and 750 µl of supernatant was transferred into a clean 2 ml Collection Tube. Then 1.2 ml of Solution C4 was added to the supernatant and vortexed for 5 sec. 675 µl of the supernatant was loaded onto an MB Spin Column and centrifuged at 10 000 × g for 2 min. The flow-through was discarded and the same was done with the rest of supernatant. 500 µl of Solution C5 was added and centrifuged for 2 min at 10 000 × g. The flow-through was discarded and centrifuged again for 2 min at 10 000 × g. The MB Spin Column was placed in a clean 2 ml Collection Tube which remained 10 min with the tube opened lid to evaporate the rests of ethanol. Then 100 µl of miliQ water was added to the middle of the membrane and centrifuged for 30 sec at 10 000 × g to elute the DNA. The concentration of eluted DNA was measured immediately by fluorometer Qubit 4 and chemistry Quibit dsDNA BR Assay Kit (Invitrogen by Thermo Fisher Scientific) according to the protocol as follows. The working solution was prepared by mixing 199 µl of Qubit buffer and 1 µl of Qubit reagent per each sample, mixed by vortex and prepared 198 µl aliquots of the working solution to the 0.5 ml Qubit Assay Tubes (Invitrogen by Thermo Fisher Scientific). Then 2 µl of each sample was added to the tubes, incubated for 2 min at laboratory temperature and measured. DNA was stored at −20 °C in 1.5 ml microcentrifugation tubes for downstream applications.

3.6 Sequencing and sequence processing

3.6.1 Sample preparation for sequencing (16S rRNA amplicons)

The DNA was diluted in deionized water with respect to the previously measured concentration. The final volume was 25 µl with a concentration of at least 10 ng/µl in 1.5 ml microcentrifuge tubes and the caps were fixed with parafilm. DNA samples were shipped for sequencing to Novogene Company Limited (Hong Kong) to a separate facility in Cambridge (United Kingdom) on wet ice and were delivered within 24 hours.

3.6.2 DNA amplicon sequencing

Total bacterial communities in 24 samples were characterized by amplification and high-throughput sequencing of 16S rRNA gene V4 region fragments using the Illumina platform. The quality check, PCR amplification, sequencing and initial bioinformatic processing was done by Novogene Company Limited (Hong Kong), the details are as follows. Primers were 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3'), while each sample contained a unique barcode. PCR amplifications were performed using 5 µl 5 × Q5 buffer, 0.5 µl PCR Nucleotide Mix (10 mM), 1.5 µl BSA (10 mg/ml), 0.25 µl Q5 High-Fidelity DNA polymerase, 1 µl forward primer (10 pmol/µl), 1 µl reverse primer (10 pmol/µl), 5 µl 5 × Q5HighGC Enhancer, 1 µl DNA template (approximately 5–50 ng) and H_2O to a total volume of 25 µl. PCR conditions were as follows: 4 min at 94 °C, 25 cycles of 30 sec at 94 ºC, 1 min at 50 ºC and 75 sec at 72 ºC, followed by 10 min at 72 ºC. PCR products were mixed in equal concentrations and purified using MinElute PCR Purification kit (Qiagen, Hilden, Germany).

3.6.3 DNA sequence processing

Bacterial 16S rRNA amplification and sequencing produced 1 791 431 reads. Sequence processing was performed according to Pérez-Valera et al. (2022) as follows. DNA sequences were denoised and de-replicated in QIIME2 2019.7 (Bolyen et al., 2019) using DADA2 (Callahan et al., 2016) in R statistical software version 4.1.2 (R Core Team, 2020). Taxonomy was assigned with the "classify-sklearn"

algorithm from QIIME2 against SILVA 138 (Quast et al., 2013). After the taxonomy assignment, sequences whose taxonomy identification related to chloroplast, mitochondria, eukaryote, archaea or remained unassigned were purged from downstream analyses. DNA sequences that did not properly align against the SILVA v138 template in "mothur" (Schloss et al., 2009) and whose identification was not possible at the level of phylum were also eliminated. A total of 32 818 amplicon sequence variants (ASVs) were generated. The relative abundance of each ASV was normalized according to the 16S rRNA copy numbers with the function "rarefy_rrna" of MicEco (10.5281/zenodo.1169176) for R. The data obtained were used to describe the taxonomic composition of the bacterial groups present and to infer the values of bacterial traits.

3.6.4 Bacterial traits

The 16S rRNA gene copy number, genome size, and GC content were estimated per ASV with PAPRICA (Bowman and Ducklow, 2015), and community weighted means (CWM) were calculated with the "weimea" package (Zelený, 2018) for R. Phenotypic traits values represented by motility, sporulation ability and Gram staining were also inferred per ASV using the database BactoTraits (Cébron et al. 2021) and expressed as the ratio of the observed binary trait counted from community weighted means for the taxa found in the database.

3.7 Analyses of soil properties

3.7.1 Soil reaction (pH)

Soil reaction was analysed following standard procedures, as described in Pérez-Valera et al. (2018). An 8 g air-dried and grounded aliquot of each soil sample was weighed into a glass bottle and enriched with 40 ml of distilled water. Then the bottles were shaken on a shaker (IKA KS 260 basic, Germany), at 150 rpm for 10 min and stand at laboratory temperature for 5 h. The pH was measured with pH meter (WTW pH 526) and combined pH electrode with liquid electrolyte SenTix 61 (pH range 0:14) at laboratory temperature.

3.7.2 Soil salinity (electrical conductivity)

Electrical conductivity (EC) is a method of measuring soil salinity, indicating the intrinsic ability of the medium to conduct an electric current and it is used as an expression of the total concentration of dissolved salts in aqueous solution. EC was analysed following standard procedures, as described in Pérez-Valera et al. (2018). The saturated extract from previous pH measurement was obtained by filtrating the soil solution trough a paper filter (Grade: 4b) and electrical conductivity was measured by a conductometer (Hanna instruments HI98192) at 25 °C and expressed in µS/cm.

3.7.3 Dry matter

Dry matter on mass basis was determined according to the standard gravimetric method ISO/DIS 11465:1993. Aliquots of approximately 2 g were collected immediately after microcosm sampling in standard aluminium vessels of known weight. The samples were weighted and placed in a dry-air oven for 5 h at 105 °C (the samples were exposed to the 105 °C for 4 h). After cooling, the samples were weighted again, and the dry matter content was calculated.

3.8 Microbial activity

3.8.1 Basal respiration

Basal respiration is used as an indicator of microbial activity during the experiment. It was estimated according to Šimek et al. (2011) as aerobic CO_2 production after 1, 4 and 24 h at the 25 g sample of moist soil, sampled immediately after microcosm destruction. Emitted CO_2 was determined by GC (Agilent Technologies 6850 Network GC system equipped with a 0.53 mm x 30 m x 25 µm 19095P-MS6E column and thermal conductivity detector). The values, recalculated per 1 h and 1 g of dry soil during the 24 h period, were used in this study as basal respiration.

3.8.2 Enzymatic activities

All the enzymatic activities were analysed following standard procedures, as described in Pérez-Valera et al. (2019b).

β-glucosidase activity

The activity was measured in two 0.500 g aliquots from each soil, one as a control. As a standard, 0.5 ml of p-nitrophenol was used in concentrations 10, 20, 50, 100, 200 μg/ml. Then 2 ml of MUB-HCl buffer solution pH 6 was added to each sample, control and standard and 1 ml of distilled H_2O was added to 0 standard and to all controls and samples**,** while to the rest of the standards, 0.5 ml of distilled water was added. To the samples, 0.5 ml of the solution p-nitrophenyl-β-d-glucopyranoside 25 nM (substrate) was added. The samples, controls, and standards were incubated at 37 °C for 60 min. The tubes were then cooled on ice for 5 min. 0.5 ml of $CaCl_2$ and 2 ml of extractant solution (THAM-NaOH pH 12) was added to samples, controls, and standards and, also 0.5 ml of substrate to the controls and standards. Then, all the tubes were vortexed for a few seconds and 1 ml aliquots from well shaken samples were centrifugated for 5 min at 5 000 rpm. The samples were diluted when it was necessary as to be within the limits of the standard curve. The absorbance was measured at 400 nm in the volume of 200 μl by BioTek Synergy 2 SL Microplate Reader (BioTek instruments, USA). The results are presented as μmol PNP/g/h meaning units where 1 unit of activity represents 1 μmol of substrate hydrolysed at 37 °C in one hour per g of dry soil.

Alkaline phosphatase activity

The activity of alkaline phosphatase was measured as the β-glucosidase activity, with three modifications. The reaction pH was adjusted to a higher pH by adding 2 ml of MUB buffer solution pH 11, a solution of 25 mM p-nitrophenyl phosphate was used as substrate and 2 ml of 0.5 M NaOH was used as extractant.

Urease activity

The activity was measured in two 2.000 g aliquots from each soil, one for urease activity and one as a control. Borate buffer (10 ml, pH 10) was added to each sample and control and 1.75 ml of urea solution (substrate) to the samples only. The samples, controls and remaining substrate were incubated at 37 °C for 120 min. The tubes were then cooled on ice for 5 min and 1.75 ml of substrate was added to the controls and 15 ml KCl-HCl (7.4 %) to samples and controls. The samples, controls and standards

were vortexed for a few seconds and then shaken for 30 min at 200 rpm. Then 2 ml aliquots were centrifugated in the 2 ml tubes for 5 min 5 000 rpm. An aliquot of 0.5 ml of clear extract was taken from each of the tubes with samples and controls and pipetted into an empty tube. Standards were prepared by pipetting 0.5 ml NH_4Cl at different concentrations (5, 10, 15, 20 and 25 µg/ml) into the corresponding standards (0.5 ml KCl-HCl for the blank). Then 4.5 ml of distilled water was added to the samples, controls and standards, and then shaken. Then 2.5 ml Na/NaOH salicylate and 1 ml of 0.1 % sodium chloroisocyanide was added to samples, controls, and standards. The samples, controls, and standards were incubated for 30 min in the dark. The absorbance was measured at 690 nm in the volume of 200 µl by BioTek Synergy 2 SL Microplate Reader. Urease activity is presented as µmol $N\text{-}NH_4^+$/g/h, where 1 unit of activity represents 1 µmol of substrate hydrolysed at 37 °C in one hour per g of dry soil.

3.9 Statistical analyses

Statistical analyses were performed according to Pérez-Valera et al. (2022) as follows. The bacterial composition was analysed by non-metric multidimensional scaling (NMDS) generated using Bray-Curtis's dissimilarities with the "phyloseq" package (McMurdie and Holmes, 2013) for R. Compositional shifts were evaluated by permutational analysis of variance (PERMANOVA) (Anderson, 2005), performed using the function "adonis" in "vegan" package (Oksanen et al., 2015) for R. Bray-Curtis's dissimilarity matrix was included as the dependent variable, and treatment, time, and their interaction as independent variable factors. The relationship of bacterial composition and functional traits was tested by performing similar PERMANOVAs with the trait values as additive variables. The alpha diversity was calculated as the Shannon index with the function "diversity" in "vegan" for R.

3.10 Construction of plots

Figures for data visualization were plotted with "ggplot2" (Wickham, 2016) in R Studio. Statistical comparisons among each treatment-time combination were conducted using one-way analysis of variance (ANOVA) and *post hoc* Tukey HSD tests in R, using variables regarding soil properties, bacterial activity, or traits as

dependent variables in independent models. Logarithmic transformation was applied to improve the model assumptions except for the 16S rRNA copy number, Gram positive ratio, and sporulation in which it was not needed.

4 Results

Results of the analyses obtained by examining 24 samples are presented, including 6 source samples used for the inoculation of the microcosmos (soil and excrement, SI and EI, three replicates of each) and 9 samples (3 treatments F – filtrate, S – soil, E – excrement; three replicates of each treatment, with respect to the transects) in each time point sampled after 14- and/or 56-day incubation (labelled T1 and T2).

4.1 Bacterial composition and diversity

4.1.1 Bacterial relative abundance

The relative abundances of bacteria at the phylum level in the source soil and in the inoculated soils after 14 and 56 days obtained by high-throughput sequencing of the 16S rRNA gene is showed in Figure 4.1 and Table 4.1. The most abundant phyla in the source soil used for inoculation of treatment S were Proteobacteria followed by Acidobacteria, Actinobacteria and Verrucomicrobia. While Proteobacteria were the most abundant taxa in S at 14 days of incubation, Acidobacteria lost the position and were displaced among the less abundant taxa. Over time, abundance of Actinobacteria increased only slightly, but Firmicutes gained dominance, together with Bacteroidota, which were more abundant than in the source soil. On the contrary, Verrucomicrobia were representing almost the same part of the community after 14 days as well as 56-day incubation. A closer look at treatment E showed that Proteobacteria became abundant after 14 days of incubation and became even more abundant after 56 days, when they had greater contribution to the bacterial community composition. Firmicutes were the most abundant phylum in the source excrement and were successfully introduced into the soil environment but decreased over time. The abundance of Actinobacteria after inoculation into the soil environment represented a slightly higher relative proportion of the community after 14 days of incubation. Bacteroidota, which were highly abundant in the source excreta, almost disappeared after 14 days, only to reappear after 56 days, but not as abundant. In the control treatment F, Proteobacteria dominated after 14 days of incubation followed by Firmicutes. After 56 days of incubation, the abundance of Proteobacteria increased and that of Firmicutes decreased.

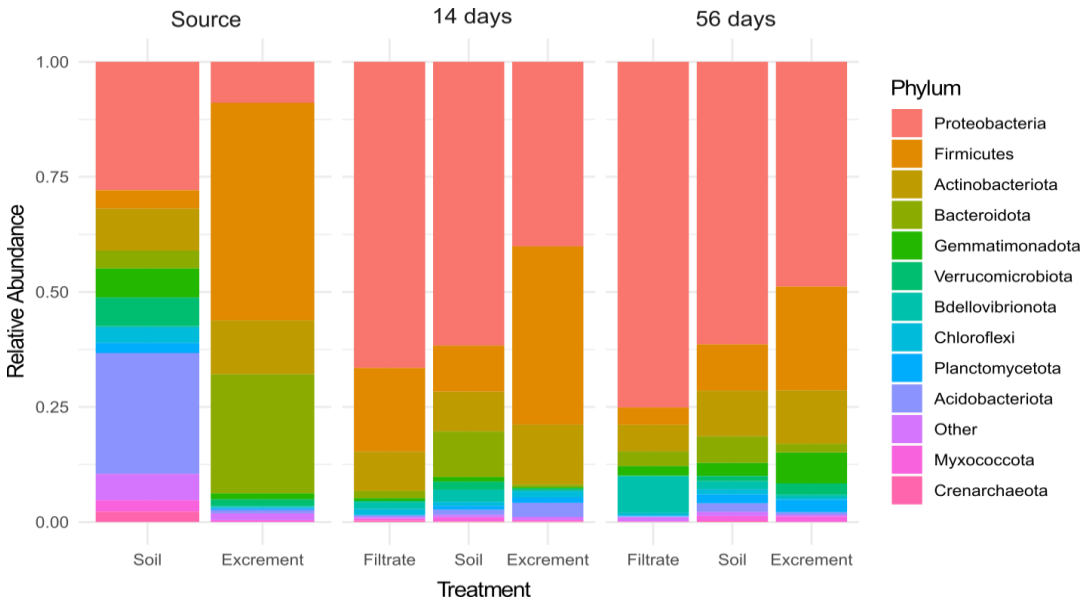

Figure 4.1: Relative abundance of bacteria at the phylum level

Table 4.1: Relative abundance of most important phyla across the experiment in %.

	T0		T1			T2		
Phylum	EI	SI	E	S	F	E	S	F
Proteobacteria	8.9	27.9	48.8	61.7	66.5	40.1	61.4	75.1
Firmicutes	47.3	3.9	38.8	10.0	18.2	22.6	10.1	3.7
Actinobacteria	11.7	9.2	13.0	8.6	8.6	11.6	9.9	5.8
Bacteroidota	25.9	3.9	0.6	9.9	1.6	1.9	5.8	3.2
Acidobacteria	0.8	26.3	3.1	1.2	0.4	0.5	1.8	0.1
Verrucomicrobia	1.5	6.2	0.4	1.8	0.3	2.5	1.1	0.1

The relative abundance of bacteria at the class level is shown in Figure 4.2. Table 4.2 illustrates shifts at the class level, particularly between Gammaproteobacteria and Alphaproteobacteria, as the trend over time differs for all three treatments. In both source environments, the ratio between Alphaproteobacteria and Gammaproteobacteria was similar. While Alphaproteobacteria were slightly more abundant in both environments, this changed when the community was inoculated into the γ-irradiated soil. After 14 days of incubation, Gammaproteobacteria was the most abundant class in treatment S and control F, followed closely by Alphaproteobacteria in all three treatments. After 56 days of incubation, the ratio changed rapidly in the control treatment, and the progression was also evident in treatment E but was not as significant. Another interesting class is Bacilli, while they were not very abundant in

the source samples, after 14-day incubation they became abundant in all treatments, in the treatment E Bacilli were the most abundant taxon.

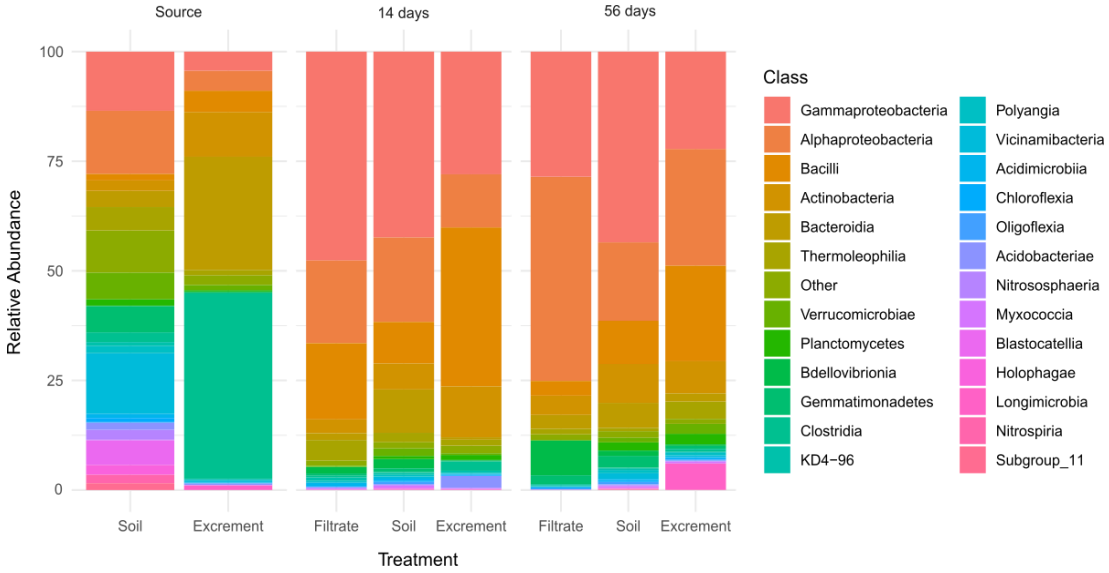

Figure 4.2: Relative abundances of bacteria at the class level

Table 4.2: Relative abundances of selected bacterial classes (%).

	T0		T1			T2		
Class	EI	SI	E	S	F	E	S	F
Gammaproteobacteria	4.3	13.5	28.0	42.4	47.7	22.2	43.5	28.5
Alphaproteobacteria	4.6	14.4	12.1	19.3	18.9	26.6	17.9	46.6
Bacilli	4.9	1.5	36.3	9.5	17.4	21.8	9.9	3.3
Bacteroidia	25.9	3.7	0.6	9.9	1.6	1.9	5.7	3.2

4.1.1 Bacterial diversity

A comparison of the alpha diversity expressed by the Shannon index is shown in Figure 4.3. The differences between treatments S and E were significant after 14 days of incubation, but after 56 days, the values were similar for both treatments. A significantly lower diversity was observed in the control treatment inoculated with filtered soil solution after 14 days of incubation, which decreased even more with time. While the Shannon index remained almost the same for treatment S, it increased in treatment E. The communities in S treatments showed lower diversity levels than those from the source soil on day 56.

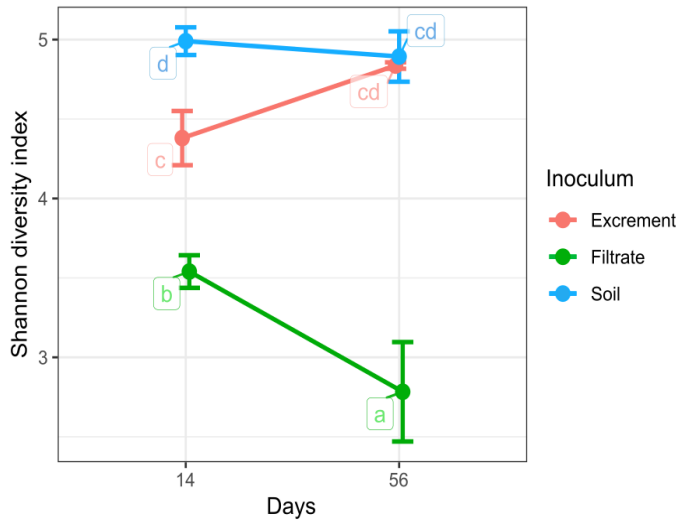

Figure 4.3: Shannon index representing alpha diversity during the experiment. The diversity of the original communities was 5.67 ± 0.02 for soil and 4.22 ± 0.15 for excrement (different letters indicate statistical significance of differences between samples; points represent arithmetic means; error bars and ± indicate SD, n = 3)

The non-metric multidimensional scaling (NMDS) (Figure 4.4) shows the differences in the bacterial community composition across treatments and incubation times and is analysed through Bray-Curtis's dissimilarities. The bacterial community composition within treatments was similar, while there were differences in the bacterial composition over time. On day 14, treatments E and S shared 40.1 % of the taxa while only 28.2 % on day 56. The comparison of treatments S and F showed a similar pattern. After 14 days, the similarity was 21.1 %, but after 56 days it was only 13.2 %. Over time, there were 68.1 % shared taxa between time points in treatment S, and only 51.9 % shared taxa in treatment E. The analyses also showed that bacterial communities tended to be more related to the source soil or excrement, especially in treatment S (21.0 % of taxa shared after 14 days) as compared to treatment E (12.1 % of taxa shared after 14 days). Source samples were the most consistent, as 82.0 % of taxa were common within the soil inoculum and 83.7 % within the excreta inoculum. The bacterial communities differed most markedly between the two original sources, with only 4.7 % of taxa being shared. The highest variability within the treatment was measured in the control samples, with only 43.0 % of taxa shared after 14 days, and 50.9 % of taxa shared after 56 days. Samples in treatment E were different to each other after 14 days of incubation, as only 54.8 % of taxa were shared. After 56 days, data were more consistent, with 71.6 % of taxa shared. Samples in treatment S were

more consistent over time (77.4 % of taxa shared at T1 and 73.8 % at T2). The PERMANOVA analyses showed that dissimilarities between samples during the experiment were more dependent on treatment (p = 0.001, R^2 = 0.404), as treatment explained 40 % of the dissimilarities, and less on time, with 23 % of the dissimilarities explained (p = 0.001, R^2 = 0.235). The interaction of treatment and time explained 22 % of the dissimilarities (p = 0.001, R^2 = 0.216).

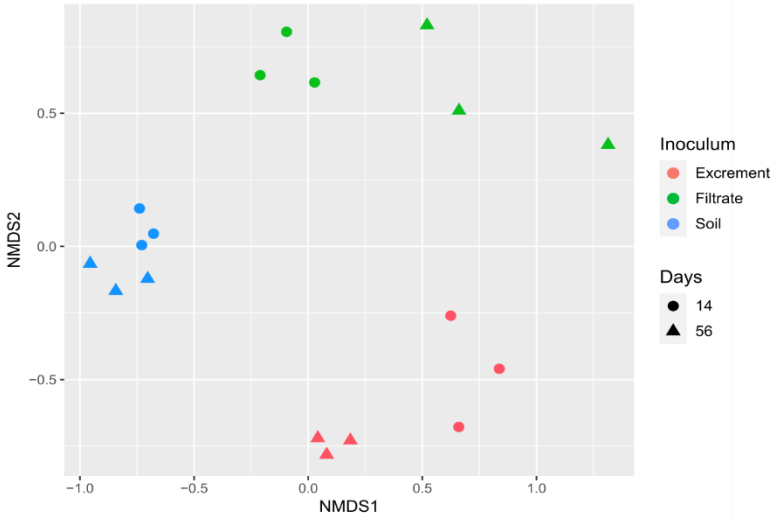

Figure 4.4: Non-metric multidimensional scaling (NMDS) plot of the bacterial community composition in treatments and time (Stress 0.07). Axes do not represent any measured parameter; instead, they form a 2-D space plot based on Bray–Curtis dissimilarities.

4.2 Trait analyses

Trait analyses were in this work represented by community weighted means (CWM) of three genotypic (16S rRNA copy number, genome size and GC content) and three phenotypic (motility, sporulation ability and bacterial cell wall type) bacterial traits to assess changes in bacterial life history strategies during the experiment.

4.2.1 16S rRNA copy number

The copy number of the 16S rRNA gene in the identified taxa from the 16S amplicon libraries was chosen as an indicator of bacterial growth rate and is evaluated in Figure 4.5a. After 14 days of incubation, the copy number of the 16S rRNA gene was higher in all treatments compared to the source samples, with significantly higher values in treatment E. Also, after 56 days of incubation, the values in treatment E were still significantly higher than in the other treatments, although it showed a decreasing trend

as well as the control treatment F. Treatment S had the lowest 16S rRNA copy numbers after 14 days of incubation and did change over time. PERMANOVA showed that the 16S rRNA copy number in bacteria was both time- and treatment-dependent, with 20.9 % and 20.5 %, (p = 0.001, R^2 = 0.209; p = 0.001, R^2 = 0.205, respectively) explained by these interactions.

4.2.2 Genome size

Changes in the predicted genome size during the experiment are shown in the Figure 4.5b. The average genome size showed differences related to treatment, rather than incubation time, as PERMANOVA showed that treatment explained 21 % (p = 0.001, R^2 = 0.210) of the differences, while time explained only 12 % (p = 0.003, R^2 = 0.127). There was high variability within the treatment F, but the genome size was consistent in treatments S and E. There were not significant differences between the treatments but in the treatment S, the genome size was slightly higher than in treatment E in both sampling time points, with growing tendency. The opposite pattern could be observed in treatment F as the tendency was decreasing in time.

4.2.3 GC content

The ratio of predicted GC bases in the bacterial genomes is shown in Figure 4.5c. There was significant variation between treatments and over time. The GC content was shown to be more dependent on time rather than on treatment, while 21.1 % of differences were explained with time and just 14.9 % with treatment (PERMANOVA, p = 0.001, R^2 = 0.211 p = 0.001, R^2 = 0.149, respectively). Also, variability within treatments was high, particularly in the control samples. When comparing the treatments, after 14 days of incubation, treatments S and F behaved similarly, but treatment E showed lower values. After 56 days, however, there was a shift and treatment E showed much higher values than treatment F and an even greater difference was noted for treatment S. During incubation, treatment E showed an increasing tendency and treatment S a decreasing tendency; treatment F showed no significant change over time. The GC contents of both source samples were high throughout the experiment, with only treatment E showing such a high ratio at 56 days of incubation.

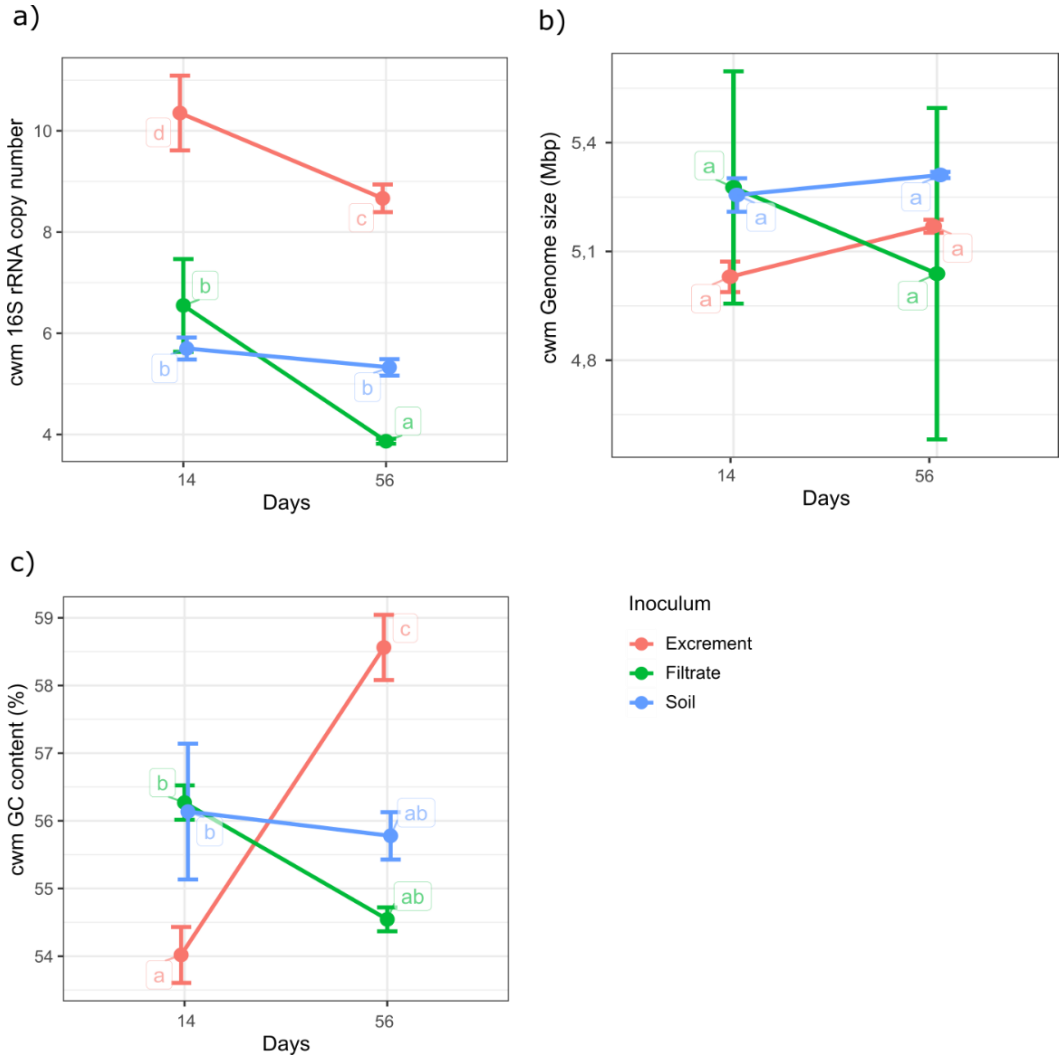

Figure 4.5: Genotypic traits expressed as community weighted means (CWM) of the trait across bacterial communities in the treatments and control at two time points a) Copy number of 16S rRNA gene; source soil 3.00 ± 0.25 and source excrement 5.17 ± 0.24. b) Genome size; source soil 4.94 ± 0.04 Mpb and source excrement 3.79 ± 0.08 Mpb. And c) Percentage of GC content in bacterial genomes, for source soil 58.7 ± 0.6 % and for source excrement 57.9 ± 0.2 % (different letters indicate statistical significance of differences between samples; points represent arithmetic means; error bars and ± indicate SD, n = 3).

4.2.4 Motility

The proportion of potentially motile bacterial community was comparable for treatments S and E at both time points, although much higher compared to the source community, as shown in Figure 4.6a. In treatment F, the number of motile taxa was even higher and increased further with time, with almost 80 % of the taxa with the trait information being motile after 56 days incubation. Motility showed to be more dependent on time rather than on treatment, while 19.7 % of differences is explained

with time and just 10.6 % by treatment (PERMANOVA, p = 0.001, R^2 = 0.197, p = 0.01, R^2 = 0. 106, respectively).

4.2.5 Sporulation

The higher levels of bacteria capable of sporulation were observed in treatment E after 14-day incubation, with significant differences compared treatment S. While the abundance of sporulating bacteria in treatment S did not change over time, there was a decrease in treatment E and also in treatment F, where the decrease was even more pronounced and the final abundance was even lower than in treatment S, as shown in Figure 4.6b. Sporulation ability was more dependent on time than on treatment, with 17.1 % of the differences explained by time and only 8.2 % by treatment (PERMANOVA, p = 0.001, R^2 = 0.171 p = 0.05, R^2 = 0.082, respectively).

4.2.6 Bacterial cell wall type

Type of cell wall in the identified bacteria was shown as occurrence of Gram positive bacteria across the experiment. Figure 4.6c shows that the highest number of Gram positive bacteria was in treatment E immediately after 14 days of incubation and was significantly higher than in treatment S. However, the number of Gram positive bacteria in treatment E decreased over time, but remained almost the same in treatment S, although with a slightly increasing tendency. The greatest changes were observed in treatment F, where there was a significant drop in the abundance of Gram positive bacteria over time, with less than 10 % Gram positive taxa after 56 days incubation. In the source soil, the abundance of Gram positive bacteria was close to 15 %. The type of cell wall was more dependent on time than on treatment, because 20.7 % of differences were explained by time and just 11.1 % by treatment (PERMANOVA, p = 0.001, R^2 = 0.207, p = 0.002, R^2 = 0.111, respectively).

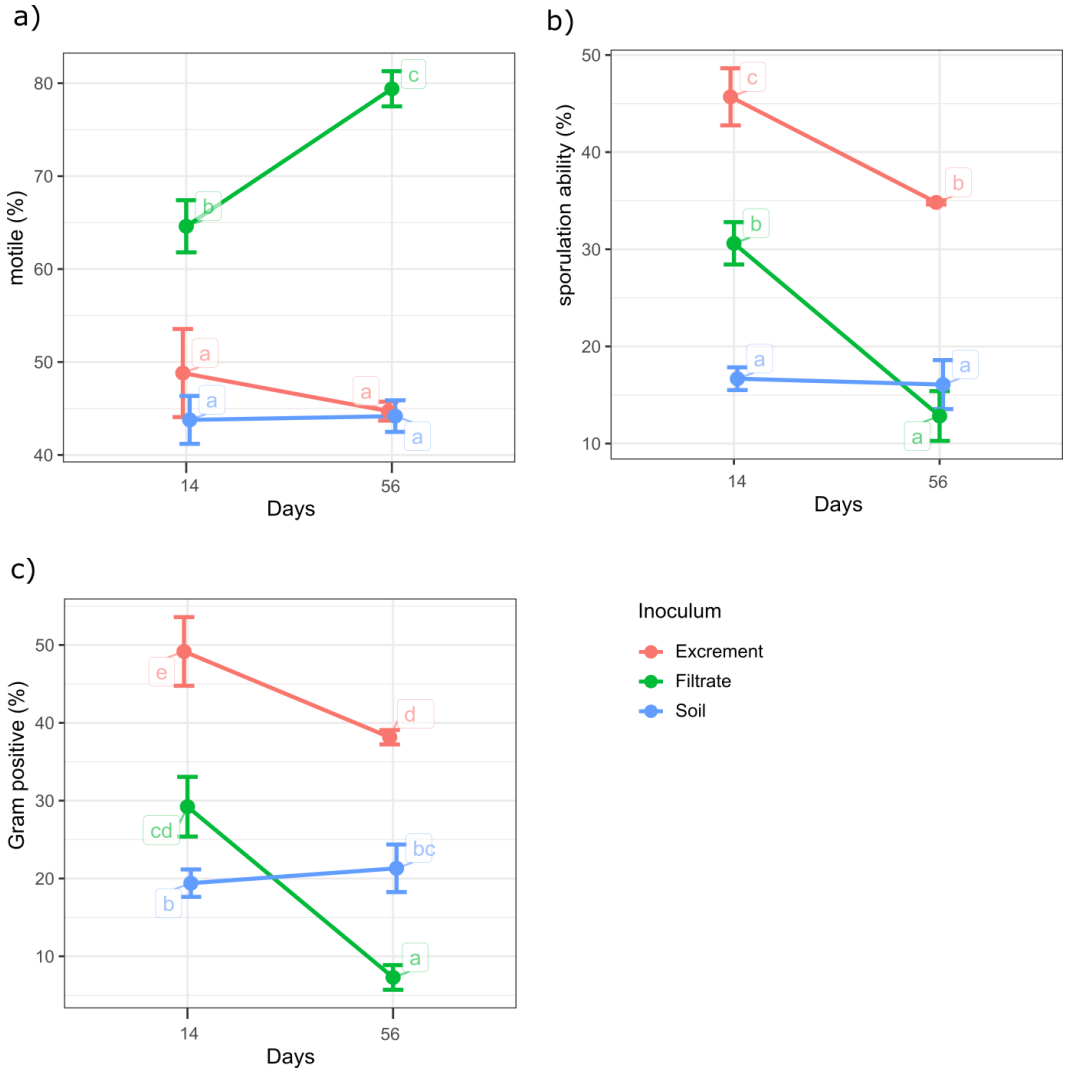

Figure 4.6: Phenotypic traits expressed as community weighted means (CWM) of traits across bacterial communities in the treatments and control at two time points a) Potential motility; source soil 18.8 ± 0.2 % and excrement 8.8 ± 1.2 %; b) potential sporulation ability, source soil 13.4 ± 0.2 % and excrement 17.9 ± 0.6 %; c) Gram positive bacteria occurrence in the experiment compared to identified taxa for this trait, in source soil 14.4 ± 0.4 % and in source excrement 36.6 ± 0.2 %. (different letters indicate statistical significance of differences between samples; points represent arithmetic means; error bars and ± indicate SD, n = 3).

4.3 Soil properties

4.3.1 Soil reaction (pH) and electrical conductivity (EC)

Soil pH significantly differed across treatments (Figure 4.7a), with treatments E and F showing higher values than those of treatment S, whose pH values were close to the source soil. Although pH tended to decrease over time, the differences were non-significant. After 56 days, pH in treatment S showed similar values to the source soil.

The electrical conductivity (Figure 4.7b) in treatment S showed significantly higher values when compared to the other treatments, and after 56 days the difference was even bigger. The values tended to increase over time for all samples and showed the opposite tendency to the pH values. Treatment S showed significantly different values from treatments E and F, and this difference widened over time. Compared to the source soil, EC values tended to increase for all treatments, heading away from the values measured in the source soil.

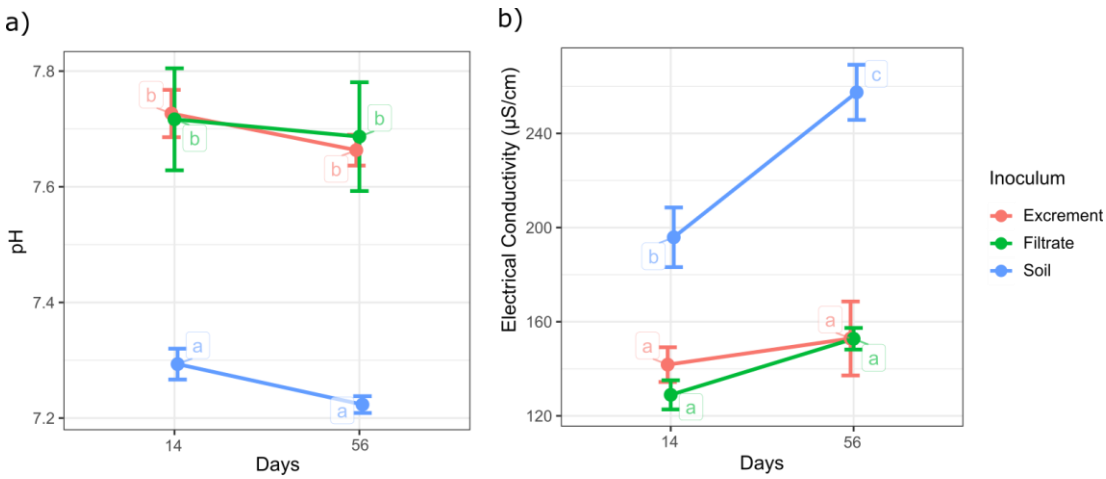

Figure 4.7 Soil reaction and soil salinity expressed as pH (a) and EC (b) in the treatments at two timepoints. pH of source soil was 7.19 ± 0.16 and excrement 6.68 ± 0.4. EC of source soil was 110.9 ± 8.1 µS/cm and excrement 1705 ± 119 µS/cm (different letters indicate statistical significance of differences between samples; points represent arithmetic means; error bars and ± indicate SD, n = 3)

4.4 Microbial activities

4.4.1 Basal respiration

The release of CO_2 from the soil during the experiment was measured as basal respiration. Figure 4.8a shows that higher basal respiration was in treatment S than in treatment E after 14 days incubation. After 56 days of incubation, the respiration levels in treatment S were still significantly higher than those in treatments E and F. Basal respiration tended to decrease in all samples over time. When compared to the source soil, the respiration was higher in treatment S after 56 days but slightly lower in samples inoculated with excreta and the control samples inoculated with filtrate.

4.4.2 Enzymatic activity

Of the three enzymatic activities measured during the experiment, urease activity showed the greatest variation between treatments over time as is shown in the Figure 4.8b. There were not differences between samples after 14 days of incubation and urease activity was low in all samples. After 56 days of incubation, however, there were significant differences between treatment E, which showed the highest values, and treatments F and S, which remained low. Compared to the source soil, the activity after 14 days of incubation was much lower in all samples, but it showed an increasing tendency in treatment F and was even 2 times higher in treatment E than in the source soil.

β-glucosidase activity showed no significant changes over the course of the experiment, either over time or between treatments. Figure 4.8c shows that after 14-day incubation, all treatments had similar values, but after 56 days of incubation, they had a different pattern. While treatments S and E showed a slightly increasing tendency over time with the similar values, control treatment F showed a decreasing tendency. The highest values of the soils measured had the source soil and no experimental samples reached these values until the 56 days of incubation.

Also, alkaline phosphatase activity did not show any significant differences across the experiment (Figure 4.8d). The values were comparable in all treatments of 14- and 56-day incubation, and even if the tendency is increasing during the time, this was not significant. None of the treatments could reach the levels of the source soil during the experiment. There was a high variability within the treatments.

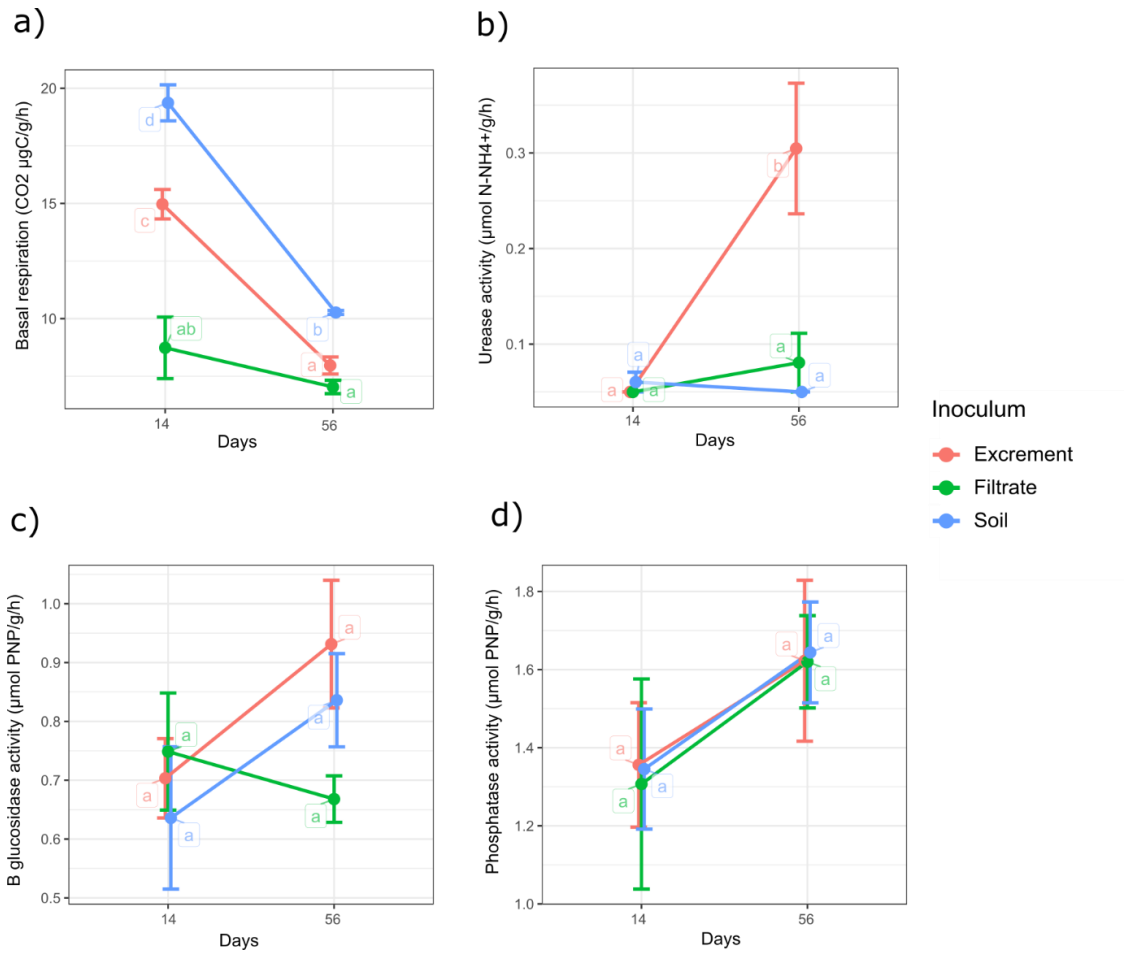

Figure 4.8 Microbial activity presented as a) basal respiration of CO_2 in the period of 24 hours expressed as amount of C (µg) released per hour, when basal respiration of source soil was 8.4 ± 1.2 and of excrement 2468.9 ± 29.4. and b, c, d) enzymatic activities as units, when 1 unit of activity represents 1 µmol of substrate hydrolysed at 37 °C in one hour per g of dry soil. Values of source soil and excrement are as follows (respectively) b) 0.15 ± 0.02 and 0.62 ± 0.05; c) 2.00 ± 0.22 and 32.47 ± 3.19; d) 2.32 ± 0.56 and 105.6 ± 19.0 (different letters indicate statistical significance of differences between samples; points represent arithmetic means; error bars and ± indicate SD, n = 3).

5 Discussion

5.1 Overview

Elevated levels of microbial biodiversity in soil are important for soil functions. Agricultural practices that harm soil microorganisms, on the other hand, cause soil degradation and have an impact on soil fertility. Organic fertilizers, such as fresh excrement or manure, improve soil characteristics while simultaneously introducing exogenous bacteria into the soil which relationship to soil biodiversity is unclear. Understanding the community assembly and the effects of soil bacteria on soil attributes, such as fertility, quality and productivity, is essential for preserving biodiversity and sustainable agriculture. This study is focused on the community composition, diversity, and life history strategies of bacterial communities during colonization of sterile soil, as well as their impacts on soil characteristics and microbial activity. Sterile arable soil was used as a basal substrate in experimental soil microcosms, with microbial populations reintroduced from two different sources, namely soil and cow excrement. According to the findings, bacterial composition and diversity were highly influenced by the inoculation source, and at the same time, the bacterial communities in different treatments significantly altered soil characteristics and bacterially mediated functions. This study contributes to a better knowledge of how the initial bacterial pool influences bacterial biodiversity in soil, and how this biodiversity, in turn, may influence changes in soil fertility and bacterial activity via diverse life history strategies and traits using simple bioinformatic tools.

5.2 Bacterial composition, diversity, and life history strategies

Bacterial communities in the microcosms, containing γ-irradiated soil inoculated with cow excreta or soil, mostly showed dominance of Proteobacteria (mainly Gammaproteobacteria and Alphaproteobacteria), Firmicutes and Actinobacteria. These groups are normally abundant in soil (Delgado-Baquerizo et al., 2018). However, the fact that many members of these groups are fast-growing species suggests that they may be early colonizers (Ortiz-Álvarez et al., 2018), which could explain their over-representation in our experiment in the first stages (i.e., after 14-day incubation). Indeed, our data based on analysing the average number of 16S

rRNA gene copies per cell, point out in this direction. This was especially observed when inoculating excreta, which resulted in taxa with the highest average 16S rRNA copy numbers, as it occurred in the source excreta as compared to the source soil. Elevated 16S rRNA copy numbers could provide taxa with adaptative advantages that could be important during the early stages of colonization and succession. For example, Nemergut et al. (2016) found more than 9 copies/individual during primary succession, and their results were validated in different environments, such as boreal forest soil after fire (Whitman et al., 2019) or glacier foreland (Kim et al., 2017). They suggest that these taxa may take advantage of their ability to grow fast (copiotrophy) but also the lack of competition (empty niches) and availability of resources (Nemergut et al., 2016). Our data also suggest that motility could be a key characteristic of microorganisms during initial colonization and early succession in soil, as we observed that approximately half of the taxa had motile abilities compared to 10–20 % of the community in the source material. The analysis of other traits, such as the average genome size or GC content, also confirms our findings. In contrast to excrement communities, soil communities are characterized by larger genome size, which is likely selected for due to resource limitation (Merhej et al., 2009; Chuckran et al., 2022). Larger genome size could provide bacteria (mainly oligotrophs) with more metabolic tools to cope with resource limitation (Barberán et al., 2014). Schmidt et al. (2018), observed that bacteria with large genomes and initially high 16S rRNA copy numbers were successful colonizers, but the trend changed over time in favour of taxa with even larger genomes but lower 16S rRNA copy numbers. In our experiment, the genome size in all treatments was more comparable to the source soil than to the excreta, indicating the presence of taxa able to draw from different sources or a good ability to adapt to new conditions. On the other hand, Gram-positive bacteria, which are considered more as K-strategists, i.e., oligotrophs, were very abundant in the samples inoculated with excreta after 14 days of incubation. This feature may explain the concomitant higher abundance of sporulating bacteria (Drenovsky et al., 2010), with the abundance of both traits decreasing similarly with time in treatments E and F, but remaining the same in treatment S.

Although γ-irradiation is the recommended method of soil sterilization because it preserves soil structure and most of the soil properties (McNamara et al., 2003), the death of living cells usually releases labile C that can promote the initial dominance of copiotrophic taxa, that are able to utilize the available carbon. Once readily available

resources are depleted, resource-specialized taxa, usually oligotrophs, which are more abundant in resource-limited soils, may gain a selection advantage (Fierer et al., 2007). The average GC content was lower in all treatments than in the source material, which would also be consistent with higher C availability. Chuckran et al. (2022) suggest that greater GC content may be a selection advantage when carbon sources are partially unavailable. It could also explain the spike in the GC-rich community for the excreta treatments when carbon sources were depleted. In contrast, the soil-origin community was more stabilized.

Despite the overall dominance of Proteobacteria, Firmicutes and Actinobacteria in our microcosms, bacterial taxa varied depending on the source of inoculation. While control microcosms, consisting of γ-irradiated soil inoculated with sterile soil solution, were dominated mainly by Proteobacteria and Firmicutes, microcosms inoculated with soil and excreta had lower numbers of Proteobacteria and higher numbers of Bacteroidetes and Firmicutes. The diversity was similar across time for E and S treatments, but lower for F, indicating that γ-irradiation and sterile solution inoculation show less diversity than soil and excrement inoculum treatments, confirming the effectiveness of sterilization. Indeed, microcosms inoculated with soil showed communities more like those of the source soil, while microcosms inoculated with excreta showed communities more similar to source excrement. This highlights the importance of the initial microbial pool during early colonization and succession (Ortiz-Álvarez et al., 2018), which results in different communities in similar soil environments despite some studies suggesting that soil microbial composition may depend entirely on soil properties (Rousk et al., 2010; Kim et al., 2016; Bang-Andreasen et al., 2017). Calderón et al. (2017) confirmed the importance of soil properties in modulating colonisation but also showed that the establishment of complex microbial communities in degraded soils is strongly influenced by the nature and strength of interactions between species. In addition, it is also suggested that physicochemical properties can be overcome if the right combination of competing species is ensured, allowing the community to be resilient towards a stable equilibrium (Calderón et al., 2017).

5.3 Enzymatic activity and microbial impact on soil properties

Our results showed important changes in soil properties (i.e., pH and electrical conductivity) among treatments, despite the soil microcosms being similar in the beginning and using small inoculation volumes to ensure sufficient microbial biomass with the least impact on soil properties. This suggest that changes in soil pH and electrical conductivity were driven by the biotic communities. Compared to the filtered control, excreta inoculation had no effect on pH, but inoculation with a soil solution decreased pH. This could be related to the decomposition abilities of microorganisms present and use of C, humic acid transformation, or either dissolution or precipitation of rock and soil minerals depending on the type of bacteria and the available energy and nutrient sources (Or et al., 2007). The community from the source soil appeared to be the only one with these capabilities, given how pH and conductivity differed in treatments S from E and F, which were comparable. This reinforces the importance of the presence of a bacterial community that is able to not only survive in the soil but also draw from more diverse sources, thus allowing multiple taxa to coexist leading to greater diversity and better ecosystem services.

No changes in β-glucosidase and phosphatase activities were observed, which may be due to the availability of resources due to γ-irradiation. However, with respect to nitrogen cycling, there was an increase in the urease activity in the excrement-treated samples after 56 days of incubation. This might indicate that nitrogen content is becoming limiting, as readily available nitrogen may have been depleted and the bacteria released urease to obtain a less available form from organic matter (Adetunji et al., 2017). This also suggests that not only the composition of the microbial community, but also how readily available resources are in the soil determines how the microbiome performs functions and ecosystem services in the soil.

Conclusions

Based on the diversity and composition of bacterial communities as well as data on functional traits and soil properties, it was concluded that:

1. The composition, diversity, and life history strategies of bacterial communities in soil microcosms depends mainly on the inoculation source, with smaller differences depending on the incubation time. Although the composition of bacterial communities shows elevated differences to that from the original sources, soil microcosms inoculated with excreta are slightly more similar to excrement inoculum, and those inoculated with soil more similar to soil inoculum.

2. Sterile soil microcosms inoculated with microorganisms from different sources have higher 16S rRNA copy numbers, smaller genomes, and higher potential motility than those from the original sources, suggesting that bacterial communities are dominated by taxa showing copiotrophic strategies. The differences in functional traits between treatments also highlight the role of the bacterial pool during colonization and succession.

3. Excreta bacteria can establish a living community in a sterile soil but are less active than those from soil. This suggests that soil bacteria have a higher potential to alter the soil environment than bacteria from excreta.

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ASV – Amplicon Sequence Variant

CWM – community weighted mean

DNA – deoxyribonucleic acid

EC – electrical conductivity

NMDS – non-metric multidimensional scaling

PCR – polymeraze chain reaction

PERMANOVA – permutational multivariate analysis of variance

rRNA – ribosomal ribonucleic acid

OTU – operational taxonomic unit

SOM – soil organic matter

UV – ultraviolet