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"If you fall, I'll be there - The ground"- *unknown*

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General Introduction

Why are we interested in soil?

Soil is our solid foundation. It grounds us. It is the basis for the houses we build and for the paths that connect us. Soil fuels all terrestrial food webs by being essential for the growth of higher plants and thus supports more than 95% of all food that is produced globally (FAO, 2015). There are 24 soil "ecosystem services" humans benefit from, including water purification, fresh water supply, climate regulation, fiber and food production, as well as cultural and recreational services (Millennium Ecosystem Assessment, 2005). Humanity therefore strongly depends on soil which makes it of utmost importance to understand its functioning. While we have found ways to investigate the deep interior of the earth (Ma et al., 2003; Lin & Oglivie, 2019) and the other planets in the galaxy (Brennan et al., 2020) which are physically inaccessible for us, we seem to be just beginning to identify the organisms, discover the structure, and understand the processes in the upper 20 cm of the ground we walk on every day.

As part of the outermost surface of the earth, soil separates us from the inner layers of the globe (Stanley, 2001), while it connects us to the surrounding ecosystems, constituting an interface with the oceans. Soil is where the hydrosphere, the atmosphere, the lithosphere, and the biosphere interact (Wall, 2012). A multitude of essential chemical and physical processes occurs at these interfaces. Regarding its volume, soil itself is composed of air, water, mineral compounds, and organic matter. From the latter, about a fifth is "living", including microorganisms, soil fauna and plant roots. The rest is considered soil organic matter (SOM, also humus in the older literature), or dead organic matter at different levels of decomposition and transformation (Kalev & Toor, 2017). SOM mainly consists of microbial remains, i.e. organic material of plant origin, which has been taken up and transformed by soil microorganisms (Gleixner et al., 2001; Fierer 2017). SOM chemically interacts with the charged surfaces of the soil minerals causing hot spots of microbial decomposition (Rasmussen et al., 2018).

Carbon (C) cycling in soil is of main global interest. Soil represents the largest terrestrial C reservoir and the largest pool of organic C globally, containing about 1,500 Gt of this important element (Jackson et al., 2017; Stockmann et al., 2015; Post et al., 1982).

Autotrophic C is transformed by heterotrophic soil organisms, either used for growth or mineralized for energy generation and subsequently released to the atmosphere as CO_2 or CH_4. The remains of heterotrophic microbes make up most of the soil organic matter, together with chemically altered plant litter (Gleixner et al., 2001; Fierer 2017). These transformation processes, as well as the nutrient cycling in soil, are dependent on environmental conditions, such as temperature and soil moisture (Allison et al., 2010; Post et al. 1985; Yang et al., 2013). This puts soil at the center of our interest with regard to Global Change (Schlesinger & Andrews, 2000). An increased loss of the greenhouse gases CO_2 and CH_4 from soil may possibly lead to a positive feedback on global warming - a major concern of the present (IPCC, 2013).

Why don't we know more about soil yet? Why is it so challenging to decipher soil processes and their controls?

Soil is arguably the most heterogeneous habitat on earth (Tibbett et al., 2020) - it is a well-structured rather than a well-mixed system. At the landscape level, soils differ in age, vegetation cover, and bedrock material. On a pedon scale, the spatial heterogeneity is expressed as distinct layers - the soil horizons. How many of these layers are distinguishable at site, as well as their extent and development vary in soils (Schoeneberger at al., 2012; Thompson et al., 2012; IUSS Working Group WRB, 2015; Zhang et al., 2020). Within a horizon, the proportions of inorganic and organic components might differ, causing heterogeneity in texture and physical properties of the respective soils. This further affects the occurrence and abundance of soil organisms by shaping pore space and influencing its water and O_2 concentrations (Nannipieri, 2020). On a microscale, the presence of clay minerals, oxides, and hydroxides strongly influences the properties of a soil and the rate of its biogeochemical reactions (Gleixner et al., 2001). This immense complexity across all scales complicates the process of finding universal answers to general scientific questions concerning soil.

Approximately 30% of the world's surface are covered by land (The World Factbook, 2020). The soil below these 150 Mio. km^2 contains the major players of element cycling which mediate processes whole ecosystems rely on - the microorganisms (Vogel et al., 2009). Taxa from the three domains of life regulate the concentration and availability of all essential elements through their metabolic activity. Terrestrial prokaryotes are just a few

µm in size and exist since hundreds of millions of years. They inhabit so-called soil microsites, 3-dimensional structures varying in physical and biochemical conditions, forming niches, and constituting the spatial heterogeneity in soil (Madigan et al., 2009). This combination allowed the diversification of microorganisms and has led to an overwhelming genetic and biological diversity. One gram of a typical soil contains more than 10^9 cells and 10^5 to 10^7 different taxa (Zhou et al., 2020; Tibbett et al., 2020). There are more microbial cells in one ton of soil than there are stars in our galaxy (Gans et al., 2005). The biological and spatial variability of soil, developing over a long period of time led to what is regarded the biologically most complex habitat on earth (Tibbett et al., 2020).

To investigate processes within soils, we usually measure pools and metabolic parameters at a cm to m scale. This is an extremely large volume or area (mm^3 - cm^3 or dm^2 - m^2) compared to the size or volume of microorganism (0.2 - 100 µm or 0.005 μm^3 – 0.2 mm^3 (Madigan et al., 2009; Christensen et al., 1999; Tibbett et al., 2020). If for example, the heterotrophic activity of the microorganisms in a soil shall be assessed, the soil CO_2 production is measured in the absence of roots. This is usually performed with soil chambers covering 0.1 - 0.5 m^2 of the soil surface or in incubations with several to several hundred cm^3 (Davidson et al., 2002; Fang et al., 2020). The resulting soil respiration is then an expression of the community respiration of many billions of microbial cells and their interactions, and of a plethora of various microsites differing in environmental conditions. This prevents to link the contribution of specific microbial taxa or interactions of taxa to the respective soil processes, such as the degradation of organic material (Fierer, 2017). Which of the present microorganisms exactly interact, will remain unclear, because their spatial distribution is not understood, thus limiting a mechanistic understanding of large-scale fluxes (Fierer, 2017).

In addition, only about 0.3% of the total soil microbes are cultivated yet (Amann et al.; 1995; Vogel et al., 2009; Martiny, 2019). It is still unclear, with which environmental parameters the remaining 99.7% are confronted at their microsite. But the mimicking of the required organic substrates and inorganic nutrients, as well as the environmental conditions like pH, water content, or O_2 concentration, is essential for the successful cultivation of microorganisms in the lab. It becomes even more complex, when the taxa of

interest rely on “quorum sensing” and with this on a certain number of cells present for cell division and growth (Gupta et al., 2017; Xia et al., 2019; Nannipieri, 2020). But microorganisms do not only depend on cells from their own kind. Since they often occur in consortia in soil, it is crucial for some strains to be co-cultivated with their syntrophic partners which promote or even enable growth by providing essential substances or allow to degrade complex substances in “teams” (Madigan et al., 2009; Lewis & Ettema, 2019). The details of such dependencies are still not fully explored, and this lack of understanding contributes to the low cultivability of soil microorganisms and to large uncertainties regarding their role in soil processes.

The implementation of biomolecular methods during the last years, drastically increased our knowledge concerning the identity of microorganisms from soil, even if they are not yet cultivated (Nannipieri et al., 2020). Amplicon sequencing targets unique areas within the variable coding regions of the ribosomal DNA, which are characteristic for a certain organism. From short reads and assembled contigs this leads to lists of strains, providing information about the identified organisms and their cultivated “relatives”, present in the respective samples (Widder et al., 2016; Fierer, 2017). Metagenomics based on shotgun sequencing of extracted DNA , termed metagenome-assembled genomes (MAGs), allows to decrypt the genetic information of (some) community members from soil samples (Hugerth et al., 2015; Parks et al., 2017; Kroeger et al., 2018; Wilkins et al., 2019). Nevertheless, sequencing data alone do not allow linking specific microbes to a process. First, the DNA present in soil does not reveal, if a strain is living or not, since extracellular or relic DNA, i.e. DNA that has not (yet) been degraded is also found in soil (Carini et al. 2020). It is additionally unclear, if a strain whose DNA was found, is actively contributing to a soil process or not (Nannipieri et al., 2020; Blagodatskaya & Kuzyakov, 2013; Breitwieser et al, 2019). The existence of a sequence cannot tell if the respective gene is actually expressed, or, when expressed, if also translated. The steps of transcription, translation, and post-translational modification stand between a genetic potential and the potential role in a process (Sergaki et al., 2018; Nannipieri et al., 2020). Additionally, a process not only depends on the enzymes produced by an organism, but also on the availability and accessibility of the respective substrates the enzymes act on (Gleixner et al., 2001; Schnecker et al., 2019). So even 16S-based amplicon sequencing or

metagenomic information cannot inform, to which processes a specific organism contributes. Further, the details of species interactions have yet to be explored from genetic information. Microbes have been shown to interact on average with a surprisingly low number of about 100 neighboring cells (Raynaud & Nunan, 2014; Widder et al., 2016). In general, most of the available C substrates are high-molecular-weight substances, which must be processed extracellularly before they can be taken up. Microbes produce enzymes which are released to their environment and depolymerize the substrates to smaller molecules (such as mono- and dimers) outside of the cell, which are then incorporated through uptake systems in microbial cell membranes. The products from degradation are thus public goods and a range of microorganisms have evolved as "cheaters", which do not contribute to SOM depolymerization by actively excreting extracellular enzymes, but nevertheless take up the products of the community (Dunham, 2007; Widder et al., 2016). So far, it is still largely impossible to link the produced substances found in the soil to their producers. Information concerning details of such interactions of soil microorganisms is hard to infer from sequencing data.

To make things even more complex, many microorganisms are known to work together, performing a so-called "co-metabolism". Microbial interactions such as competition, amensalism, parasitism, commensalism, mutualism, and neutralism are usually defined by the impact of the interaction on the fitness of the organism involved: Co-metabolism specifically means the degradation of substances which do not support growth of the degrading organism but are nevertheless processed in the presence of a primary energy source (Campbell & Reece, 2008; Perez-Garcia et al., 2016). The range of the sum of capabilities from the single taxa becomes broader through these metabolic interactions with other organisms (Madigan et al., 2009). These emerging properties within and between consortia are in the center of the scientific interest and yet have to be unraveled for soil (Baveye, 2009; Widder et al., 2016). These knowledge gaps complicate the understanding of the regulation of microbial soil processes. Additionally, all soils are different. There is not *one* set of organisms who fulfill a certain role (Fierer, 2017; Nannipieri et al., 2020). It is therefore often difficult or almost impossible to reproduce experiments, because the highly diverse soil communities permit to work with exactly the same species and interactions twice.

How were complex processes investigated in the past, and which approaches have been successful before? From the prokaryotic model organisms to the model ecosystems

Complexity does not only concern ecosystems or ecosystem compartments, such as soil, or microbial communities. It is also found on the level of the single cell, concerning the regulation of the cell metabolism, movement, signaling and more (Campbell & Reece, 2008; Madigan et al., 2009).

An effective way to study complex processes and structures was to make use of model organisms or systems. A model organism can be understood as a non-human "species" which serves as a surrogate for more complex organisms by being simply or traceably structured in a certain biological aspect of interest (Akeny & Leonelli, 2011; Dietrich et al., 2014). A model organism is chosen to have certain characteristics, such as a high mutation rate, genetic manipulability, short generation time, high fertility, mapped genome, etc., which allow investigating biological processes in detail, and to later infer the gathered knowledge to other organisms, with a more complex or unknow structure (Akeny & Leonelli, 2011; Dietrich et al., 2014). Growing *Escherichia coli* (*E. coli*) in pure culture was probably one of the first and still widely used approaches in this context. Blattner and colleagues deciphered *E. coli's* complete genome 1997, enabling the study of gene regulation of organisms in the environment (Blattner et al., 1997). Researchers are still investigating *E. coli's* genetic and metabolic potential today. With the tools of genetic modification and gene editing, gene functions were established, for example through knock-out techniques (Santiago et al., 2008; Spiegel et al., 2019), or the size of a minimum essential genome was assessed by reductive manipulation (Kurokawa & Ying, 2020). *E. coli* was and is also used as a model organism to study evolution in the lab (Blount et al., 2008). In another model system, *Bacillus subtilis* (*B. subtilis*), genome analysis shed light on the importance of bacteriophages, their role in horizontal gene transfer, and the spread of bacterial pathogenesis (Kunst et al., 1997). Various open databases resulted from the cooperation of scientists and made this Gram+ bacterium becoming an important target of metabolic engineering. It is now used for industrial applications as "cell factory" and for the development of pharmaceuticals and bio-products worldwide (Gu et al., 2018). The successful implementation of such techniques, addressing organisms in pure cultures in

the lab, is nevertheless applicable to cultivable taxa only. Additionally, respective growth media are designed to fully cover the nutrient requirements of the taxa in culture. It remains unclear, which strategies would be pursued to deal with limitations or how interaction with other strains in a structured environment like soil would influence the cellular regulations of the organism (Hatzenpichler, 2020). Such research questions are of main scientific interest and can only be answered by using other experimental systems.

Cells with distinct functions, which communicate and act coordinately to fulfill essential processes are not only found in soils. They are the basic requirement for the functionality of every multicellular organism. The use of biologically more complex model systems than *E. coli* or *B. subtilis* revealed, how cells communicate with each other and how these interactions influence the shape and fate of a whole organism. Groundbreaking insights into fertilization and early embryogenesis were possible through related experiments with *Danio rerio* (van Eeden et al., 1996; Streisinger et al., 1981), *Drosophila melanogaster* (Lewis, 1978; Hughes et al., 2018; Otto & Payseur, 2019), *Arabidopsis thaliana* (Hamamura at al., 2011), *Caenorhabditis elegans* (Brenner, 1974; Link & Jantsch, 2019), and more. Research projects using model systems revealed the role of transposable elements and RNA interference (Fire et al., 1998; Tomari & Zamore, 2005) as well as the genetic regulation of apoptosis (Gumienny et al., 1999; Kuranaga, 2011), neurogenesis (Herbomel et al., 2001) and organogenesis (Baker & Woollard, 2019). The pioneer work of Shimomura (1979; 2009) and Chalfie and colleagues (1994) with multicellular model systems allowed later the use of the green fluorescent protein (GFP) as labelling tool. The photorespiratory pathway and the development of floral structures were explained in *Arabidopsis* (Woodward & Bartel, 2018), while Sturtevant's work with *Drosophila* was crucial for the development of genetic maps (Sturtevant, 1913), and the experiments of Chen and colleagues (2002) led to a basic understanding of gene regulation in the context of behavioral biology. The outstanding suitability of some species as model systems is not only expressed in the essential knowledge gain they enabled, but also in their long-lasting implementation and the extension of the applicable fields since their introduction for example to disease, cancer, and drug research (Arvanitis et al., 2013; Sieber et al., 2019; Maynard & Weinkove, 2020).

To study communities and their interactions, model ecosystems were of great value too. Different plant species were cultivated together in pots in the glasshouse or in field settings, where they were exposed to the natural climate. Such artificially designed communities helped to identify interactions between plant species and of plant communities with their environment. They were of great value and extensively used when exploring the relationship between plant diversity and ecosystem functions, such as productivity (Tilman et al., 2001; Tilman et al., 2006), or element cycling and trophic interactions (Roscher et al., 2004). Other studies focused on removal experiments, which for example tried mimicking non-random distinction (Díaz et al., 2003) to gain insights into the relation between (functional) diversity and ecosystem services in the field of conservational biology.

Lawton and colleagues went one step further with the introduction of the "ecotron" concept (1993), that allowed putting parts of complex ecosystems from the field into a controlled environment. In such an experimental design it was possible to investigate complex food webs, including consumers and predators, during community development (Lawton et al., 1993), by controlling many environmental factors. Ecotron experiments can, however, also allow exposing artificial communities to desired environmental conditions and to repeat the experiments under exactly the same abiotic conditions when analyzing processes on a community level (Lawton, 1996). Such a managed system allowed for example a better understanding of how plant diversity influences the water regime over the course of the day, provided predictors for modelling water and energy fluxes (Milcu et al., 2016), and produced evidence regarding how functional plant groups will potentially deal with weather extremes in the future (Milcu et al., 2016).

How can micro-heterogeneity in a complex system be handled experimentally, and how can questions be addressed at this "inaccessible" scale?

Although researchers were able to control the plant composition in the ecotron studies, the identity of the microorganisms inside the soil, remained unknown and uncontrolled (Lawton et al., 1993; Lawton, 1996; Milcu et al., 2016). Plants are not isolated individuals but naturally live in close association with consortia of microorganisms, the same applies to insects and the soil fauna (Guerrero et al., 2013; Six, 2013). Together with

their host, the microbiome forms the "holobiont" (Zillber-Rosenberg & Rosenberg, 2008, Gilbert et al., 2012). Part of the variability observed within and between experiments, may well have been caused by the inability to control the microbiome in these experimental approaches. Even though the character of the relationship of the host and its microbiome is not always exclusively beneficial, the interplay between them is significant for both sides. This was shown for a broad range of interactions, from the well described nutrient acquisition by bacteria and fungi in exchange for C compounds from plant hosts (Madigan et al., 2009; Bonfante & Genre, 2010), to microorganisms influencing the plants' flowering time (Wagner et al., 2014), nutrient acquisition, development, and fitness (Hardoim et al., 2015). For full functionality of the light organ, the squid *Euprymna scolopes* depends on bacteria as well as the wasp *Asobara* for the development of its ovaries (Gilbert et al., 2012). Even the evolutionary adaptation to the environment is dependent on the interaction of microbes and higher organisms. It was the symbiosis of plants with fungi, which allowed to conquer terrestrial ecosystems (Redecker et al., 2000) and the association with endophytic microorganisms enabling the extensive diversification of the insects (Six, 2013; Guerrero et al., 2013). Some plants cannot even be cultivated after being sterilized but need their associated microbiota for growth, adding further evidence to the importance of a holistic view of organisms (Vandenkoornhuyse et al., 2015). The microbiome of different plant species or of different plant organs (e. g. phyllosphere vs. rhizosphere) within a host species, was found to be similar at a genus and order level, but not necessarily below (Vorholt, 2012; Zillber-Rosenberg & Rosenberg, 2008; Bettenfeld et al., 2020; Browne et al., 2016). During the process of identifying the players of a microbiome (Schlaeppi & Bulgarelli, 2015; Tian et al., 2020) it was shown, that there's not only interaction between the host and the microorganisms but also among the prokaryotic taxa which build the associated consortium. The respective strains are partly inherited and partly a subset from the surrounding environment (Gilbert et al., 2012). Microorganisms from air significantly participate in the composition of the phyllosphere, the ones from soil have a greater share in the rhizosphere community (Sánchez-Cañizares et al., 2017). The microbiome assembly is genetically regulated by both partners - the hosts and the microbiota (Guerrero et al., 2013; Bodenhausen et al., 2014). With respect to the complexity and biodiversity in soil, it is not surprising that the identity of the microbiome-forming microorganisms from this heterogeneous part of the terrestrial ecosystem is not

yet fully described (Vandenkoornhuyse et al., 2015). It were again the model organisms which helped understanding the regulations and controls of the holobiont's formation and functioning (Martino & Leulier, 2017). By using model organisms, scientists could also trace the spread of microbes through the soil matrix when using fungal hyphae, roots, and moving organisms like earthworms and nematodes as transport vehicles (Yang & van Elsas, 2018) to encounter their hosts.

A very successful approach was and still is, to make us of sterilized organisms, which are re-colonized by reduced microbial communities of known origins, i.e. gnotobiotic organisms (Rawls et al., 2004; Kirk, 2012; Basic & Bleich, 2019; Miebach et al., 2020). By doing so, it was for example shown that the microbiome shapes *Drosophila*'s mating preferences over more than fifty generations (Sharon et al., 2010) or that xylophagous insects are only capable through their gut microbiota to process and digest woody material (Guerrero et al., 2013).

The gastrointestinal tract represents another highly diverse and complexly regulated system. The research field studying a host and its gut microbiome is very active and successful in the recent past. The intestine is the area of nutrient uptake from food sources and of interaction of an organism, its microbiota, and their surrounding environment (Gilbert et al., 2012; Burns & Guillemin, 2017; Blum et al., 2019). The cell number of microorganisms inhabiting the gut is high and can be compared to the one from soil (Blum et al., 2019). Only the fine regulation of interactions within the nutrient-processing prokaryotic network inside the gut enables the host to survive and grow (Gilbert et al., 2012; Burns & Guillemin, 2017). Both, gut and soil have in common, that the investigated system harbors an incredibly high cell density within a small inhabitable and dynamic area which leads to a broad spectrum of tightly connected and regulated interplays between the environment and the associated microorganisms as well as among microbiome-community members (Burns & Guillemin, 2017; ; Shapira, 2017; Kim & Jazwinski, 2018; Blum et al., 2019). The respective interactions are shaped by a neutral, beneficial, auxiliary, or competitive character (Petrof & Khoruts, 2014; Kim & Jazwinski, 2018; Vitorino & Bessa, 2018). The resulting outcomes of these interactions do not only affect a host's nutrition but influence many more aspects of its well-being (Petrof & Khoruts, 2014; Martino et al., 2017; Shapira, 2017).

The biodiversity of the gut microbiota follows the hierarchical complexity of the host organism and the diversity of its nutrition. The more complex the host and diverse its diet, the more complex is its microbiome (Martino et al., 2017; Adair et al., 2018). The connection between a healthy gut and a healthy host is evident for most organisms by now (Adair et al., 2018; Zhang et al., 2017; Zehavi et al., 2018; Moon et al., 2018; Shetty et al., 2019). Great interest especially concerns the human gut microbiome and its impact on various human diseases (Petrof & Khoruts, 2014; Liwinski & Elinav, 2019). Explaining gut-related processes and feedbacks is already challenging in the well-studied model systems *C. elegans* (Shapira, 2017) or *D. rerio* (Gilbert et al., 2012; Burns & Guillemin, 2017). Due to the complexity of their intestinal microbiome the investigation is still ongoing. In contrast, 10^{13} to 10^{14} active cells from microorganisms can be found in the human gastrointestinal system. Its microbiome is composed of about 1,000 different bacterial strains (Lagkouvardos et al., 2017; Kim & Jazwinski, 2018; Blum et al., 2019). Consequently, the community processes are even more complexly regulated there compared to abovementioned "simpler" model organisms. This leads to challenges similar to the ones from soil science.

How can we investigate the most diverse and complex systems?

The same principles that led to the establishment of prokaryotic model organisms, can also be successfully applied to the more complex environments. There simplifying the system of interest opened the field of "gnotobiology" (*gnotos:* known; *bios*: life; *logos*: study). For employing gnotobiotic model systems, germ-free organisms must be created, raised under sterile conditions, and subsequently be inoculated with an artificial microbial community, with a reduced complexity (Kirk, 2012; Basic & Bleich, 2019). The experimental conditions and procedures in context with gnotobiotic models are highly standardized and therefore allow to reproducibly study microbe-host interactions (Basic & Bleich, 2019). Gnotobiotic experiments pointed at the influence on the host's immune system through the microorganisms (Selber-Hnativ et al., 2017), directly and indirectly increasing the number of circulating lymphocytes in the host's vascular system, and the microbiota affecting the development of various chronic diseases such as Asthma, Crohn's disease, and many more (Selber-Hnativ et al., 2017). Gnotobiotic model organisms allowed the deeper investigation of eating disorders and highlighted that the gut microbiome can

"manipulate" the host's behavior concerning his nutrition and food preferences (Selber-Hnativ et al., 2017). The significance of the work with these organisms is evident regarding diseases causing severe gastro-intestinal deficiencies, and that related experiments led to the development of fecal transplants as treatment for life-threatened patients (Petrof & Khoruts, 2014). Nevertheless, some important basic questions are unanswered yet (Cani, 2018) and the challenge remains to classify emergent properties within communities.

Interestingly, various results from such gnotobiotic experiments suggested a shared core microbiome for related species (Vorholt, 2012; Bai et al., 2015; Shapira, 2017; Zhang et al., 2017; Garrido-Oter et al., 2018; Voges et al., 2019; Bettenfeld et al., 2020; Miebach et al., 2020) and fueled researchers' attempts to identify and define a potential "minimal-microbiome", a host-associated community of as many members as necessary but not more, to ensure a stable functionality together with a healthy host (Clavel, 2017; Elzinga et al., 2019). The complexity of the considered "minimal networks" is reduced and allows functional studies with a manageable number of participants (Clavel et al., 2017; Zhang et al., 2017). This further enables a deeper investigation of regulations, processes, and feedbacks between members and the host (Clavel et al., 2017; Zhang et al., 2017).

The results from experiments with gnotobiotic organisms with a reduced microbial diversity were promising, because they allowed the investigation of a complexly regulated process while at the same time being able to link processes and interactions to the respective microorganisms and to identify their role. Successful attempts were also conducted with reductionist approaches from natural communities during the last decade. Wagg and colleagues applied the concept to soil and reduced guilds of organisms in microcosm experiments (2014). They used communities filtered through different mesh sizes to exclude several groups of organisms from experimental setups (i.e., fungi, mycorrhiza, and nematodes from the small microflora and -fauna). They could show how plant performance, litter decomposition, nutrient cycling, and other ecosystem services changed with the loss of biodiversity below-ground. Their results provide support for connecting certain functional traits of a community to the respective groups or abundance of certain guilds. Zehavi and colleagues used a comparable reductionist approach (2018), by decreasing the cell density in environmental samples through dilution. By doing so, they could considerably increase the proportion of cultivable microbes from the known rumen

microbiome, concerning abundant as well as rare taxa. A similar study following such a top-down perspective allowed a natural seawater community to self-assemble followed by dilution series to obtain pure cultures. Doing so, researchers could track the fate of community processes and functions while the sample's biodiversity was decreased step-by-step (Yu et al., 2019).

The reduction of the biotic complexity of environmental samples has already been applied in various disciplines, giving insights into microbial interactions, feedbacks, and providing additional hints of emerging properties arising from communication of microorganisms (Szabó et al., 2007; Peter et al., 2011; Philippot et al., 2013; Burns & Guillemin, 2017). But one important riddle remains unsolved: When diluting environmental samples, the surviving species may involve uncultured and un-sequenced taxa with unknown physiology.

A possible solution for many challenges in soil ecology: The Synthetic Soil approach

The goal of the present study was, to create a Synthetic Soil, consisting of an artificially produced abiotic soil and an *in-silico* designed and controlled synthetic microbial community, representing as many of the biotic functions of an intact soil as possible. More specifically, the objectives of the study were to construct a Synthetic Soil that would (i) support an active microbial community over a longer time period (at least four weeks), and (ii) lead to the decomposition of soil organic matter, and resulting microbially-mediated soil processes, such as soil respiration and nitrogen mineralization.

Based on decades of experience in building artificial soils, designing co-cultures and in-vitro synthetic communities, and in the mechanistic understanding of how microbial interactions are controlled (gained through the investigation of gnotobiotic systems), we here present a proof-of-concept study of such a Synthetic Soil.

An *in-silico* designed minimal community of genome-sequenced soil microorganisms was added to an artificially assembled sterile soil. By combining the fundamentals of the ecotron studies and gnotobiology, we intended to build an abiotically *and* biotically fully controlled and stable experimental platform - the first such Synthetic Soil. The design of the microbial community and the artificial soil should ensure the repeatability of the

experimental system. Such a system should be manipulable regarding the soil properties and the functional characteristics of the organisms it contains. It should also only harbor organisms for which genetic compliments are fully known (i.e., which are fully sequenced) to be able to understand the contribution of individual species and consortia to soil functions.

We here report on conducting a proof-of-concept experiment, demonstrating the performance of the novel Synthetic Soil and its general applicability to fundamental biological questions.

In the long-term, this new approach should serve as a model soil ecosystem, which could also be a basis for a synthetic ecosystem, including autotrophic organisms and complex food-webs.

Material & Methods

The Experimental Set-up

For this study, single sterilized organic and inorganic components were combined to an artificial soil, which was inoculated with a reduced and *in-silico* designed synthetic community (SynCom). Via consecutive sampling, soil parameters were evaluated and the development of the community in the soil was assessed over time. Four different experimental groups were designed (Tab. 1): Group "A0" contained the Artificial Soil which was not inoculated with a microbial community and remained sterile. In group "AS" the artificial soil was inoculated with the synthetic community. Group "AN" contained the artificial soil, inoculated with a natural community (NatCom), obtained from garden soil, and in group "NS" sterilized garden soil was inoculated with the synthetic community.

Table 1 The four different experimental groups and their properties.

Soil	Community	Experimental Group	No. of Replicates
Artificial (A)	-	A0	5
Artificial (A)	Synthetic (S)	AS	9
Artificial (A)	Natural (N)	AN	5
Natural (N)	Synthetic (S)	NS	5

The Need for Sterility

Working with Pure Cultures

All work during this study, which concerned cultures of bacteria and fungi, was performed with sterile equipment inside a laminar flow (Lamsystems, BMB-II-LAMINAR-S-1,2 SAVVY), inside a hood for fungal work (Erlab, Captair Smart 321) or under a flame (Campingaz Butane / Propane tank CV 470 plus and Labogaz 470 / 206). To ensure that a sterile environment was maintained, a proper cleaning protocol was followed before, during, and after handling of samples. Before working with the laminar flow, its interior was surface sterilized with the integrated UV light for 30 minutes. All benches and surfaces used, were cleaned with 70% Ethanol beforehand. The same was done for equipment and gloves in use. After the UV radiation, the laminar flow was opened, and the air flow engaged. All inside surfaces were cleaned with 70% Ethanol before bringing in working

material which had been surface sterilized ahead of use. When working with the laminar flow was finished, 70% Ethanol was used again followed by applying a fungicidal disinfectant foam (Ecolab, Incidin, OxyFoam S) to neutralize present spores. After 15 minutes of incubation, UV radiation was performed again. Before and after working in the hood for handling fungi or under a flame, all used surfaces were cleaned with 70% Ethanol. The hood and the laminar flow were tested for fungal contaminations regularly by using a test kit (Orion, Hygicult Y&F; data not shown). No contaminations were detected in the laminar flow. In the second test of the fungal hood, cell growth was detected on the incubation strips of the kit (not shown) and therefore all manipulations concerning fungi were performed under a flame outside of the hood.

All equipment necessary for working with the cultures or with the samples was autoclaved at 121°C, surface-sterilized with 70% Ethanol or sterilizing wipes (Bode Chemie GmbH, microbac tissues) before use.

The Isolator

To ensure gas exchange, while preventing contamination from surrounding air, the samples were incubated inside the two sterile chambers of an isolator (NKP Isotec, ISO-Type 2D; Fig. 1). The upper and lower compartment were properly cleaned from visible dust and particles with a broom and the two ports were closed. By using the attached sleeves, the whole interior was sprayed with a disinfectant (Antiseptica, Descogen Liquid r.f.u., 1 L). Afterwards, both ports were also sterilized via the dedicated port openings. High pressure was then applied, with the internal air flow system connected to HEPA (high efficiency particulate air) filters and the disinfectant was incubated for several days until completely dry. Spraying was repeated several times to ensure a germ-free environment. The air pressure in the chambers was at any time kept higher compared to the surrounding to prevent the system against entering spores or organisms. Equipment needed for handling, was fit into a smaller chamber which was connected to the isolator's port system (Fig. 1), after only the outer lid of the port was opened while the inner door stayed shut. When the connection was established, the equipment inside the smaller chamber was surface sterilized by being sprayed with the disinfectant. After 30 minutes of incubation, the equipment was brought into the chamber, the inner port door was shut, the small chamber was detached, the outer lid of the port was re-attached, and the port's surface

was sprayed with disinfectant. When something had to be brought out of the sterile compartment, the inner port door was opened, the equipment was placed in the port area, the inner door was shut, and then the outer port access was opened to bring the equipment out. The outer lid could then be re-attached and the whole port area was again surface sterilized by spraying it with disinfectant again. Following this protocol ensured, that the inner chamber was never in direct contact with the surrounding air. All equipment used inside the isolator was autoclaved at 121°C and / or surface-sterilized with 70% Ethanol and sterilizing wipes before use.

Figure 1 The isolator (right side and left side; a + b) providing a sterile environment within its lower and upper chamber by filtering incoming air via a HEPA (high efficiency particulate air) filter. Both chambers have a round-shaped port system to which a smaller chamber (c + d) can be attached to bring equipment in after being disinfected. Picture credit b: Carolina Urbina Malo, MSc.
Total size of the isolator: height = 209 cm, length = 225 cm, width = 100 cm.

The Incubation Jars

Autoclavable glass jars (Wheaton w216913; Wheaton w216921 with Microlink PP, solid white lids 70-400) of two different sizes (volume small jar approx. 200 mL; volume big jar approx. 350 mL) were used for the incubations (Fig. 2). Holes with a diameter of 20 mm were drilled into the lids. Glass beads of three different sizes (VWR collection, diameter: 6 mm, 8 mm, and 10 mm) were used as spacers at the bottom to later prevent the soil from

becoming anoxic. The mesocosms, their lids, and the glass beads were rinsed with MQ water and left to dry over night. They were then covered with 0.2M HCl, and after 45 minutes of incubation washed with MQ water to remove acid remains. After drying, the mesocosms were assembled as follows: approximately 10 glass beads of each size were added to a jar to cover its bottom. Mesh bags (The Press Club, 7.62 x 15.24 cm, 25 micron) were added to the small jars, and to the large jars (12.7 x 12.7 cm), and with 2 small pieces of autoclavable adhesive tape (Deemark, PVC indoor; Scapa) attached to the mesocosm's rims (Fig. 2). The screw caps were attached, and their holes were covered with autoclavable wool (Sera, filter wool for mechanical prefiltration). In the end, 22 small and 16 large mesocosms were set up and put into a small container which was sealed with autoclavable adhesive tape and stored until γ-irradiation, after being autoclaved at 212°C.

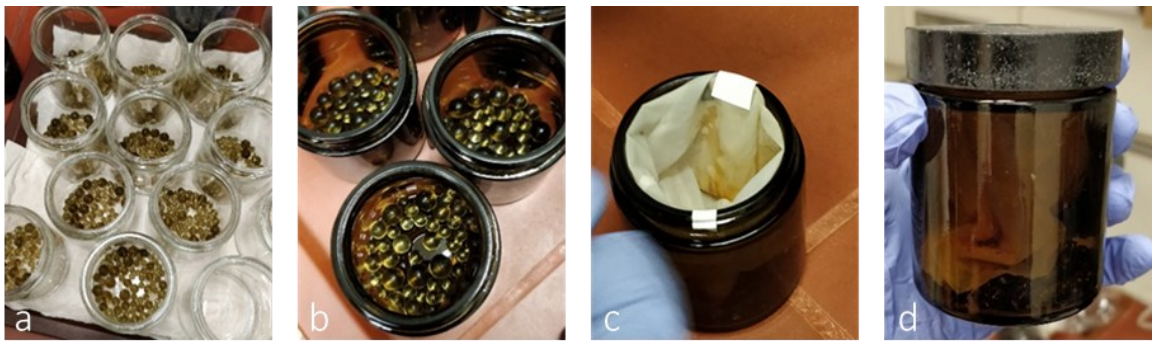


Figure 2 The mesocosms used for the incubation of the sample groups. a: large jar with glass beads covering the bottom, volume ca. 350 ml, height = 9 cm, r = 3.5 cm; b: small jar with glass beads covering the bottom, volume ca. 200 ml, height = 7 cm, r = 3 cm; c: small jar with mesh bag added which later contained the soil, adhesive tape attached to its rim; d: closed small jar containing the glass beads and the mesh bag, wool was later added to the hole in the lid; pictures were taken before γ-irradiation and after HCl-washing.

The Timing of the Experiment

All steps concerning the preparation of living and non-living components of the experimental groups (Fig. 3) had to be perfectly timed in advance, mainly to lower the risk of contamination.

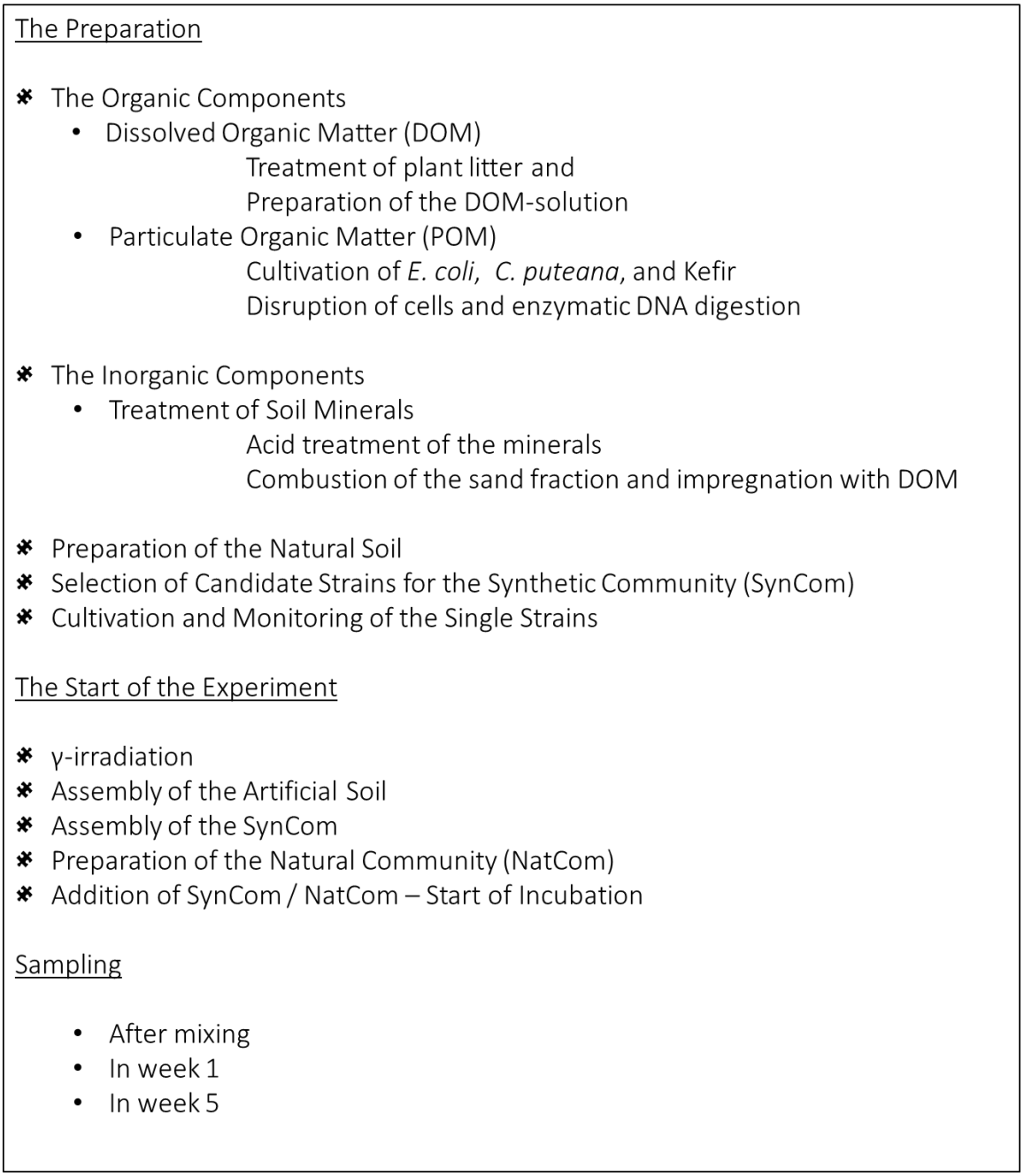


Figure 3 Overview of the preparational steps prior and during the experiment.

The Organic Components

Plant biomass (leaves and stems) was harvested from a permanent grassland at the Austrian Research and Education Centre in Gumpenstein, Styria. The plant biomass

consisted mainly of the following species: *Arrhenatherum elatius, Festuca pratensis, Lotus corniculatus, Trifolium pratense*, and *Taraxacum officinale*. The cut and dried (60°C) plant material was milled and sieved into three size classes (1 - 0.250 mm, 0.250 - 0.063 mm, < 0.063 mm). The dried plant biomass is termed plant litter hereafter. The plant litter size classes were added separately to autoclavable plastic bags and covered with MQ water (Merck, Milli-Q ultra-pure water; filtered through 0.22 µm pore size). The content was autoclaved for 15 minutes at 1 bar and 121°C (CertoClav Sterilizer, portable tabletop autoclave). The liquid was decanted, and the plant biomass stored at 37°C for 24 hours to allow potentially present intact microbial cells to recover and metabolize to then be more vulnerable to further treatments, comparted to their resting stages. The autoclaving procedure was then repeated to finally destroy active organisms on the litter surface. The solution resolved after autoclaving contained high concentrations of dissolved organic matter (DOM) from the plant material. The content of the autoclavable bag from the < 0.063 mm fraction was transferred to three 50 mL tubes (Greiner bio-one, Cellstar PP 50 mL, conical) and centrifuged at 8,000 RPM (revolutions per minute; Eppendorf 5810 R) for 15 minutes to separate particles from the liquid. The liquid phase from the tubes was finally added to 2 L autoclaved MQ water inside the laminar flow, to create the DOM-solution, and the remaining plant litter was stored at -20°C until further processing.

In addition to the plant litter and DOM, also cell wall fragments from pre-treated bacteria and fungi were added as soil organic matter (particulate organic matter = POM). More specifically, we used cell wall fragments of the Gram-negative bacterium *Escherichia coli*, the fungus *Coniophora puteana* (Basidiomycota), and a fast-growing Kefir culture, consisting of yeasts and *Lactobacilli*, which form a polysaccharide matrix.

E. coli strain K12 DSM 498 was reactivated from a stock stored at -80°C in glycerol, by incubating it at 37°C on petri dishes containing LB medium (Suppl. Tab. 5). After cell growth, the plates were kept at 4°C. To start biomass build-up, cells were transferred to fresh plates and incubated at 37°C for 6 days. 5 Erlenmeyer flasks (Duran, wide neck, 1 L) were filled with approximately 500 mL LB medium each and were autoclaved at 121°C. Inside the laminar flow, one inoculation loop of cells was added to each of the flasks before being incubated in the dark at 28°C (Pol-Eko), horizontally shaking at 100 rpm (rotations per minute). The cultures were harvested after eleven days. With sterile pipette tips (Greiner

bio-one, 25 mL pipette, 2/10 mL, sterile) the cell-containing medium was transferred to 50 mL tubes which were then centrifuged for 10 minutes at 3,700 RPM to separate the liquid phase from the biomass. The supernatant was decanted, and the cells were collected in three tubes using a sterile pipette tip (Biotix, 1250 µL tips, racked, filtered, sterilized). Autoclaved MQ water was added until the 45 mL markings of the tubes which were then centrifuged again at 5,400 RPM for 10 minutes. The supernatant was discarded, and the biomass collected in a single 50 mL tube. Cell washing with autoclaved MQ water was performed two more times to properly exclude remains of the culturing liquid. About 13 g fresh cell mass were retrieved from 2.5 L LB medium and were stored at -20°C until further processing.

Coniophora puteana (*C. puteana*) strain MB 209 (kindly provided by Dr. Katja Sterflinger, BOKU, University of Natural Resources and Life Sciences Vienna) was grown on Agar plates containing malt extract medium (Suppl. Tab. 5). Pieces of the fungal biomass were transferred to fresh plates and incubated at room temperature. For the experiment, fungal cells were transferred with a sterilized spatula to three autoclaved 1 L Erlenmeyer flasks containing 0.5 L sterile malt extract medium each. The liquid cultures were incubated in the dark at 28°C, horizontally shaking at 100 rpm. After 14 days of cultivation, the fungal biomass was poured into a mesh (49 µm pore size), which was washed with MQ water and put into a funnel before. After drainage of medium, the cells were transferred to three 50 mL tubes with a sterilized spatula. They were centrifuged at 5,400 RPM for 10 minutes to separate the liquid from the solid phase. The supernatant was poured off and autoclaved MQ water was added to the tube to wash the fungal cells from the medium. After a second centrifugation step, the biomass was collected in a single 50 mL tube followed by two more washing and centrifugation steps until the supernatant became clear. Approximately 15 g fresh cell mass were retrieved from 1.5 L malt extract medium and were stored at -20°C until further processing.

Part of the biomass was gained from cultivation of a Kefir culture, a fermenting community of different yeasts (e.g., *Saccharomyces* or *Candida*) and various *Lactobacillus* strains (Gulitz et al., 2011). They were selected due to their ability to build new biomass in a short period of time, since they produce a matrix from polysaccharides and proteins, and their cultivation requirements could easily be met (Suppl. Tab. 5). Approximately 400 mL Kefir

starting culture were cultivated in 4 L shares (Westside, Gallone, 5 L). Whenever the added sugar had been completely metabolized, part of the biomass was used to start a new fermentation in fresh liquid and the rest was frozen at -20°C. To separate the liquid from the harvested cells, the biomass was transferred to 50 mL tubes and they were centrifuged at 4,800 RPM for 5 minutes before being frozen at -20°C. Centrifuging was repeated once after thawing, and once more at 4,800 RPM for 10 minutes. The biomass containing tubes were stored at -20°C until further processing.

The plant litter and the cells from *E. coli*, *C. puteana*, and the Kefir culture were thawed and autoclaved three times at 121°C, 1 bar for 15 minutes to destroy the intact cell membranes and to make cell walls become leaky. The content of each tube was treated with DNAse I (40 mg DNase I in 50 mL filter-sterilized MQ water + 5 mL 5 µM $MgCl_2$ + 5 mL 0.1 mM $CaCl_2$) and incubated over night at 37°C to digest present DNA. The enzyme was then heat-inactivated by autoclaving at 121°C, followed by centrifugation at 5,400 RPM for 15 minutes to collect the cell wall fragments in the pellet. The successful DNA digestion was verified through DNA extraction by using the FastDNASpin Kit for Soil (MP Biomedicals) and the PicoGreen dsDNA Quantification Kit (Invitrogen Molecular Probes) with a spectrophotometer. The supernatant from the tubes was stored at -20°C until γ-irradiation.

Table 2 Components of the organic fraction added to approximately 500 g mixture of artificial soil minerals.

Fraction	Component	Amount fresh [g]	Treatment
POM	Plant litter	13	autoclaved, DNAse-treated, γ-irradiated
POM	*E. coli*	6	autoclaved, DNAse-treated, γ-irradiated
POM	*C. puteana*	15	autoclaved, DNAse-treated, γ-irradiated
POM	Kefir	7	autoclaved, DNAse-treated, γ-irradiated
DOM	Plant litter solution		autoclaved, filtered

POM = particulate organic matter; DOM = dissolved organic matter

The Inorganic Components

The mineral components (Tab. 3) were obtained from the companies “LB Minerals, s.r.o.”, Czech Republic (sand, silt), “Clariant SE BU Functional Minerals”, Germany (clay), “Lanxess Germany” (Fe-oxides), and “Sasol Performance Chemicals”, Germany (Al-oxides).

In a first step, sand, silt, and clay were sieved to different size classes (> 2 mm, 2 - 1 mm, 1 - 0.250 mm, 0.250 - 0.125 mm, 0.125 - 0.063 mm, < 0.063 mm).

We used very coarse and coarse quartz sand, a primary Na-K Feldspar and an Al-Fe Silicate in the fine and very fine sand size classes, a K-Feldspar in the silt fraction, and Montmorillonite, a common 2:1 silicate mineral with the general chemical formula $(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2$ in the clay fraction (Tab. 3). Additionally, we also used the Fe- and Al-oxides, Goethite and Boehmite to construct the artificial soil.

Acid treatment of Minerals

We observed in a preliminary experiment that pH values tended to increase during incubation (data not shown). Therefore, all mineral components except oxides were exposed to various acid-treatments and washing steps (Suppl. Tab. 3) to lower the pH of the soil at the beginning of the incubation.

For this purpose, approximately 10 g from all mineral components (except oxides) were weighed into 50 mL tubes. All components had been exposed to γ-irradiation before (see below). While handling under a flame, 15 mL of 0.2M HCl were added to each tube, 20 mL to Montmorillonit. Their content was properly mixed, and the tubes let to rest for 15 minutes. The reaction with acid caused formation of bubbles in the tubes of Montmorillonit and Feldspar dust, indicating the presence of carbonates being released as CO_2. The tubes were centrifuged at 5,000 RPM and the supernatant was decanted. For washing off the acid, MQ water was added to the 45 mL marking on the tubes, they were shaken a few times for proper mixing and let to rest for five minutes followed by centrifugation at 5,000 RPM for 5 minutes. After decanting the MQ water, the washing step was repeated twice. Before the supernatant was decanted after the 3rd washing step, the pH was determined (Sentron pH meter; Suppl. Tab. 3). Approximately 1 g of each component was weighed into a scintillation vial (Sarstedt, 20 mL), 5 mL MQ water were added, and the vial was turned repeatedly upside-down for 2 min by hand. The pH of the acid-washed components was determined after a resting time of 30 minutes (Suppl. Tab. 3). The 50 mL tubes were meanwhile covered with aluminum foil to protect their content but at the same time allow evaporation of HCl remains, and were kept in a drying oven (Memmert, UF260 plus) at 65°C over night. On the next day, the content of the tubes was washed another time and the pH

of the supernatant was determined (Suppl. Tab. 3) before 1 g was weighed into a fresh scintillation vial, to determine pH in the component's slurry (Suppl. Tab. 3). When the pH of the supernatant and of the slurry became more similar, this indicated that most of the added HCl was finally washed off.

To test the pH development of the combined components, they were mixed inside 50 mL tubes in proportions that corresponded to the proportions they had in the artificial soil (Tab. 3). Tube 1 contained all mineral components. They were γ-irradiated, HCl-treated, and 4x washed with MQ water; oxides and plant litter (both autoclaved and γ-irradiated) were additionally added. Tube 2 had the same content but the smallest sand fraction (0.063 - 0.125 mm) was only γ-irradiated, not HCl-treated. In tube 3, Feldspar dust was only γ-irradiated and in tube 4 Montmorillonit (Suppl. Tab. 3). After the components were homogenized and each tube contained approximately 5 g of the mixture, they were let to rest for 15 minutes followed by a pH determination (Suppl. Tab. 3). The test soils were kept at 4°C for two days and pH was determined again to investigate the development over time (Suppl. Tab. 3).

To further evaluate the outcome of the acid treatments of single components and to test their various possible combinations, four more washing steps were applied to Feldspar dust and Montmorillonit (MQ water until 45 mL marking on the tube, centrifugation at 5,000 RPM once, and at 9,000 RPM three times) to measure how much the pH would finally increase in the supernatant and in the slurry (Suppl. Tab. 3). The remaining 3.5 g of assembled components from tube 1 (all components γ-irradiated, HCl-incubated and 4x washed) were then treated with 25 mL 50mM $NaHSO_4$ (then tube 1a; Suppl. Tab. 3) to test a further pH decrease without considerably influencing the nutrient and mineral composition of a future artificial soil. After the pH measurement and incubation over night at 18°C, 4 more washing steps and a last determination of pH were performed (then tube 1b; Suppl. Tab. 3). At the same time, another set of assembled test soils was set up for comparison. Tube 5 contained all mineral components and plant litter which were all γ-irradiated before. Only the smallest sand fraction (0.063 - 0.125 mm) was HCl-treated and 4x washed. In tube 6 this was the case for Feldspar dust instead of sand, in tube 7 for Montmorillonit, and in tube 8 for Feldspar dust and Montmorillonit. Tube 9 contained the same combination as tube 8 but the acid-treated components were washed 8 times. After

the first pH measurement, 15 mL 50 mM $NaHSO_4$ were added to 2 g of the mixture from tube 9 and it was treated like tube 1a and further like tube 1b respectively (see above and Suppl. Tab. 3).

The mineral fraction of the artificial soil of this study was finally composed of Feldspar dust and Montmorillonit, pre-treated with 0.2M HCl, and washed 6 times, including drying in between. Both components had mean values around pH 6 (data not shown) after the treatment.

Impregnation with DOM-solution

To remove remains of organic material, the four components of the sand fraction (0.063 - 2 mm; Tab. 3) were transferred to separate glass beakers (Duran, 1000 mL) and combusted for 4 hours at 300°C in the muffle oven (Heraeus M1100/1) before left to cool over night at room temperature. The previously prepared DOM-solution was added to each glass beaker until covering its content and left to incubate for 12 hours at 4°C. Paper coffee filters (Amaroy, brown, unbleached, size 4) were surface sterilized with UV light inside the laminar flow on the next day and the sand was filtered to let the DOM-solution drain. The impregnated soil components were stored in autoclavable bags at room temperature until γ-irradiation.

Table 3 Components of the inorganic fraction to build approximately 500 g of artificial soil.

Fraction	Fraction size [mm]	Sample	Amount fresh [g]	Company name	Treatment
Very coarse sand	2 - 1	Quartz sand	100	VL6	muffled, DOM impregnated, γ-irradiated
Coarse sand	1 - 0.25	Quartz sand	72	VL6	muffled, DOM impregnated, γ-irradiated
Fine sand	0.25 - 0.125	Na - K Feldspar	76	MZ55	muffled, DOM impregnated, γ-irradiated
Very fine sand	0.125 - 0.063	Al-Fe-Silicate	75	GEM 60	muffled, DOM impregnated, γ-irradiated
Silt	0.063 - 0.002	K-Feldspar dust	125	Casial	acid washed, γ-irradiated
Clay	< 0.002	Montmorillonite (Na-Al-Silicate)	75	Ceratosil	acid washed, γ-irradiated
Fe-oxide	< 0.125	Goethite	4	Bayferrox 910	γ-irradiated
Al-oxide	< 0.063	Boehmite	4	Pural	γ-irradiated

DOM = dissolved organic matter

Preparation of the Natural Soil

In one experimental group, sterilized garden soil was used to assess the development of the SynCom in natural settings. The soil was obtained from a garden belonging to the University Vienna. The sample was taken from the upper 10 cm below the litter layer. It was sieved to 2 mm and stones and bigger remains of plant material were removed. Approximately 40 mL of the sieved soil were transferred to five 50 mL tubes which were then autoclaved with the tabletop autoclave at 121°C at 1 bar for 15 minutes. The lids of the tubes were only loosely attached and covered with parafilm to allow air exchange. This procedure was repeated after one week to allow resting stages of surviving organisms to metabolize in the meantime and become vulnerable to heat exposure. Three days later autoclaving was repeated for the third time, the lids of the tubes were firmly closed, and they were stored at room temperature until γ-irradiation.

Selection of the Candidate Strains for the Synthetic Community (SynCom)

The candidate strains which represented the SynCom members, were chosen under different aspects. Since they had to be traceable during the incubation, only fully

sequenced strains from soil were of interest. Further, they had to be cultivable in the lab and their nutrient requirements had to be known. The composition of phyla should be comparable to the one from a real soil (Fierer, 2017; Delgado-Baquerizo et al., 2018; Nannipieri et al., 2020). Further, the used taxa should not produce toxins possibly harming the other organisms, and only heterotrophic taxa were chosen.

The detailed process of picking the candidate strains was not subject of this thesis and has been done in cooperation with Dr. Dimitri Kits and Univ.-Prof. Dr. Alexander Loy (DOME, Division of Microbial Ecology, University of Vienna). Briefly, genome-sequenced bacteria and fungi, isolated from soil and available through DSMZ (German Collection of Microorganisms and Cell Cultures GmbH) were selected with regards to clusters of orthologous groups of proteins (Tatusov et al., 2000) by the program "OrthoFinder" (Emms & Kelly, 2015). When an organism was picked, the contributing orthogroups, which relate to functional clusters (pathways) in soil, were excluded from the pool, and a new iteration was performed by the software. In the end, 16 different microorganisms were selected to build the SynCom, which covered 77% of the functional clusters present in reference soil metagenomes. (Tab. 4).

Cultivation and Monitoring of the Strains

The strains were ordered from DSMZ Germany. Some of the organisms were delivered freeze-dried and some active, some incubated on Agar, and some in their respective liquid medium. The strains were incubated at 3 different temperatures: 20°C, 25°C, and 28°C (Tab. 4) although some of the growth optima slightly deviated from these temperatures (data not shown). The cultivation of the 16 organisms required a total of ten different media (Tab. 4 and Suppl. Tab. 5). All of them were prepared as indicated in the recipes. pH was adjusted with NaOH or HCl if not indicated otherwise and determined using a pH probe (Metrohm, E510). After autoclaving for sterilization, the liquid media were stored in Schott bottles (Duran, 0.5 L, 1 L, 2 L) at room temperature and regularly checked for potential contaminations. Every medium was additionally prepared containing Agar. The poured plates were wrapped in plastic bags and stored upside-down in a plastic box at 4°C.

Table 4 Microorganisms selected as members of the synthetic community (SynCom). Organism's names, ID, MediumID, cultivation temperature, and delivery status refer to German Collection of Microorganisms and Cell Cultures (DSMZ) who delivered the taxa.

	Phylum	Organism	ID	MediumID	Cultivation	Delivery Status
Bacteria						
1	Acidobacteria	*Koribacter versatilis*	22529	1266	at 25°C	living
2	Acidobacteria	*Luteitalea pratensis*	100886	1426	at 28°C	freeze-dried
3	Acidobacteria	*Solibacter usitatus*	22595	1266	at 25°C	living
4	Actinobacteria	*Solirubrobacter soli*	22325	830	at 28°C	freeze-dried
5	Actinobacteria	*Streptosporangium roseum*	43021	65	at 28°C	freeze-dried
6	Bacteroidetes	*Flavisolibacter ginsengisoli*	18119	830	at 28°C	freeze-dried
7	Bacteroidetes	*Spirosoma spitsbergense*	19989	830	at 20°C	freeze-dried
8	Firmicutes	*Paenibacillus alginolyticus*	5050	1	at 28°C	freeze-dried
9	Proteobacteria	*Paraburkholderia xenovorans*	17367	220	at 28°C	freeze-dried
10	Proteobacteria	*Chelativorans multitrophicus*	6780	830	at 28°C	freeze-dried
11	Proteobacteria	*Labilithrix luteola*	27648	1509	at 28°C	living
12	Verrucomicrobia	*Chthoniobacter flavus*	22515	1266	at 25°C	living
Fungi						
13	Ascomycota	*Periconia macrospinosa*	62880	191	at 25°C	living
14	Ascomycota	*Trichoderma flavofuscum*	3500	129	at 25°C	freeze-dried
15	Basidiomycota	*Armillaria bulbosa*	3732	90	at 22°C	living
16	Basidiomycota	*Psilocybe cyanescens*	4999	90	at 25°C	living

The procedure to re-activate the delivered strains which were freeze-dried, was the following: The delivered glass ampoule was opened as recommended and 0.5 mL of the respective liquid medium were added to it. After 30 minutes of incubation, 150 µL from the mixture of medium and cells were used to be stored at -80°C in 150 µL Glycerol for creating a back-up if needed. The rest was split to incubate two petri dishes and to start a liquid culture with only a small amount of additional medium. The strains were incubated at the respective temperatures (Tab. 4), the plates (Fig. 4) on a shelf and the Erlenmeyer flasks on a shaker at 100 rpm. The first transfer for the actively delivered strains, was similar only lacking the step of reactivation with 0.5 mL medium. The fungi were received on Agar and small pieces of their biomass were transferred with a sterilized spatula to fresh plates and into liquid medium. All strains were continuously cultivated on plates and in liquid cultures in duplicates. Transfers to fresh medium were performed approximately every third week. Two autoclaved 100 mL Erlenmeyer flasks were filled with 45 mL sterile medium each, and 5 mL from the active culture containing the growing cells of the respective organism were added. Since the fungi grew as aggregates of biomass, small pieces were separated with a sterile pipette tip and transferred to the new flasks. Cultures from plates were transferred every second month. With an inoculation loop or a sterilized spatula, cells from the active culture were transferred to two fresh plates which were then sealed with parafilm to protect the content from contamination but allow respiration. Bacterial plates were incubated upside-down, fungal plates bottom-down.

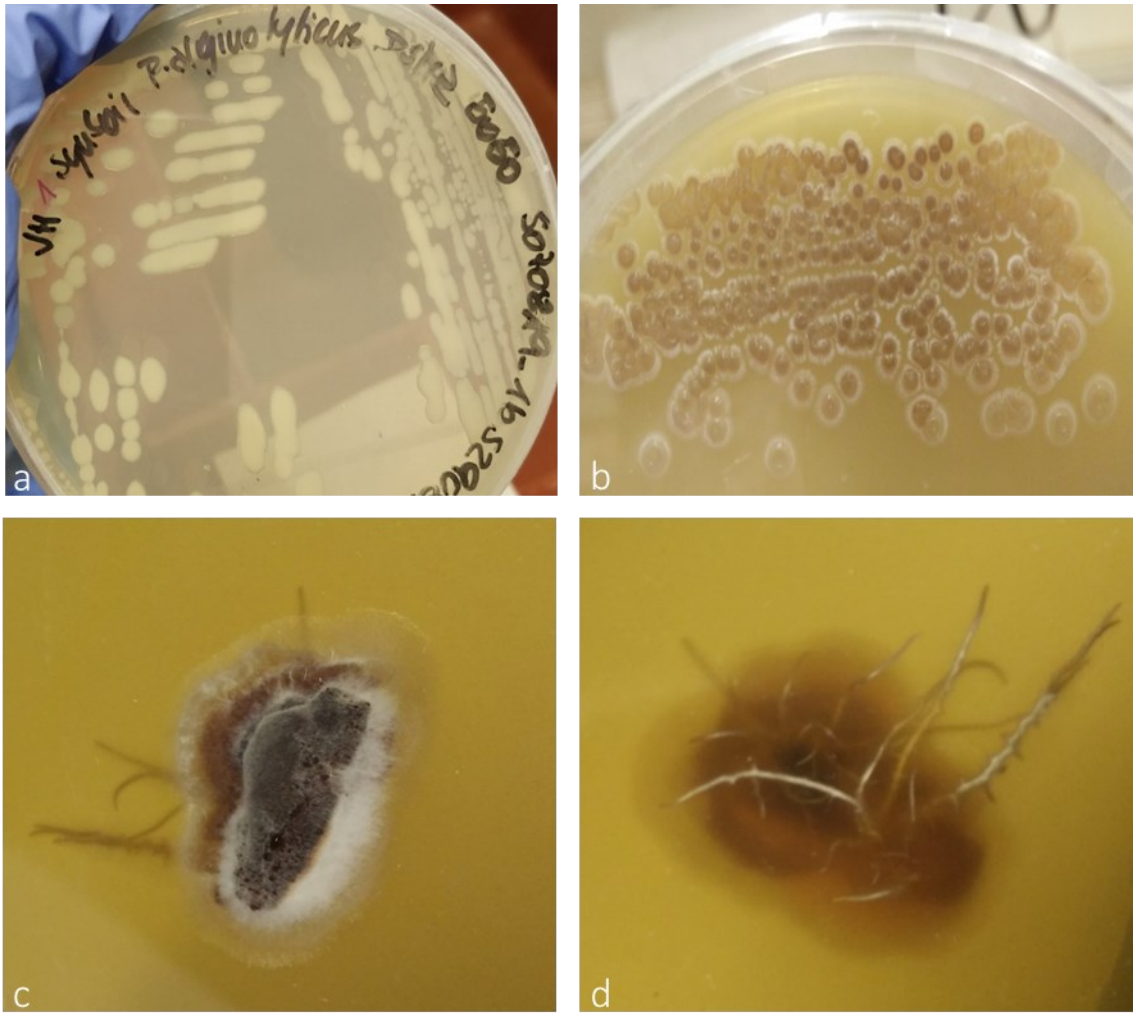


Figure 4 Three members of the synthetic community. Whitish *P. alginolyticus* (a): belonging to the Firmicutes order, growing on an Agar plate containing medium 1, diameter of the plate = 6 cm. *S. roseum* (b): belonging to the Actinobacteria order, growing on an Agar plate containing medium 65, overgrowing the surface and incrusting, diameter of the plate = 6 cm. The fungus *A. bulbosa* (c + d): belonging to the order Basidiomycota growing on an Agar plate containing medium 90; c: top-view, size of the fungus ca. 3 cm; d: the same fungus building rhizomorphs (root-like structures) seen from below.

From the second transfer onwards, the changes in optical density (OD) of the bacterial cultures were determined to evaluate growth. 300 µL of the respective media were added in triplicates to a transparent 96-well microtiter plate (Greiner bio-one, F bottom, clear) to set the OD of the liquid only. 300 µL from the replicates of the liquid cultures were then pipetted into the adjacent wells of the plate and the OD was determined by measuring the absorbance of light sent through the well with a spectrophotometer (Tecan, Infinite 200 Pro). The plate was orbitally shaken for 5 seconds with an amplitude of 1 mm before the measurement, to evenly distribute the present cells. 25 light flashes with a wavelength of 600 nm were then emitted and the absorbance was measured on 9 different spots within

each well. The OD of the medium only was subtracted from the OD of the medium containing the cells and was related to the duration of incubation [d] of the culture. Growth curves were then drawn, indicating if and how quickly the cells grew, when they entered the exponential phase of growth, and when a plateau was finally reached (Fig. 5). This measurement was not applied to fungi and the bacterium *S. roseum*. These strains grew in aggregates or optically too dense, that an OD determination could not provide reliable information concerning growth. Nevertheless, the growth of these strains was evaluated visually (bare eye). The bacteria *F. ginsengisoli*, *L. pratensis*, and *P. xenovorans* did not perform well since the beginning of the cultivations and were excluded as community members.

The microorganisms were cultivated separately, in pure cultures. The aim was to combine the single taxa to a community, when they were at the end of their respective exponential growth phases, when the machinery for cell division was still active, the metabolic rate of the cells was high, and the maximum of the biomass was already built. 500 mL fresh medium in 1 L Erlenmeyer flasks were incubated with the single strains, depending on the respective growth curves, to have all of them ready to be harvested on the same day. The incubation started 16 days before the experiment for the four fungi and the bacteria *S. roseum*, and *S. spitsbergense. S. usitatus*, *K. versatilis* and *C. flavus* followed four days later. Six days before harvesting, *S. soli* and *C. multitrophicus* were freshly cultivated, while *P. alginolyticus* and *L. luteola* needed only five days of incubation. 40 mL each from the active cultures were used for the new ones. The fungi were transferred as always, just more biomass was used for the new cultivation.

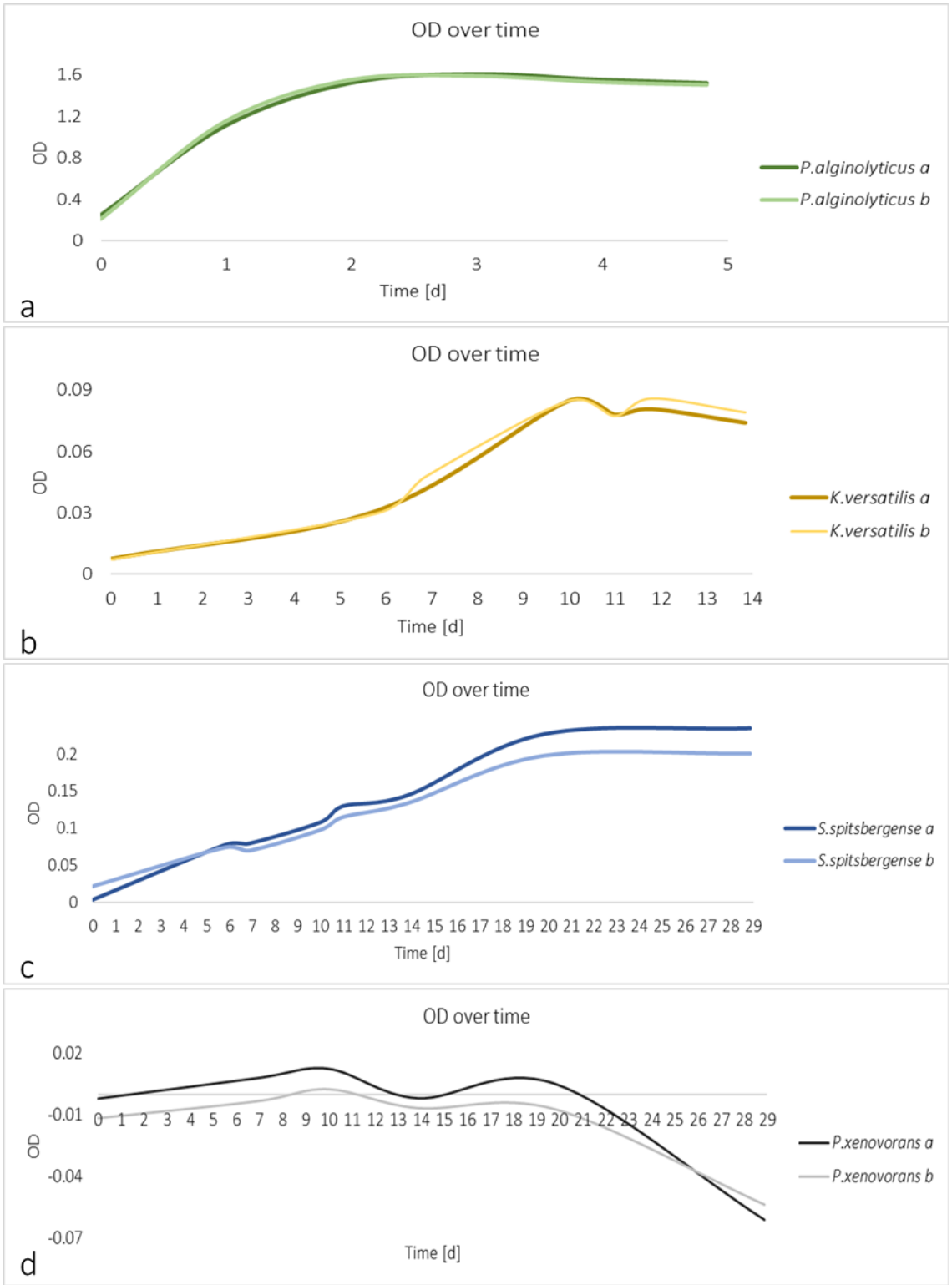


Figure 5 Exemplary growth curves of four different bacterial candidate strains evaluated with photometric measurements of the optical density (OD) in the liquid cultures. *P. alginolyticus* (a), *K. versatilis* (b), and *S. spitsbergense* (c) entered and left the exponential growth phases after a different amount of days in culture. Cultivation of *S. spitsbergense* started 16 days before harvesting its cells, *P. alginolyticus* grew quicker and *K. versatilis* lay in between of the two. *P. xenovorans* (d) did not perform well in pure culture and cell growth could not be detected via OD measurement. This strain was therefore no member of the synthetic community. The OD was determined from at least 4 different cultures of each bacterial strain (except *S. roseum*) and was always performed in duplicates (indicated as "a" and "b" in the respective figure legends).

The Start of the Experiment

γ-irradiation

The containers with the soil components and all pieces of needed equipment were additionally packed in autoclavable plastic bags (A. Hartenstein PB30; Roth Sekuroka) and stored in plastic boxes (Orthex, Smart Store). The boxes were then treated with > 25 kGy γ-radiation (Mediscan, Cobalt 60 Gammatron 1500) to further sterilize all material.

Assembly of the artificial soil

The assembly of the artificial soil was performed in one compartment of the isolator. Following our sterility protocol (see above), the treated and sterilized inorganic and organic components were combined to approximately 500 g of dry artificial soil (Tab. 2 and Tab. 3), with properties comparable to a temperate grassland soil (sandy loam Cambisol; Chinedu & Isaiah, 2016). In more detail, 320 g (fresh weight) were represented by the sand fraction, 125 g by silt, and 75 g by clay, making the Synthetic Soil a sandy loam. Then, 4 g of each oxide were added, 41 g of cell wall remains (POM), and 0.19 g sterilized MQ water per g fresh weight of the artificial soil (approximately 45% water holding capacity). The components were homogenized with a whisk in a sterilized bowl. The assembled artificial soil was sampled for later analysis, and was covered with aluminum foil (Rotilabo, thickness: 30 µm) until further use.

Assembly of the Synthetic Community (SynCom)

The harvesting of cells from the pure cultures (see above) for the assembly of the SynCom was performed as follows: The content of the flasks was transferred to an autoclaved beaker inside the laminar flow and poured into 50 mL tubes in 45 mL shares. The tubes were centrifuged at 3,900 RPM for 15 minutes. The supernatant was almost completely decanted, and the pellet was resuspended with a pipette. The biomass of each strain was collected in a single, pre-weighed 50 mL tube. It was then centrifuged for 5 minutes at 3,900 RPM, and autoclaved MQ water was added up to the 45 mL marking to wash the cells. The tubes were centrifuged again, and washing was repeated twice. After decanting the supernatant, the harvested biomass of all strains was weighed. The biomass of *K. versatilis* was so low, that it could not exactly be weighed due to the remains of MQ water, followed by *S. soli* with 0.4 g of fresh cell mass. When all cells had been weighed, 40

µL subsamples were transferred to Lysing Matrix tubes (MP, Lysing Matrix E, 2 mL tube) for later DNA extraction and were stored at -80°C. 0.4 g fresh weight from each strain were then collected in the 50 mL tube containing *K. versatilis,* and tentatively remaining biomass from single strains was stored in the respective tube at -20°C. Approximately 20 g of autoclaved tap water were added to the tube containing the combined strains, to allow homogeneous mixing. Since a contamination was detected in the cultivation flask of *S. usitatus* during the harvest, it was excluded from the experiment and the SynCom was finally built of 12 different organisms, 4 fungi (*A. bulbosa, P. macrospinosa, P. cyanescens, T. flavofuscum*) and 8 bacteria *(C. flavus, C. multitrophicus, K. versatilis, L. luteola, P. alginolyticus, S. soli, S. spitsbergense, S. roseum*).

Preparation of the Natural Community (NatCom)

One of the experimental groups was inoculated with a natural soil community to assess the suitability of the artificial soil to support microbial life. For the extraction of such a natural community, 10 g of soil, obtained from the department's garden were combined with MQ water up to the 45 mL marking in a 50 mL tube and incubated at room temperature for 2 hours on an orbital shaker (Ika, KS 501 digital) at 300 rpm. The tube was centrifuged for 20 minutes at 1,000 RPM to separate the soil particles (bottom of the tube) from the microbes in the supernatant. After transferring the liquid phase, the centrifugation was repeated once with the same settings and while using a fresh tube. To pellet the microbial biomass, centrifugation was repeated at 8,000 RPM for 20 minutes. The supernatant was discarded, and the biomass resuspended in 20 mL autoclaved tap water to not cause any osmotic stress. The content of this tube represented the "Natural Community".

Start of the Incubation

Inside the isolator, approximately 30 g of the artificial soil were added from the bowl to the mesh bags of 5 small mesocosms each. They were weighed (balances from Ohaus, CL Series), remained sterile and were kept inside the isolator. They represented the group "Artificial Soil" (A0). This chamber of the isolator was kept sterile at any time, to not risk any contamination of controls which were supposed to remain sterile.

A smaller bowl with part of the artificial soil was properly sealed and transferred to the other compartment of the isolator, where living strains could be handled. Approximately 20 g of the SynCom from the tube were added to 300 g artificial soil and both were homogenized by mixing. After being sampled for later analysis, 30 g of the mixture were transferred to the mesh bags of 9 small mesocosm each and their weights were noted. They remained inside the 2nd compartment of the isolator and represented the sample group “Synthetic Soil” (AS).

Since the plastic chambers of the isolator were transparent and it was kept in the greenhouse during the incubations, the lower compartment was covered with black plastic bags and the mesocosms were wrapped in sterilized aluminum foil (Fig. 6) to incubate the soil in the dark.

The remaining two experimental groups contained sterilized natural soil or the natural community. They were kept separately from the isolator to not have any contamination risk through potentially present spores or air-borne contamination with microbes.

The artificial soil was brought out of the isolator, and 150 g were inoculated with 10 mL of the NatCom under a flame. The homogenized mixture was sampled, and 30 g were transferred to the mesh bags of 5 small mesocosm each and their weights were noted. They represented the group “Artificial Soil + Natural Community” (AN).

Under a flame, 150 g of the sterilized garden soil were finally inoculated with 7 g of the SynCom and were sampled after mixing. Approximately 25 g were added to the mesh bags of 5 large mesocosms each before their weights were noted. They represented the group “Natural Soil + Synthetic Community” (NS).

The Isolator containing the “Artificial Soil” and the “Synthetic Soil” was brought to the greenhouse, together with the two other experimental groups which were kept in separate plastic boxes. Beforehand, three holes with a diameter of 2 cm were drilled in each long side of the boxes and were clogged with sterilized wool to allow gas exchange. The samples were incubated in the greenhouse at ca. 20°C (Fig. 6).

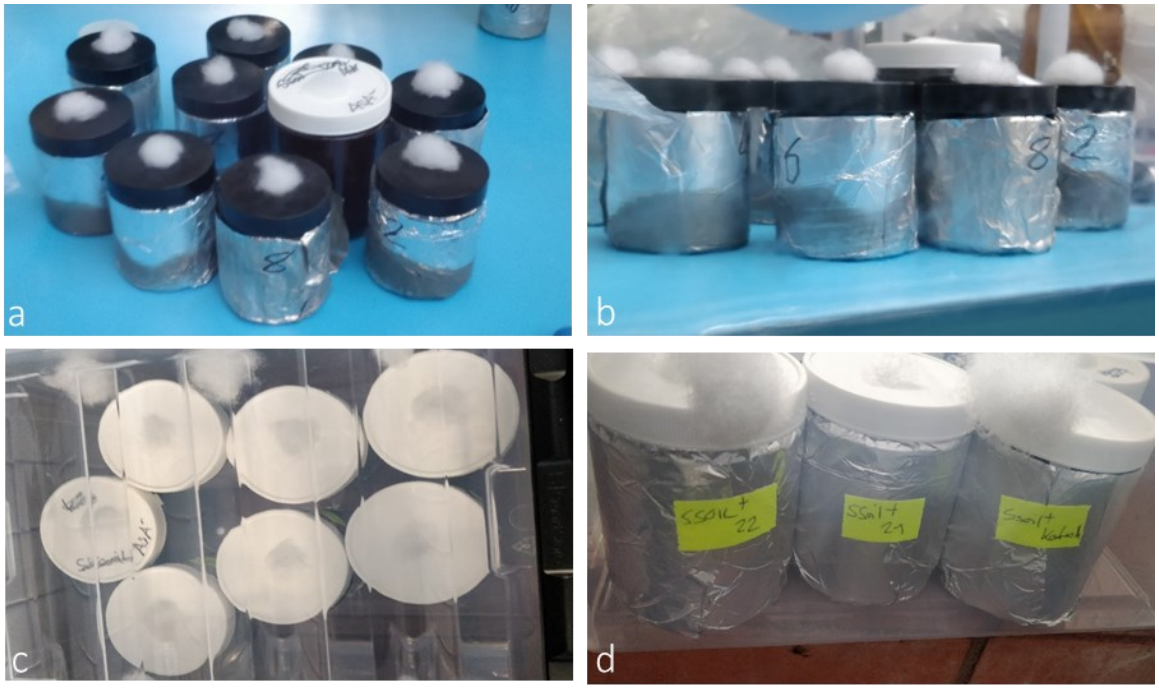


Figure 6 Mesocosms with AS and A0, incubated inside the isolator (top row, a + b) and inside closed plastic boxes (bottom row, c + d). The jars were wrapped in aluminum foil to allow incubation in the dark. The holes in the lids were covered with wool to avoid air-borne contamination while allowing gas exchange. The jars inside the boxes contained the natural community (c) and the natural soil (d). To not risk any cross-contaminations, they were kept separately from the samples inside of the isolator.

Sampling

Sampling of the soils was performed at three different time points: after mixing before the soils were split between the incubation jars (week 0), after seven days (week 1) and at the end of the experiment (week 5). At all three time points, subsamples were taken for pH determination, DNA extraction, DNA quantification, and amplicon sequencing, evaluation of NH_4^+, DOC, TDN, C_{total}, and N_{total} concentrations. In weeks one and five, soil respiration was evaluated and subsamples from the "Artificial Soil" were taken for an incubation on Agar plates to test for contamination. The dry weight / fresh weight (DW/FW) ratio was determined after mixing as well as in week five, and the enzymatic activity was evaluated after five weeks of incubation.

Soil sampling was conducted following our cleaning protocol mentioned above and was performed with sterilized equipment only. The isolator and the two boxes were brought to the lab. 10 g subsamples from the soils incubated inside the isolator were transferred to sterile 50 ml tubes for each mesocosm inside the sterile chamber and were only processed

after being brought out through its port system. The subsamples from the other two experimental groups were taken under a flame.

3 g of each soil sample were dried for several days at 65°C in aluminum dishes (Roth, Rotilabo, 28 mL and 13 mm height). After re-weighing, the DW/FW ratio was calculated (data not shown). The soils were then transferred to 2 mL tubes (Eppendorf, safe-lock tubes) and milled using a ball-mill (Retsch, MM-2000). Between 6 and 10 mg soil powder were weighed into tin capsules (IVA Analysetechnik, 5 x 9 mm) and their total C and N amounts were analyzed by using an elemental analyzer (CE Instruments, EA 1110) combined with a continuous-flow isotopic ratio mass spectrometer (Finnigan, DeltaPlus, IRMS). Due to the pronounced variation of the replicates, the subsamples were milled a second time to increase the homogeneity of the soil powder. This did not narrow the variation, but the measurements from the respective weeks were merged and treated as replicates.

For the determination of the pH, 2 g of soil were mixed in a scintillation vial with 10 mL MQ water (or 1 g of soil with 5 mL MQ water). The vials were repeatedly turned upside-down for 2 minutes by hand, rested for 30 minutes, and pH was measured in the slurry with the respective probe while shaking.

The DNA from pre-weighed and frozen samples, gained from all sampling time points as well as from the pure cultures, the SynCom, and the NatCom, was extracted and quantified by using the FastDNASpin Kit for Soil (MP Biomedicals) and the PicoGreen dsDNA Quantification Kit (Invitrogen Molecular Probes) with a spectrophotometer, respectively.

From the extracted DNA, the bacterial SSU (small subunit) 16S rRNA gene and the fungal internal transcribed spacer (ITS1) region were amplified by PCR using primers 515F-mod/806R-mod and ITS1F/ITS2 respectively (Apprill et al., 2015; Parada et al., 2016). Library preparation and sequencing services were provided by the Joint Microbiome Facility (Vienna) using a multiplexed amplicon sequencing approach. Sequencing was performed on a MiSeq platform, v3, 2x300 bp (Illumina). Paired MiSeq reads were processed using dada2 (Callahan et al., 2016) and amplicon sequencing variants (ASVs) were obtained.

The help of Leila Hadžiabdić and Dr. Hannes Schmidt from the Division of Terrestrial Ecosystem Research at the University of Vienna with DNA extractions and sequences is gratefully acknowledged.

To determine the concentrations of DOC and TDN, 2 g of soil were weighed into scintillation vials and extracted with 15 mL 1M KCl solution. After 30 minutes of incubation while shaken at 200 rpm on an orbital shaker, the extracts were filtered through glass microfiber filters and stored at -20°C until further processing. After diluting the extracts 1:5 with MQ water they were analyzed on a TOC/N analyzer (Shimadzu, TOC-VCPH /CPNTNM-1).

The KCl extracts were also used to photometrically determine the NH_4^+ concentrations of the samples, following Hood-Nowotny et al. (2010). Sample concentrations exceeding the range of the standard row were diluted 1:10 and remeasured (NS at all three time points; AS and AN in week five).

Potential enzymatic activities of 4 different enzymes (Cellobiosidase, β-Glucosidase, exo-Chitinase and Xylosidase) were determined at the end of the incubation. 1 g of soil each was suspended in 100 ml of sodium acetate buffer (50 mM, pH 7) and ultrasonicated at low energy (total energy output of 0.35 kJ). 200 µL of soil suspension and 50 µL of substrate were pipetted into black microtiter plates. After incubation at 20°C for 30 minutes, fluorescence was measured with a fluorimeter (Werfen, Tecan Infinite M200) every 30 minutes at 365 nm excitation and 450 nm emission for about 2 hours. The enzymatic activity was calculated as the increase in fluorescence over time (Kaiser et al., 2010).

The help of Leila Hadžiabdić and Dr. Alberto Canarini from the Division of Terrestrial Ecosystem Research at the University of Vienna with enzyme activity measurements is gratefully acknowledged.

1 g of soil from the “Artificial Soil” was mixed with 20 mL of autoclaved MQ water in a 50 mL tube. The content was shaken at 300 rpm for 10 minutes before let to rest for 10 minutes. 100 µL from the mixture were transferred to Agar plates (1x LB medium, 1x R2A medium and 1x malt extract medium) and incubated at 25°C to test for actively dividing bacteria or fungi. This was performed in week one as well as at the end of the experiment in week five. The plates were checked regularly. When cell growth was detected, single colonies were picked and transferred to PCR-clean 2 mL tubes 40 days after beginning of

the incubation. The tubes were stored at -20°C and were then sent off for sequencing. When this thesis was written, the samples were still under examination.

The CO_2 release from the soils was measured with a portable IRGA (InfraRed Gas Analyzer; PP Systeme EGM-4 environmental gas monitor for CO_2), by analyzing a 5 mL gas sample taken from each mesocosm under sterile conditions. Prior to the measurement, the wool inside the lids of the incubation jars was replaced by sterile septa (Supelco, gray rubber stopper, 20 mm) and changed back to a new piece of sterilized wool after the second measurement. Sampling was performed after subsamples were taken in week one and before harvesting the mesocosms in week five. Two consecutive samples were taken within 8 hours to calculate the CO_2 produced per hour in dry soil. The removed air from the first sampling was replaced by HEPA-filtered air from a gasbag, and the CO_2 release was corrected for this added air in the calculations. The CO_2 release rate in nmol CO_2 g^{-1} dry soil h^{-1} was calculated with the formula:

$$\frac{(\text{Rate [ppm } CO_2\text{ g}^{-1}\text{h}^{-1}\text{]} * (1.01325 \times 10^5\text{ [Pa]}))}{\frac{((8.31447\text{ [Joule mol}^{-1}\text{K}^{-1}\text{]} * (273.15\text{ [K]} + T\text{ [°C]}))) * V\text{ [m}^3\text{]}}{dw\text{ [g]} * 1000}}$$

Statistics and Graphics

The statistical analysis and graphic plotting were conducted by using the software R (R Core Team, 2020, Version 4.0.2) with the packages: “ggplot2”, “RColorBrewer”, “extrafont”, “lemon”, “stringr”, “plyr”, “dplyr”, “tidyverse”, “plotrix”, “nlme”, “ggpubr”, “rstatix”, “RSQLite”, “Hmisc”, “matrixStats”, “survival”, “backports”, “processx”, “Rcpp”, “usethis”, “devtools”, “multtest”, “foreach”, “igraph”, “data.table”, “scales”, “xfun”, phyloseq”, “vegan”, and “coin”, and with Microsoft Excel (Microsoft Office 365).

Results

The Synthetic Soil created in this study was composed of an abiotic artificial soil and an *in-silico* designed, reduced synthetic microbial community (SynCom). The artificially assembled soil was a mixture of sterilized organic and inorganic components. Their proportions were chosen to mimic those normally reported for temperate grassland soils.

After conducting some pre-experiments (data not shown), the Synthetic Soil was tested for its functionality and stability over time. Four different experimental groups were set up for this purpose (Tab. 1):

- The Synthetic Soil which represented the full model ecosystem approach, consisting of the artificial soil (A) inoculated with the synthetic community (S), in the following termed "AS"
- The Artificial Soil (A) which remained sterile and served as a control without a community (0), in the following termed "A0"
- The artificial soil (A) with a natural community (N) as a control for the suitability of the soil to sustain microbial life, in the following termed "AN", and
- The sterilized natural soil (N) with the synthetic community (S) as a control for the synthetic community to be able to strive in a natural setting, in the following termed "NS".

The different soil parameters of the four experimental groups were measured at three time points: after mixing of all components (week 0), seven days after incubation (week 1), and at the end of the experiment (week 5). The statistical analysis was centered around the potential differences between the Synthetic Soil and the Artificial Soil with focus on the comparison of parameters between week one and week five. Attention was further paid to the parameter variance among replicates as indicators for repeatability and controllability of the model ecosystem approach.

The Artificial Soil

We designed the artificial soil to consist of approximately 1% organic carbon at a C/N ratio of around 10. To achieve this, we added 0.7% sterilized ground plant material, and 1.5% sterilized microbial remains to the abiotic components. The mineral phase of the soil consisted of the primary minerals quartz, K-Na-Feldspar, K-Feldspar, Al-Fe-Silicates and

the secondary minerals Montmorillonite, Goethite and Boehmite. The texture of the artificial soil reflected a sandy loam with about 65% sand, 25% silt and 10% clay. Sterile water was added to approximately 45% of the water holding capacity (Tab. 3).

The artificial soil, with additions of the SynCom or a natural microbial community (NatCom) had approximately 1% C (Fig. 7), 0.1% N (Fig. 8) on average, and a molar C:N ratio of 12 (data not shown). The C and N content (C_{total}, N_{total}) did not show significant changes with time (Wilcoxon test, for detailed results see Suppl. Tab. 1). Pronounced variation among replicates was observed in all treatments in week one and week five, which points at inhomogeneous mixing.

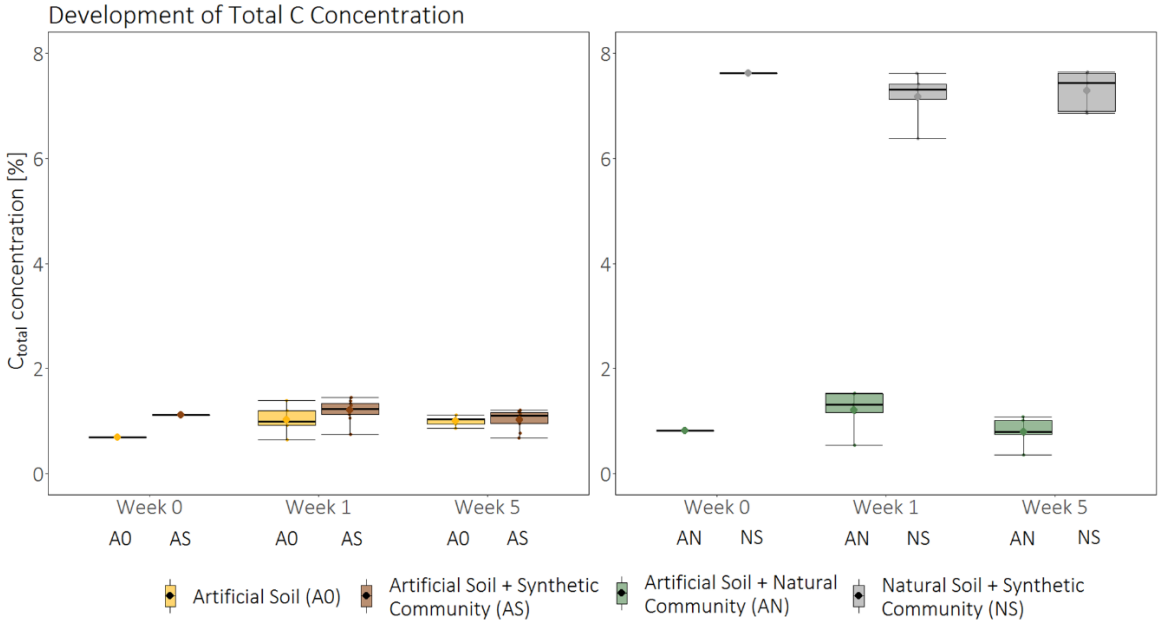


Figure 7 Development of the total C concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated, for details see Suppl. Tab. 1

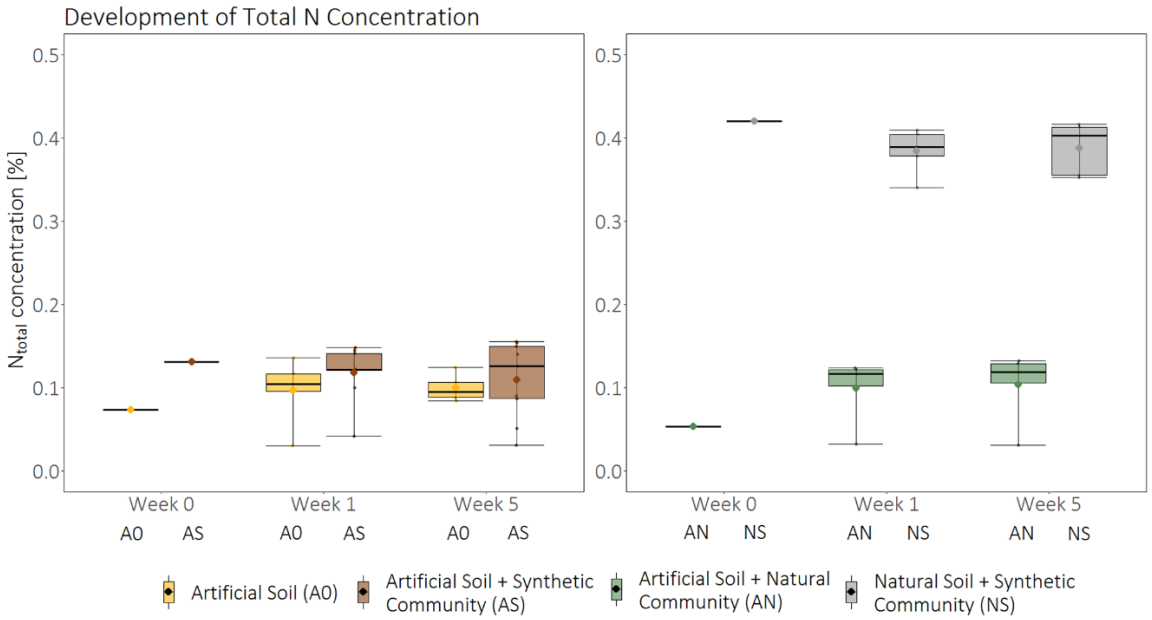


Figure 8 Development of the total N concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated, for details see Suppl. Tab. 1

Since pH has a strong effect on cell activities like organic matter decomposition, cell division, and growth, it was evaluated for all replicates from all sample groups after mixing of the components, seven days later, and at the end of the incubation after five weeks (Tab. 5). The pH of all samples containing the artificial soil was identical at the beginning of the experiment, being slightly acidic at pH 6.5. The natural soil's pH was higher and with 8.1 alkaline. The pH of all soils increased over time. This pattern was more pronounced in the artificial soil compared to the natural soil. The pH of AN and AS was 6.8 after seven days of incubation, and 7.8 and 7.4 after five weeks, respectively. By then, A0 had become neutral (Tab. 5). A statistical analysis (Suppl. Tab. 1) of the Artificial Soil and the Synthetic Soil revealed that the pH in week five was significantly higher in the latter (Wilcoxon test, $p = 0.003$ $r = 0.80$) and that its pH increase from week one to week five was also significant (paired Wilcoxon test, $p = 0.009$ $r = 0.89$).

Table 5 Development of pH over time. Values are means ± 1 standard error.
For details concerning statistical differences see Suppl. Tab. 1

Sample	W0	W1	W5
Artificial Soil (A0)	6.5	6.8 ± .01	7.0 ± .02
Artificial Soil + Synthetic Community (AS)	6.5	6.8 ± .01	7.4 ± .05
Artificial Soil + Natural Community (AN)	6.5	6.8 ± .05	7.8 ± .07
Natural Soil + Synthetic Community (NS)	8.1	8.2 ± .02	8.4 ± .01

W = Week after mixing

Pools and Processes

To gain insights into the elemental pools, the concentrations [mg g^{-1} dry soil] of the dissolved fractions (dissolved organic C = DOC; total dissolved N = TDN) were measured additionally to the total C and total N concentrations.

Concerning the dissolved fraction, the mean DOC concentrations (Fig. 9) in all samples with artificial soil were similar at the beginning of the experiment. The values decreased about 50% within the first week. Four weeks later, DOC concentrations were slightly increased in the Artificial Soil, but further decreased in the Synthetic Soil. DOC concentrations did not change from week one to five when the NatCom was present. The mean DOC concentration in NS was fourfold higher than in the other samples at the beginning, but decreased, like in all soils in which microorganisms were added (Fig. 9). A comparison of week five concentrations between A0 and AS revealed a statistically significant lower mean DOC concentration in the Synthetic Soil compared to the Artificial Soil (Wilcoxon test, $p = 0.007$ $r = 0.70$).

The TDN concentrations were similar in all groups when the experiment started (Fig. 10). Within a week, the concentrations strongly decreased in the samples with artificial soil in contrast to NS (Fig. 10). The TDN concentration in A0 remained almost unchanged in week five but increased in AS and AN 10- and 35-fold, respectively, while it remained unchanged in NS (Fig. 10). A statistical analysis showed a significant increase from week one to week

five in the Synthetic Soil (Wilcoxon test, p = 0.004 r = 0.89), and that its TDN concentration was significantly lower compared to the Artificial Soil in week one as well as significantly higher in week five (Wilcoxon tests, p = 0.001 r = 0.8 for week one and week five, respectively). These findings are in line with the NH_4^+ concentration patterns (Fig. 12). Pronounced variation among replicates was found in AS and AN in week five (Fig. 10 and Fig. 12).

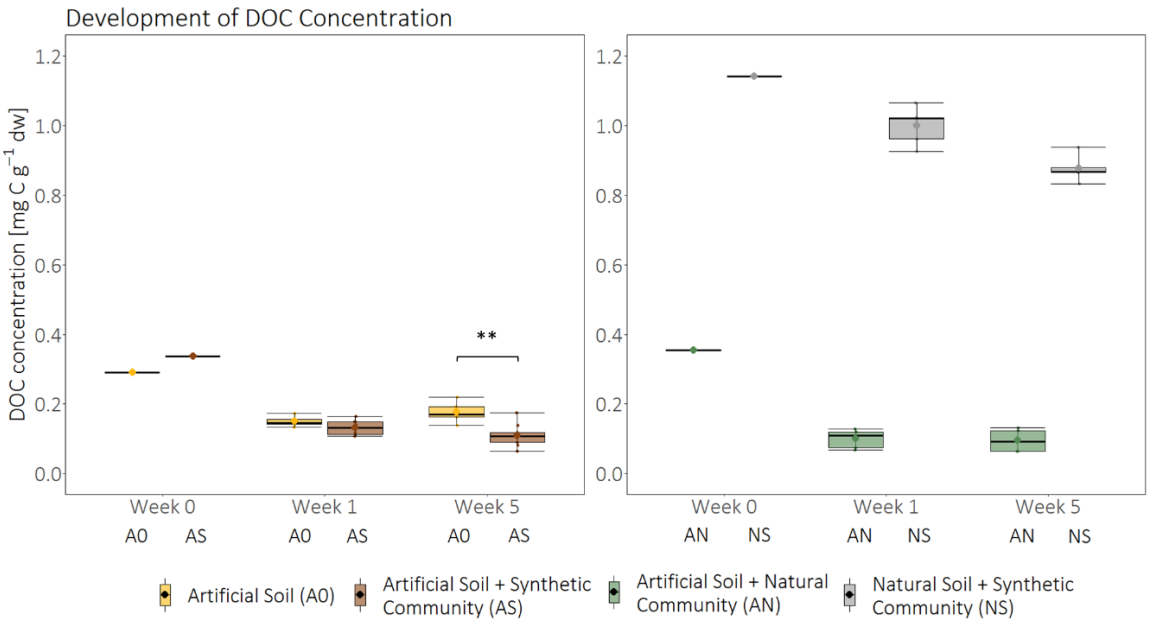


Figure 9 Development of the DOC concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 1

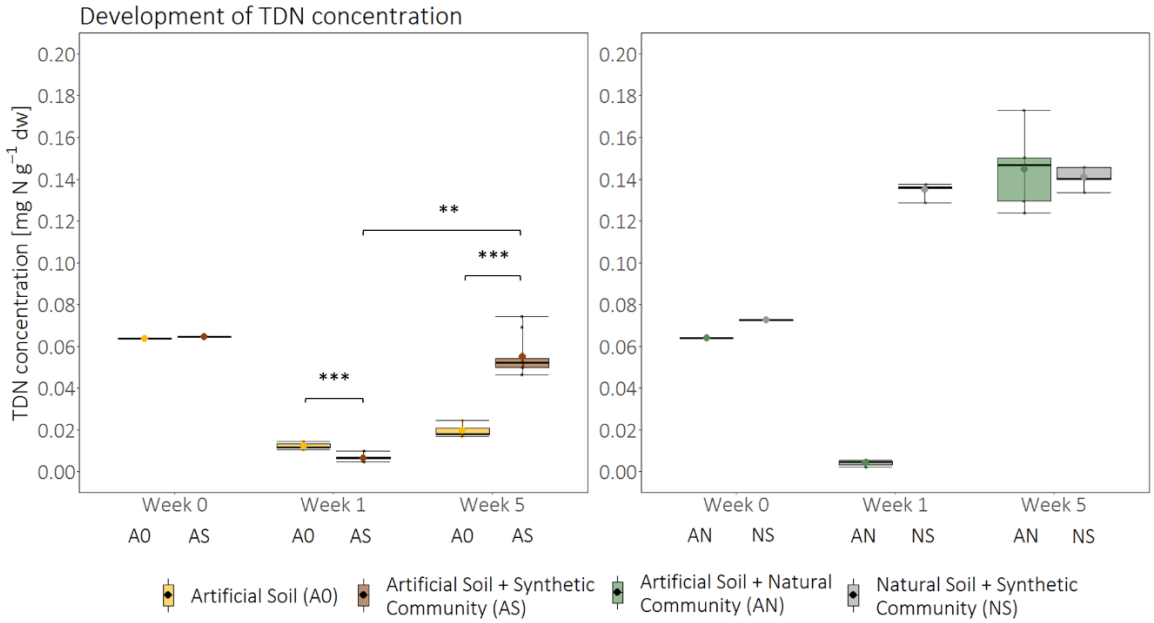


Figure 10 Development of the TDN concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details Suppl. Tab. 1

We measured microbial respiration (CO_2 exchange rate in [nmol g^{-1} dry soil h^{-1}]) as a representative process rate for microbial activity. One week after mixing, the mean CO_2 release was lowest in the Artificial Soil and five-fold higher in AN. AS and NS were very similar and intermediate between the other two groups (Fig. 11). Four weeks later, the mean respiration rates were lower than before in all groups. They decreased about 50% in AN as well as in NS. The mean CO_2 release in A0 remained lowest. The mean respiration rate of AS decreased the least and had the highest mean CO_2 exchange rate of all four groups at the end (Fig. 11 and Suppl. Fig. 1). In week one as well as in week five after mixing, the mean respiration in the Synthetic Soil was significantly higher compared to the Artificial Soil (Wilcoxon tests, p = 0.001 r = 0.80 for both weeks, respectively).

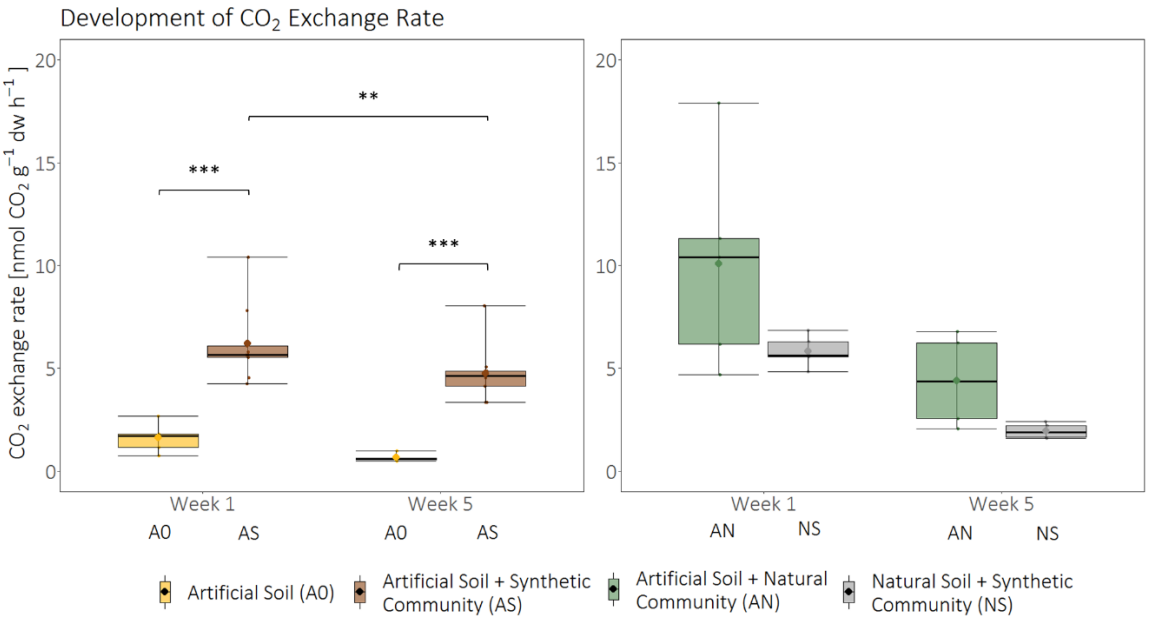


Figure 11 Development of the CO2 exchange rate over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details Suppl. Tab. 1

Changes in ammonium (NH_4^+) concentration over time can also be an indication for active microbial metabolism. When the experiment started, similar mean NH_4^+ [µg NH_4-N g^{-1} dry soil] concentrations were measured in all three groups with artificial soil (Fig. 12). In all these groups, the mean NH_4^+ concentrations dropped after a week. At the end of the experiment after five weeks, the NH_4^+ concentration in A0 was similar to the one measured at the beginning, while the NH_4^+ concentration strongly increased in AS and AN. The final concentration in AS was 8-fold (paired Wilcoxon test, p = 0.016 r = 0.89) and in AN 25-fold higher than after mixing, respectively (Fig. 12 and Suppl. Fig. 2). In week five, the mean NH_4^+ concentration in the Synthetic Soil was significantly higher than in the Artificial Soil at this time (Wilcoxon test, p = 0.001 r = 0.80). The variation among replicates was broadest in week five concerning AS and AN. The mean concentration of NH_4^+ in NS was tenfold higher than in all experimental units with artificial soil, and it further increased to approximately 90 µg g^{-1} dry soil in week five.

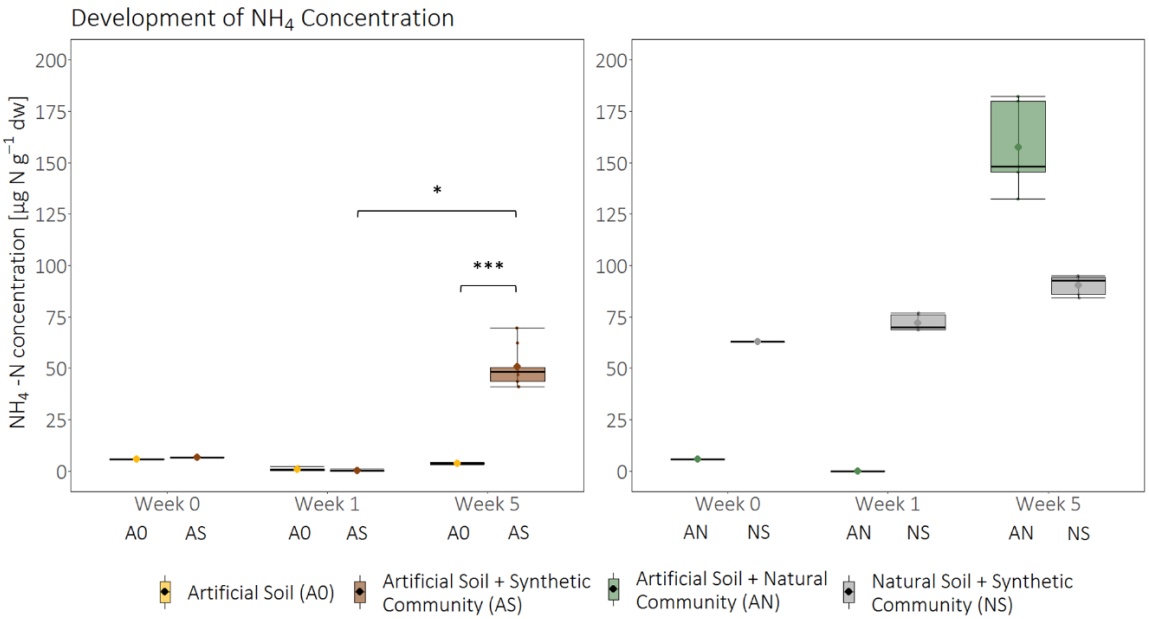


Figure 12 Development of the NH4 concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 1

Microbial Biomass and Community Composition

The development of the microbial community biomass was estimated by analyzing the total DNA concentration [µg g^{-1} dry soil] over time. Compared to the other groups, DNA concentrations were lowest in the Artificial Soil at the beginning of the experiment (Fig. 13), and remained the lowest until the end of the experiment with a mean DNA concentration of 0.5 µg g^{-1} dry soil, pointing at DNA added as microbial remains during mixing. The incubation of the soils with the two different communities (SynCom and NatCom) resulted in higher mean DNA concentrations of the respective samples at the first measurement (Fig. 13). The mean DNA concentration in AS and AN strongly increased over time. In week five, most DNA was found in AN with more than double the amount of DNA compared to AS (Fig. 13). The mean DNA concentration also increased in NS over time, but the pattern was not as pronounced and resulted in approximately 50% of the mean DNA concentration compared to AS in the end (Fig. 13 and Suppl. Fig. 3). A statistical comparison of A0 and AS revealed a significantly higher mean DNA concentration of the latter in week one as well as in week five (Wilcoxon tests, p = 0.001 r = 0.80 for both weeks, respectively; Suppl. Tab. 1) and a significant increase in DNA concentrations in AS between the measurements of week one and week five (paired Wilcoxon test, p = 0.039 r = 0.69; Suppl.

Tab. 1). A pronounced variation among replicates of AN was found in week one and in week five, caused by one extreme value at each sampling timepoint.

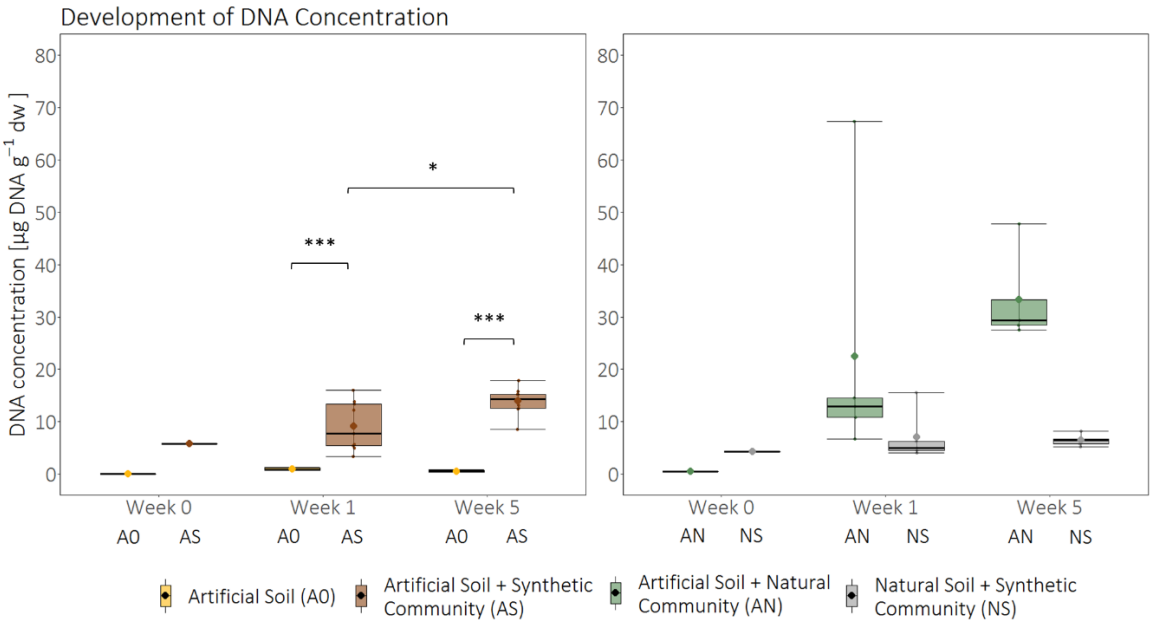


Figure 13 Development of the DNA concentration over time. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Comparison within experimental units was done with paired Wilcoxon test.
Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details Suppl. Tab. 1

Twelve different taxa were chosen to be part of the reduced synthetic community, four fungi (*T. flavofuscum, P. macrospinosa*, *A. bulbosa, and P. cyanescens)* and eight bacteria (*C. flavus, C. multitrophicus, K. versatilis, L. luteola, P. alginolyticus, S. roseum, S. soli,* and *S. spitsbergense;* Tab. 4). To assess the development of the microbial community over time and to verify if members of the SynCom are striving throughout the incubation as well as to evaluate sterility and controllability of the soils, we sequenced the 16S rRNA gene (for bacteria) and the ITS1 gene (for fungi) for all groups at all three timepoints. In the Artificial Soil, to which no community was added, only one bacterial amplicon sequence variant (ASV) was found, which belonged to the order Bacillales. This ASV was present in all samples of A0, resulting in a relative abundance of 100% in A0 (Suppl. Fig. 4). Its relative abundance of 75% in the Synthetic Soil decreased to 50% in week five. A similar pattern was found in AN Suppl. Fig. 6) but the decrease was even more pronounced, leading to only 3% relative abundance of this ASV in week five.

Although eight bacteria were part of the synthetic community, more than ten ASVs were sequenced in the Synthetic Soil at all three sampling timepoints, including at least five strains from the SynCom: *C. flavus, C. multitrophicus, K. versatilis, S. roseum,* and *S. soli* (Suppl. Fig. 5).

All taxa from the SynCom, sequenced in the Synthetic Soil, could also be identified in NS. The development of their relative abundances over time was comparable between AS and NS (Suppl. Fig. 5 and Suppl. Fig. 7). The contrast between the two groups was expressed in the total number of ASVs found. Dependent on the resolution, more than 20 additional taxa were identified during sequencing in NS. As expected, the ASVs found in AN were very different from the SynCom. Only two ASVs, a Proteobacterium and an organism belonging to the Firmicutes order were found in all three incubated groups. The remaining taxa apart from the SynCom, were unique to AN and could not be detected in the other groups (Suppl. Fig. 6). Neither in AS nor in NS, the candidate strain *S. spitsbergense* could be re-identified. All bacterial strains found in incubated samples after mixing, were also found after five weeks. The relative abundance of the ASVs showed no general pattern over time but varied dependent on the experimental groups and the ASV identity, respectively (Suppl. Fig. 5 to 7). The SynCom was however developing over time (i.e. the relative abundance of taxa changed), visible through pronounced clustering of AS according to the time incubated. No such development was visible in A0 (Fig. 14).

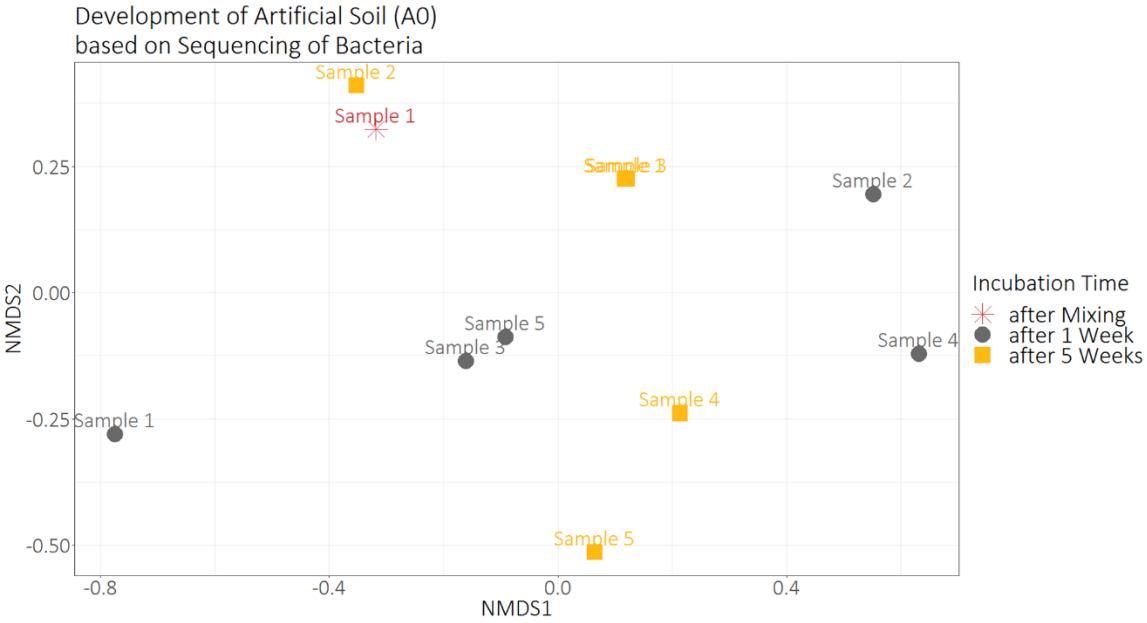


Figure 14 Development of the 50 most abundant bacterial ASVs (amplicon sequence variants) with at least 2,000 reads inside the Artificial Soil (abiotic control) over time, assessed by the 16S rRNA gene.

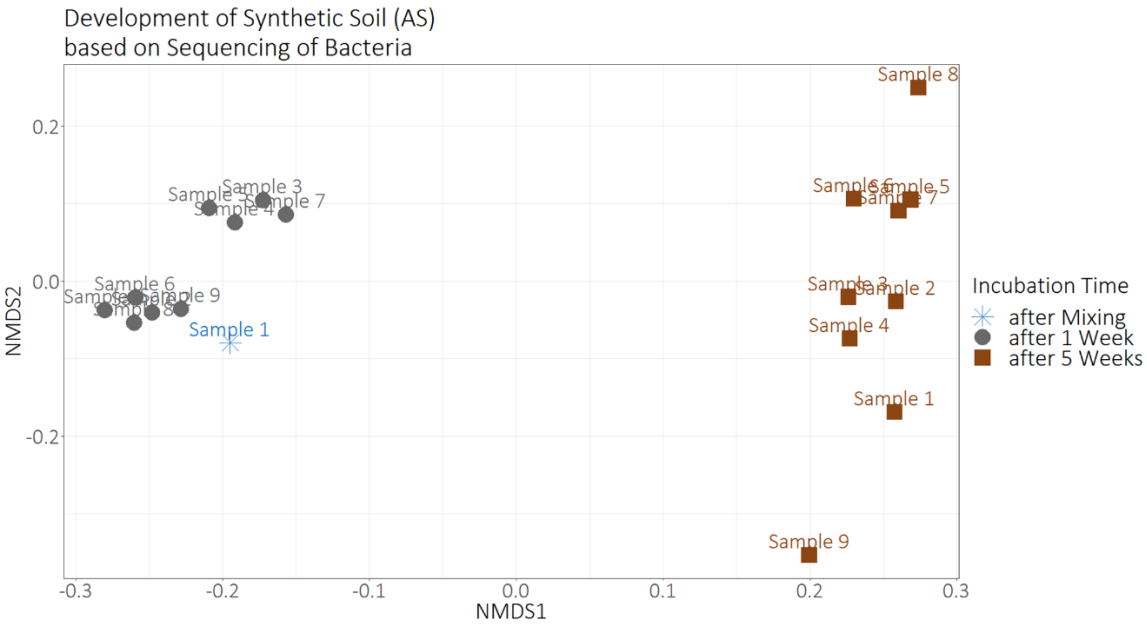


Figure 15 Development of the 50 most abundant bacterial ASVs (amplicon sequence variants) with at least 2,000 reads inside the Synthetic Soil (model ecosystem) over time, assessed by the 16S rRNA gene.

The relative abundances of fungal ASVs were very different compared to the ones from the bacteria. On a coarse overview a striking similarity could be observed between A0 and AN on one hand, and between AS and NS on the other hand (Suppl. Fig. 8 to 11). The DNA of more than ten ASVs was identified in A0, to which no community was added. The relative abundances of the ASVs did not change between week one and week five. *Alternaria sp.*, *Aspergillus flavus*, and *Aurantiporus sp*. were the most abundant identified

ASVs in A0 (Suppl. Fig. 8), as well as in AN (Suppl. Fig. 10). In both groups various additional ASVs were found but could not be classified exactly. The main difference between the taxa found in A0 and AN, was the development of their relative abundances over time. The dominant taxa in AN one week after mixing, strongly decreased in their relative abundance in week five (Suppl. Fig. 8 and Suppl. Fig. 10).

Four fungi were members of the SynCom (*T. flavofuscum* and *P. macrospinosa,* both Ascomycota; *A. bulbosa* and *P. cyanescens*, both Basidiomycota) (Suppl. Fig. 9). At least five fungal ASVs were found in AS, and four in NS (Suppl. Fig. 9 and Suppl. Fig. 11). In week one, the most abundant species in AS was *T. flavofuscum*. Its occurrence was stable and slightly increased in week five in AS, however it strongly decreased in NS. *P. macrospinosa* was also present in both groups at the end of the experiment. Although species belonging to the phylum Basidiomycota were in principle covered by the primers used (although to a lower extend than Ascomycota), they could not be found in the community, causing doubts as to whether they can be detected by the primers used. The second and third most abundant organisms in AS and NS were *Cladosporium sp.* and *Alternaria sp.* The latter was clearly the dominant species in NS and its abundance did not change over time, while it was slightly decreasing in AS with time. The abundance of *Cladosporium sp*. was comparable in AS and NS and hardly changed at the two sampling timepoints (Suppl. Fig. 9 and Suppl. Fig. 11). *Aspergillus flavus* and *Aurantiporus sp*. were found in AS, as well as in A0 and AN. In the Synthetic Soil the community was developing over time, i.e. the relative abundance of taxa changed between week one and five. No such change with time was found for A0 (Fig. 17).

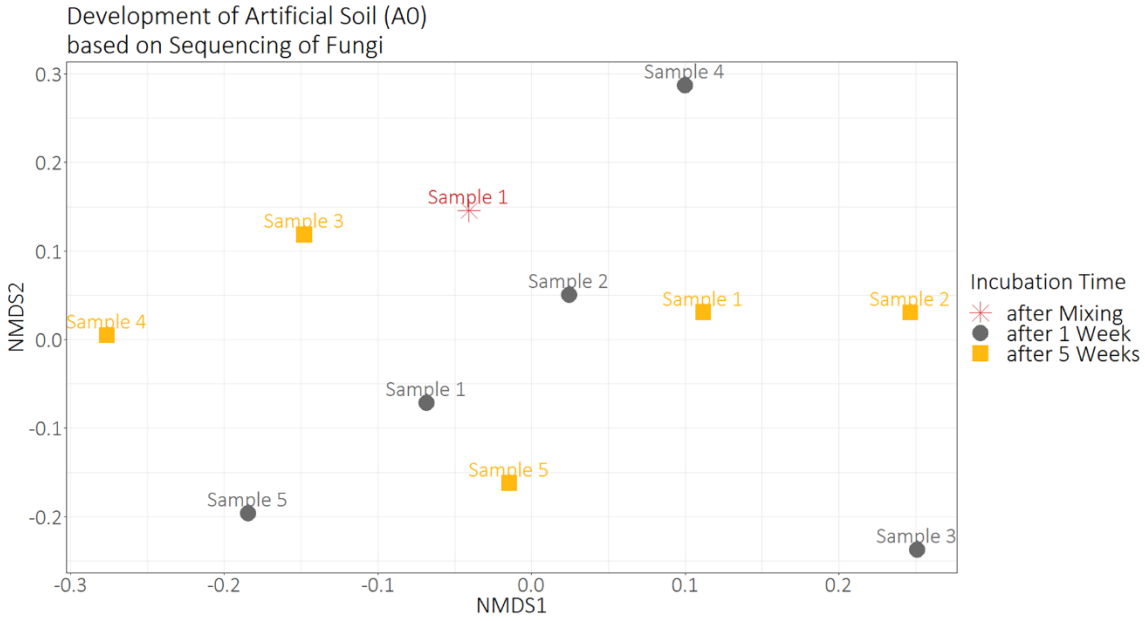


Figure 16 Development of the 50 most abundant fungal ASVs (amplicon sequence variants) with at least 2,000 reads inside the Artificial Soil (abiotic control) over time, assessed by the ITS1 gene.

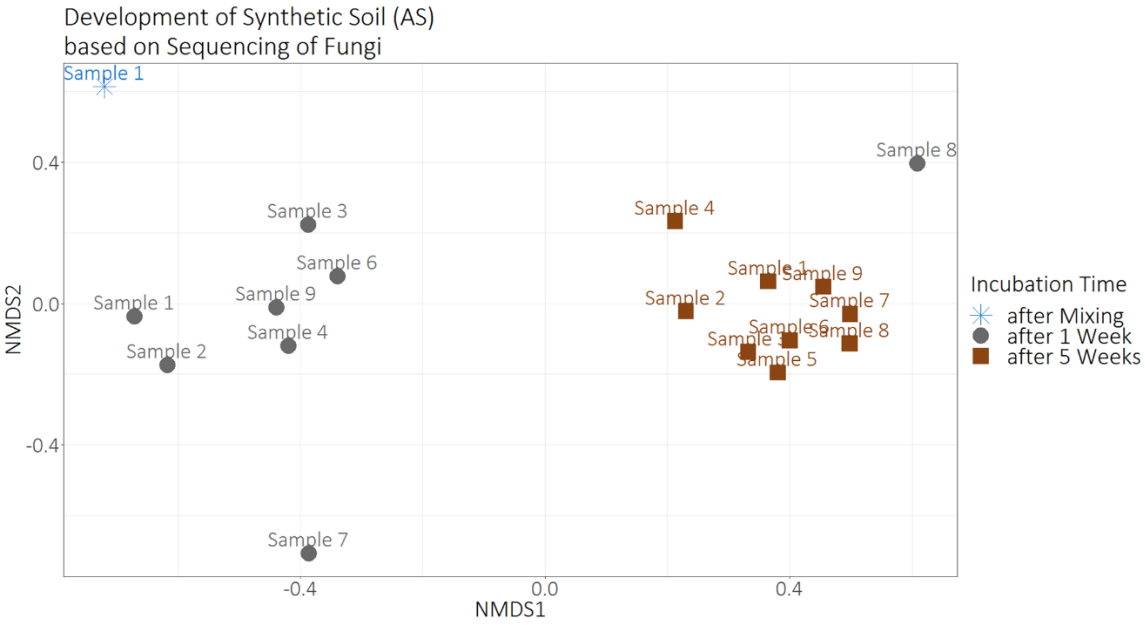


Figure 17 Development of the 50 most abundant fungal ASVs (amplicon sequence variants) with at least 2,000 reads inside the Synthetic Soil (model ecosystem) over time, assessed by the ITS1 gene.

The activities of four different extracellular enzymes (Cellobiosidase, β-Glucosidase, exo-Chitinase, and Xylosidase) were measured in all experimental groups at the end of the incubation [potential activity, µmol g^{-1} dry soil h^{-1}]. Very low activities were found in A0, as expected. In AS we found well developed enzymatic activities, which were significantly different to A0 (Fig. 18 to 21). Adding the SynCom to NS led to lower enzyme activity levels

than found in AS for three out of the four enzymes (for Cellobiosidase, β-Glucosidase, and exo-Chitinase). Only the enzyme Xylosidase was higher in NS than in AS. (Wilcoxon tests, p = 0.001 r = 0.80 for all enzymes respectively; Suppl. Tab. 1).

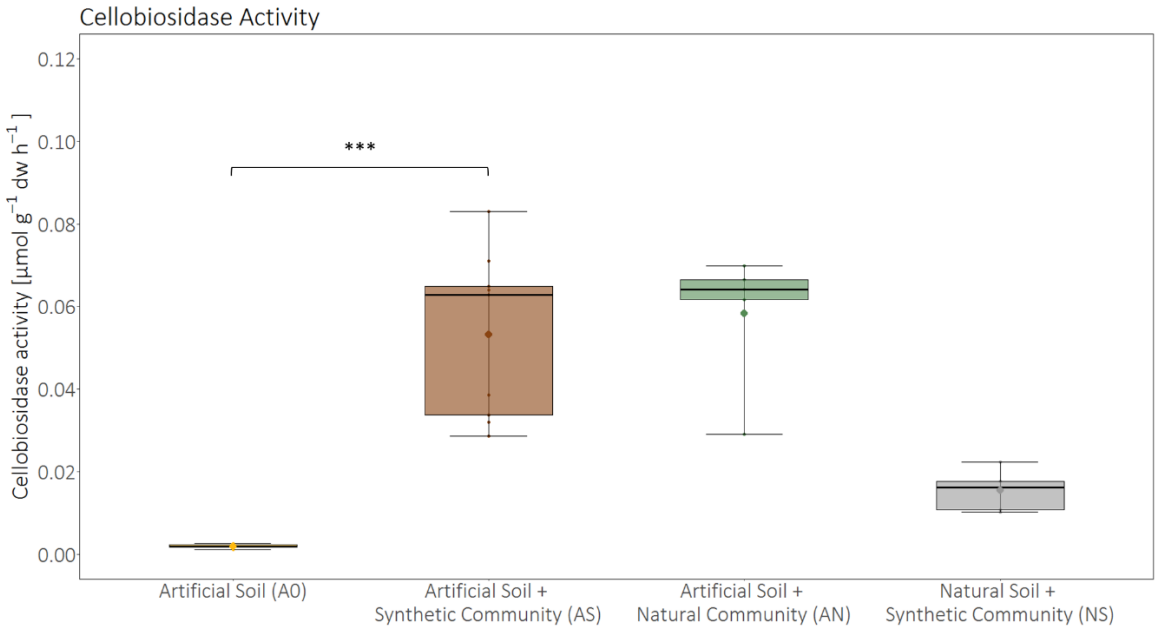


Figure 18 Cellobiosidase activity in week five. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 2

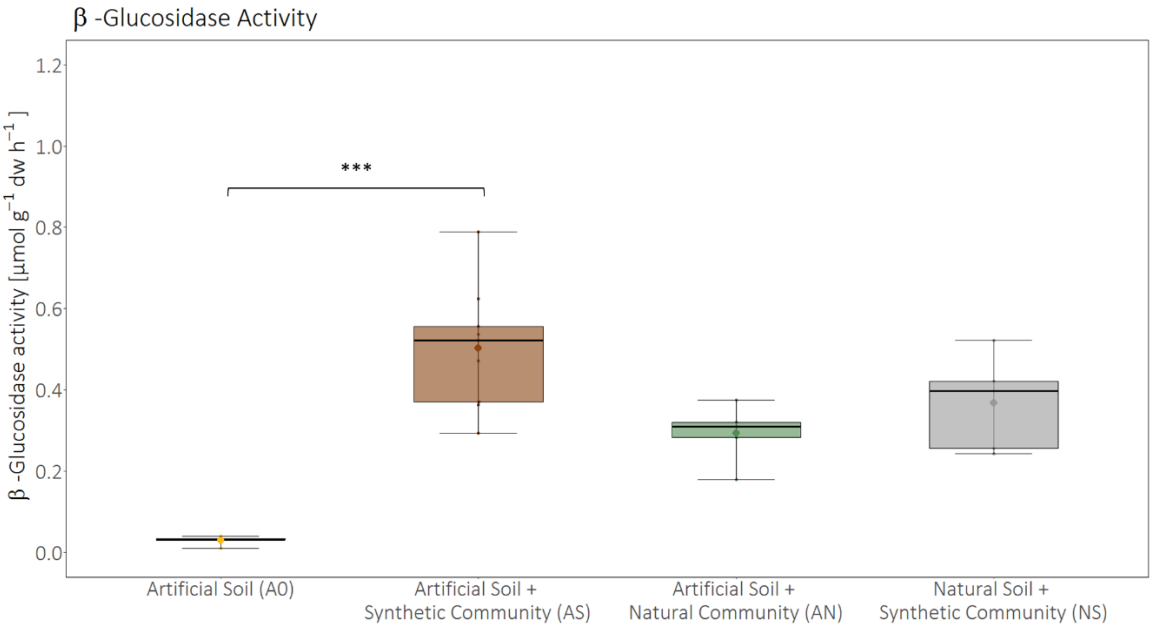


Figure 19 β-Glucosidase activity in week five. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 2

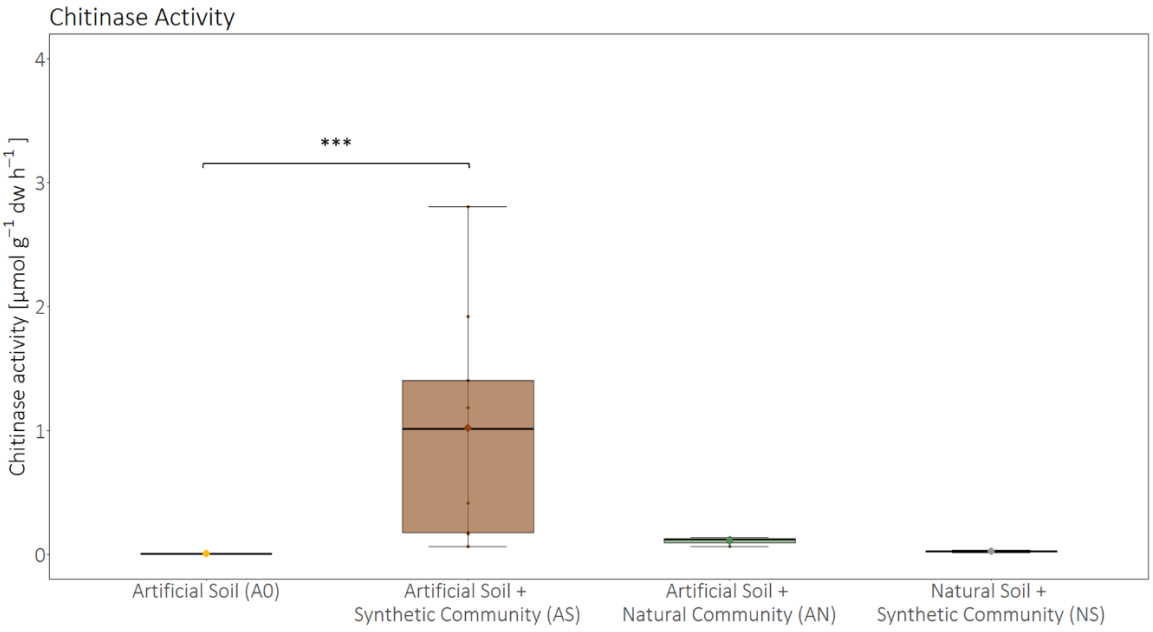


Figure 20 Chitinase activity in week five. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 2

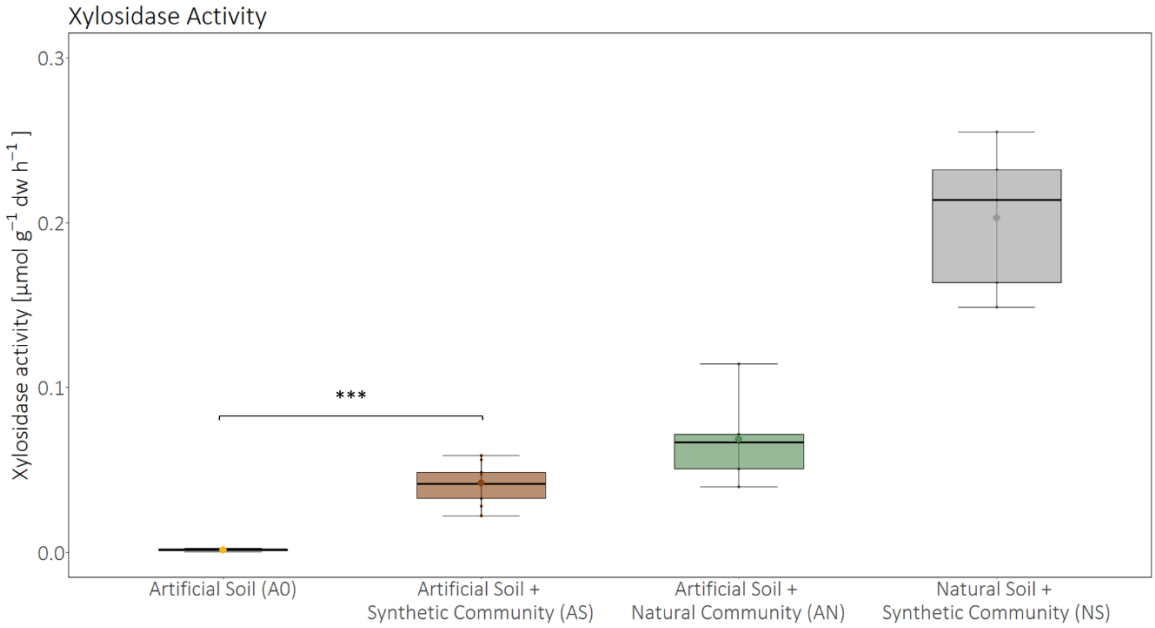


Figure 21 Xylosidase activity in week five. Whiskers show minimum and maximum values within each experimental unit. Mean (big dot) and median (black line) are given. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. Only significant differences are indicated: * p<0.05; ** p<0.01; *** p<0.005; for details see Suppl. Tab. 2

Discussion

Soils are structurally, chemically, and biologically extremely diverse ecosystems. Soil processes are caused by organisms in the µm range, but manifest themselves in the mm to m range, which demands new experimental approaches to overcome the current challenges involved, such as linking organisms and consortia to processes and having reproducible ecosystems available for ecological research. The present study introduces a novel experimental approach, a Synthetic Soil Ecosystem. A sterile soil matrix was artificially assembled and inoculated with an *in-silico* designed, reduced synthetic community of microorganisms. In a proof-of-concept experiment, an active and controllable model ecosystem developed within a few weeks, which showed basic characteristics of a natural soil. We propose that such a Synthetic Soil will be suitable in the future to address and investigate fundamental questions in soil ecology.

The "SynSoil experiment" is the first of its kind, building on previous experiments which (i) were using artificial soils with a natural, but uncontrolled inoculum (Ding et al., 2013; Vogel et al., 2014; Ditterich et al., 2016; Kallenbach et al., 2016; Pronk et al., 2017) and (ii) synthetic communities (SynComs), which are grown in petri-dishes, microfluidics devices or similar artificial environments (Celiker & Gore, 2014; Stanley et al., 2016; Bhatia et al., 2018; Cairns et al., 2018; Kong et al., 2018).

The Synthetic Soil (AS) was built in a minimal set-up to proof the general suitability of the approach. We here combined twelve components (seven minerals and five different organic materials) to construct the artificial soil and grew eight bacterial species and four fungal species in axenic cultures to construct a minimal but functional microbial community. The Synthetic Soil was then incubated for five weeks, during which a range of parameters were measured to evaluate (i) if the microorganisms grow and a microbial community establishes, and (ii) if basic functions of the Synthetic Soil, such as the decomposition of organic material (estimated by measuring the extracellular enzyme activities and respiration rates), evolve over time.

One of the basic requirements for the functioning of the Synthetic Soil approach was the presence of an active and self-sustaining microbial community inside the artificial soil.

Microorganisms need energy for growth and survival. A soil in which microbes live and strive must therefore harbor microorganisms that degrade organic matter and generate energy from it. Usually, the respiration rate is used as a proxy for energy generation of heterotrophic organisms (Madigan et al., 2012; Manzoni et al., 2012). In case of this study, significant respiration was detected in AS, which was still stable after five weeks of incubation (Fig. 11). While we also found a small CO_2 release from A0, most likely due to abiotic reasons (Fig. 11), the respiration rate of AS was by the factor of four to seven higher than of A0. These results demonstrated that the added microbial species proliferated in the artificial soil. This was encouraging, since the single community members were cultivated in liquid cultures under axenic conditions before addition to the soil, and the recipes of the liquid media (Suppl. Tab. 5) were designed to fulfill all the optimal nutrient requirements of the single strains to increase their growth. The process of cell harvest from the cultivation flasks and the combination of the pure strains with others - potentially competitors or microbes that otherwise affect their growth - to a community, followed by the transfer to the heterogeneous solid soil, meant a complete change of the microorganism's environment and could easily have caused cell death of some of the strains (Fierer, 2017). So, the growth of the strains selected for the experiment suggests a general suitability of the artificial soil to sustain a microbial life. In contrast, the incubated natural community (NatCom) was obtained from a garden soil and could be considered adapted to a soil environment. The respiration rates of the SynCom were comparable to the ones from the NatCom and both lay within the same range (Fig. 11). The CO_2 release in A0 was most likely caused by abiotic processes (Liu et al., 2019) rather than by living cells. For example, by chemical reaction of the organic material with iron oxides or at the surface of the mineral phases (Liu et al., 2019). If a relevant microbial contamination would have been present, an increase in released CO_2 in week five should have been found due to the labile substrates present in the soil supporting growth of the contaminants (Sinsabaugh et al., 2008). But the opposite was observed, the CO_2 release decreased in week five to a third of the initial one (Fig. 11).

The total concentrations of C and N in all mesocosms with artificial soil (A0, AS and AN; Fig. 7 and Fig. 8) was around 1% and 0.1% respectively, did not change over time, and were unaffected by an addition of living cells, as the amount of C and N added was small

compared to total C and N present. However, the changes of soluble nitrogen (and specifically NH_4^+ in AS) and soluble carbon (DOC) concentrations in the samples (Fig. 10, Fig. 12, and Fig. 9) from the start of the experiment to week one and then further to week five additionally supported the successful establishment of the Synthetic Soil. The pattern found was caused by the metabolic activity of the community members and can be interpreted in the context with the microbial demand for N and organic C (Soong et al., 2020). The strong decrease of DOC and NH_4^+ (Fig. 9 and Fig. 12) in the first seven days of incubation reflected the microbial immobilization of C and N. Their uptake resulted in the decrease of DOC and TDN concentrations in AS and AN during this time (Fig. 9 and Fig. 10). TDN consists of inorganic N, such as NH_4^+, but also of organic N. If microbes are C limited, the organic N taken up, would be deaminated, the C used for growth, and NH_4^+ exuded, i.e. mineralized. Soil microorganisms are generally thought to be rather C than nutrient limited (Soong et al., 2020). The total C content of the artificial soil of 1% (Fig. 7) and a C/N ratio of 12 indicates a microbial C limitation as microorganisms have on average a C/N ratio of 8 and a carbon use efficiency of 0.3 (Sinsabaugh et al., 2013; Mooshammer et al., 2014; Zechmeister-Boltenstern et al., 2015). Our results on TDN and NH_4^+changes over time are therefore consistent with the SynCom actively mineralizing N during the degradation of SOM in the Synthetic Soil Ecosystem (Fig. 12). In contrast to what was expected, the concentrations of DOC and TDN also decreased in A0 (Fig. 9 and Fig. 10) during the first week but stayed constant thereafter. The most likely reason for these changes are sorption processes, i.e., binding and releasing of the respective compounds to and from the mineral surfaces after mixing of the organic and inorganic components of the artificial soil at the beginning of the experiment (Pronk et al., 2017).

The considerable increase of the DNA concentration (Fig. 13) supported the previous findings and indicated cell division in AS and AN within the first seven days. Between week one and week five further growth was observed, given the significant increase in DNA concentration in AS and AN (Fig. 13). This strengthened the assumption that the artificially assembled soil supported microbial growth. While the DNA concentrations in the Synthetic Soil increased, the respiration rate actually decreased over time (Fig. 13 and Fig. 11, Suppl. Fig. 3 and Suppl. Fig. 1). This suggests, that the biomass of the SynCom increased after week one until an unknown time point before the end of the experiment when potentially the C

limitation restricted growth and respiration, while the DNA of the dead microorganisms may not have been immediately degraded due to the lack of sufficient extracellular DNAses. So, while DNA concentrations at the end of the experiment would represent cumulative growth, the respiration is measured as a rate at a specific time point.

In the Artificial Soil, the DNA concentration could be used to assess the sterility of the system. The small amount of DNA found in A0 at the beginning of the experiment was expected. Although the added dead organic material was DNAse-treated before use, it was likely that DNA, albeit not the intact microorganisms, would be remaining in the soil. The increase within the first seven days of incubation on the other hand, points to the presence of living cells and could constitute a contamination. However, we did not find a further increase of DNA in A0 between week one and week five. The details will be discussed below in context with the results from amplicon sequencing and the incubation of sterile soil subsamples on Agar plates.

Evaluating the enzymatic activities (Fig. 18 to 21) helped gaining additional insights into the development of the microbial potential to decompose soil organic matter in the Synthetic Soil Ecosystem. The tested hydrolases targeted different substrates of plant and microbial origin: chitin, cellobiose, β-glucan, and xylan. Chitinases decompose chitin and similar compounds, which are components of fungal cell walls (Sinsabaugh et al., 2008; German et al., 2011; Jian et al., 2016). A high activity of this enzyme was found in AS, about ten times more than in NS, suggesting that the SynCom in the artificial soil was more strongly degrading microbial cell walls compared to the natural soil, which was richer in organic matter. β-Glucosidases degrade β-glucans of various origin, including smaller breakdown products of plant cell walls (Sinsabaugh et al., 2008; German et al., 2011; Jian et al., 2016), while Cellobiosidase degrades the di-saccharide cellobiose, a breakdown product of cellulose (German et al., 2011). Finally, Xylosidases are degrading plant hemicelluloses of the xylan type (Sinsabaugh et al., 2008; German et al., 2011; Jian et al., 2016).

The pH optima of the enzymes are within the acidic range (Niemi & Vepsäläinen, 2005; Sinsabaugh et al., 2008; German et al.,), and were measured at pH 7, deviating from the pH of the samples (Tab. 5). Nevertheless, the results show that all four tested enzymes were expressed and functionally present in the Synthetic Soil, indicating that the SynCom

actively degraded plant and microbial material to soil organic matter, a pre-requisite for a healthy heterotrophic soil community.

Even the observed increase in pH of AS during the incubation (Tab. 5) indicated that microorganisms were active, leading to an increase in soil pH through the substances they produce and excrete while growing (Lauber et al., 2009; Ramirez et al., 2014; Maestre et al., 2015; Delgado-Baquerizo et al., 2018). The intracellular pH of bacteria is neutral. A smaller pH difference between inside and outside of the cell requires the microbes to increase the H^+ excretions by H^+-pumping membrane bound ATPases to establish a proton gradient, that is large enough for the uptake of nutrients from the environment (Lauber et al., 2009; Ramirez et al., 2014; Maestre et al., 2015; Ratzke & Gore, 2018). It has been shown that pH is one of the major abiotic drivers for the distribution and composition of microorganisms in soil across various scales (Lauber et al., 2009; Ramirez et al., 2014; Maestre et al., 2015; Delgado-Baquerizo et al., 2018) and that bacteria are known to have a narrower pH optimum compared to fungi (Lauber et al., 2009; Rousk et al., 2010; Delgado-Baquerizo et al., 2018). Various studies investigated the pH optima of microorganisms and identified pronounced patterns in how they react to respective pH shifts (Lauber et al., 2009; Rousk et al., 2010; Maestre et al., 2015). Such shifts in AS might have affected the members of the SynCom in different ways: Indirectly, since a changing pH can alter C and nutrient availability and their decomposition rates, and directly, since a shift in pH can act as stressor towards present microorganisms (Lauber et al., 2009; Rousk et al., 2010; Delgado-Baquerizo et al., 2018; Ratzke & Gore, 2018). The pH of the Artificial Soil (A0) also changed over time and became neutral (Tab. 5). This slight pH increase was most likely caused by abiotic processes, when cations were exchanged between the soil organic matter and the charged surfaces of the soil minerals (Yan et al., 1996; Olowoboko et al., 2018; Rasmussen et al., 2018). Our results emphasize the importance of improving the controllability of the soil pH, to optimize the conditions for microbial growth and development in the Synthetic Ecosystem in the future.

In summary, the synthetic community was able to build up biomass (indicated by the increased DNA concentration; Fig. 13 and Suppl. Fig. 3) and showed sustained respiration rates (indicated by the stable respiration; Fig. 11 and Suppl. Fig. 1) after the first week of incubation while the concentration of DOC was already exploited after seven days (Fig. 9).

The production of extracellular enzymes to replenish the DOC pool was a necessity which the SynCom clearly developed over the five weeks of incubation. The connection between the enzymes produced and the microorganisms which released them was not subject of this study but shall be targeted in future investigations.

As already stated above, an absolute requirement for the establishment of synthetic ecosystems is the absence of microbial taxa that do not belong to the SynCom. As observed in various studies before, it is a big challenge to sterilize natural soil (Bárcenas-Moreno & Bååth, 2009; Gebremikael et al., 2015; O'Brien et al., 2017; Al-Najjar & Albokari, 2019). In fact, up to date we are not aware of any soil that could be completely sterilized. The most likely reason is that many microorganisms are spore-forming and spores are extremely persistent (Lennon & Jones, 2011; Madigan et al., 2012). We therefore decided to compile an artificial soil from its single components, which are easier to sterilize, compared to a natural soil. To achieve the lowest number of surviving cells possible, autoclaving in combination with γ-irradiation were the tools of choice for the components of the artificial soil, as well as for the natural soil which was sterilized for the purpose of comparison (for treatment details see method section). It was evident in almost all evaluated parameters that the properties of the artificial and the natural soil differed. Even though we did not per se try to mimic the composition of the garden soil, the sterilization treatment might have caused additional substantial changes in its characteristics, further increasing the differences between the two soils. This likely led to different constraints for microbial growth and a meaningful direct comparison of the development of the SynCom in the two different soils was therefore not possible.

One of the major concerns in context with establishing a Synthetic Soil Ecosystem was its controllability regarding the microbial community over time. We used amplicon-sequencing of the 16S rRNA genes to track the development of the microbial community composition and to verify the sterility or possible bacterial contamination of the Artificial Soil and the other experimental groups over time, respectively.

In all artificial soils, i.e. in A0, AS and AN, amplicon sequencing was showing the presence of an ASV, identified as *Domibacillus* (Suppl. Fig. 4 to 6). This taxon was not found in NS though. When organisms are classified via sequencing, their amplified genetic code is compared to existing sequences. Depending on the reference data base, a classification

can vary on the species level and above (Hugerth & Anderson, 2017; Breitwieser et al, 2019). Further, the results from the amplicon sequencing technique indicate the occurrence of the respective DNA, but do not provide information whether a certain taxon is functionally present in a sample (Breitwieser et al, 2019; Carini et al. 2020; Nannipieri, 2020). The organism identified as *Domibacillus* belongs to the class Bacilli, to which also the order Lactobacillales belongs (NCBI, August 2020). The organic matter of the artificial soil was put together from plant material and the remains of microbial biomass, which were three times autoclaved and DNAse-treated. A considerable proportion of this dead microbial biomass was (besides *E. coli* and *C. puteana*) represented by a Kefir community, commonly used for fermentation processes. The dominant bacterial taxa of Kefir belong to the order Lactobacillales (Gulitz et al., 2011). Given, that its DNA was found in all samples with artificial soil (including A0) in relatively high amounts, suggests that *Domibacillus* might actually have been a *Lactobacillus* strain which was a component of the organic matter added, rather than a living organism. Interestingly, the relative abundance of the *Domibacillus* reads decreased in AS and AN but not in A0, suggesting that the DNA was actually degraded during the experiment when active organisms were present. Thus, the sequences from this taxon can be regarded as background DNA, stemming from incomplete digestion of DNA from dead bacterial cells, which were used as organic matter in the artificial soil.

From eight bacterial strains which were added as members of the SynCom, five were unambiguously re-identified (*Chthoniobacter flavus, Chelativorans multitrophicus, Koribacter versatilis, Streptosporangium roseum,* and *Solirubrobacter soli*; Suppl. Fig. 5). The same taxa were also found in the SynCom added to the natural soil (Suppl. Fig. 6). The strains *Labilithrix luteola* and *Paenibacillus alginolyticus* were not detected although they also were members of the SynCom we added. When the respective axenic cultures were Sanger-sequenced (data not shown), *Labilithrix luteola* was classified as *Bosea* and *Paenibacillus alginolyticus as Micrococcus*. *Bosea* and *Micrococcus* sequences were also detected during amplicon-sequencing of AS and NS. *Labilithrix* and *Bosea* both belong to the phylum Proteobacteria and are relatively closely related (NCBI, August 2020); it is therefore likely that they were just misclassified and *Bosea* is indeed *Labilithrix*. However, *Paenibacillus* and *Micrococcus* are originating from different phyla (NCBI, August 2020) and

it is therefore very unlikely that they were misclassified. Rather it may be possible that we cultivated a contaminating organism (*Micrococcus*) instead of *Paenibacillus*.

While some taxa developed well in AS (*Chelativorans multitrophicus, Koribacter versatilis, Streptosporangium roseum, Bosea*) others did not (*Chthoniobacter flavus, Solirubrobacter soli, Micrococcus*) strive. One strain (*Spirosoma spitsbergense*) was not detected at all, indicating that it was not competitive under the applied conditions. It was especially interesting that *K. versatilis* performed well in AS, although the amount of its biomass added to the SynCom was lower compared to the other strains used (see method section). These results demonstrate that the artificial soil was in principle a suitable habitat as it supported microbial growth over the course of the incubation time, and that a community established which differed (quantitatively) from the original composition. In both soils incubated with the SynCom it was evident that seven out of eight members were able to grow together as a community, even when - in case of NS - additional proliferating organisms were present (Suppl. Fig. 5 and Suppl. Fig. 6).

The development of the fungal strains was monitored via amplicon-sequencing of the ITS1 gene. Only two out of the four fungal taxa from the SynCom added to AS and NS, were found again (Suppl. Fig. 9 and Suppl. Fig. 10). While the added Ascomycota, *Periconia macrospinosa* and *Trichoderma flavofuscum* were identified, the two strains belonging to the Basidiomycota, *Armillaria bulbosa* and *Psilocybe cyanescens* were not detected. This is most likely because the primers targeting the ITS 1 sequence did not cover these specific Basidiomycota. The relative abundance of *Trichoderma flavofuscum* in AS remained stable between week one and week five, while the one from *Periconia macrospinosa* decreased (Suppl. Fig. 9). Additional fungal species were identified in AS, as well as in A0, indicating a contamination of unknown origin (Suppl. Fig. 8 and Suppl. Fig. 9). Possibly, the added dead microbial biomass also contained fungal DNA, as described above for *Domibacillus* or *Lactobacillus*. This could for example have been the case with the DNA found from *Aurantiporus* and *Aspergillus flavus*, as their relative abundances remained unchanged in week five compared to week one in A0 (Suppl. Fig. 8). However, fungi are also known to give several sequences for the same strain, and it could well be, that the sequences from the possible contaminations found are in fact sequences from the fungi that we added.

Further research here (i.e., sequencing of the pure fungal cultures) is necessary to resolve this question.

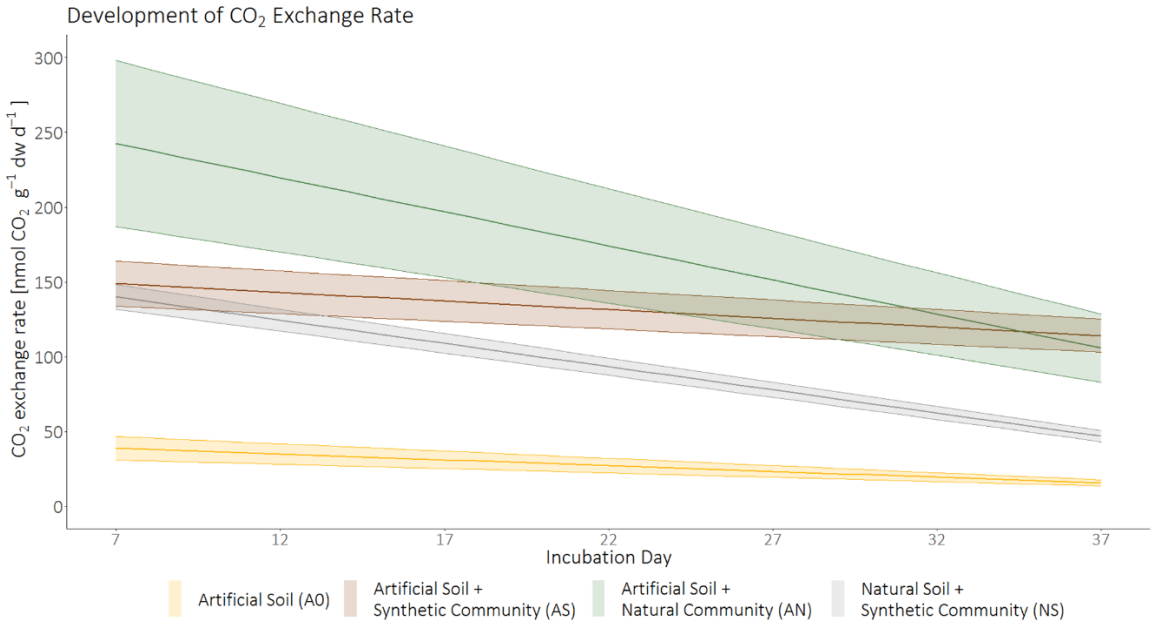
Like in AS, both Ascomycota were identified in NS, but the two Basidiomycota were not detected. As mentioned above, sterilizing the natural soil is almost impossible and does not kill all fungal spores. Thus, a meaningful interpretation of the results from amplicon-sequencing of NS is not possible. This was certainly expected and is one of the reasons we decided to work with an artificially assembled soil in the "SynSoil project".

Our attempts to see whether a synthetic soil can be prepared and kept uncontaminated by unwanted microorganisms, were ambiguous. We did find low amounts of sequences from bacteria (*Bradyrhizobium, Paraburkholderia*) and from fungi (*Alternaria, Aurantiporus, Aspergillus flavus, Cladosporium herbarum)* that did not belong to the Synthetic Soil or the Artificial Soil, respectively. However, whether this was relic DNA, stemming from the organic matter additions, or if it was part of a growing community, remains unexplained. Apart from sequencing, A0 was also incubated on Agar plates, to see if organisms were actively growing. Minimal cell growth was detected in replicates from week one and week five (Suppl. Fig. 12). The identification process of the colonies is still ongoing. However, after judging them by their macroscopic properties (color, size, shape, preferred medium) it seems that the colonies found in all replicates, belonged to a single taxon, which was already present at the beginning (Suppl. Fig. 12). The cause and potential source of contamination remain ambiguous and it will be one of the main tasks for future Synthetic Soil Ecosystems to avoid contaminations.

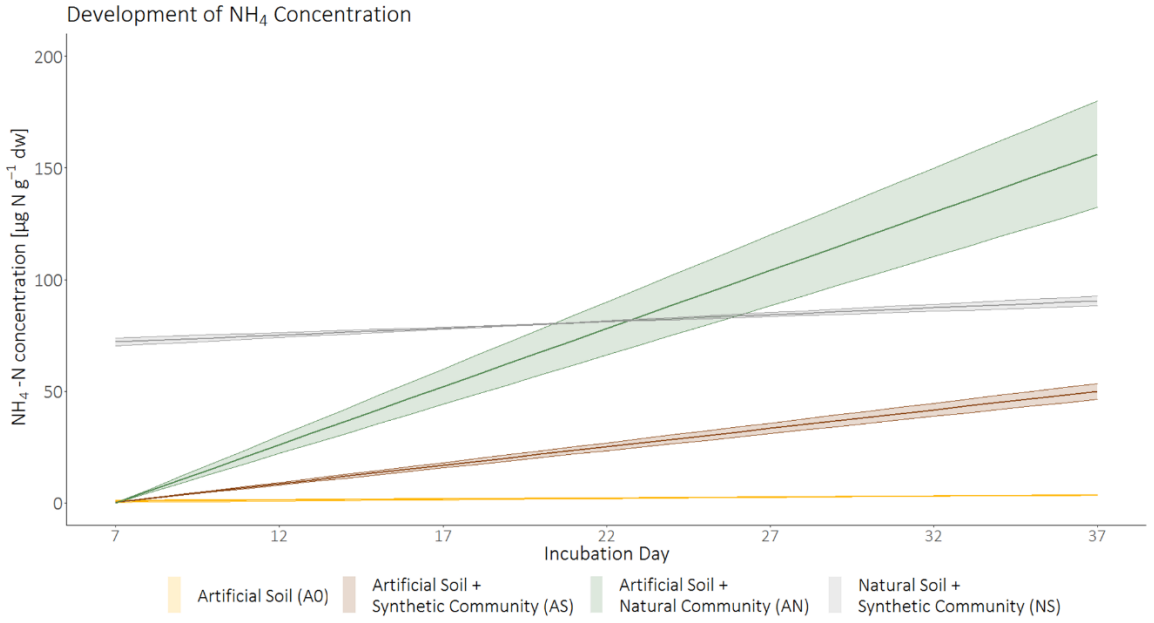
In summary, in this study we present a Synthetic Soil Ecosystem, which was assembled from single, sterilized, abiotic components and inoculated with an *in-silico* designed, minimal microbial community. After incubating the Synthetic Soil for several weeks, an actively metabolizing soil community developed, indicated by an increase in microbial DNA, combined with stable respiration rates over time. The decomposition of organic matter through bacteria and fungi - a requirement for a "healthy" soil - was indicated by the production of extracellular enzymes, an increase in soil pH, and the depletion of DOC coupled with the increase of NH_4^+ during the mineralization of organic N from soil. Amplicon sequencing of bacteria and fungi allowed to track the relative abundances of the single organisms and indicated the development of a soil community over time. In

conclusion, the Synthetic Soil approach we presented in this study, promises to allow constructing a powerful model ecosystem in the future, enabling the investigation of soil functions, linking them to respective soil organisms and addressing fundamental questions from soil ecology.

Supplementary Material

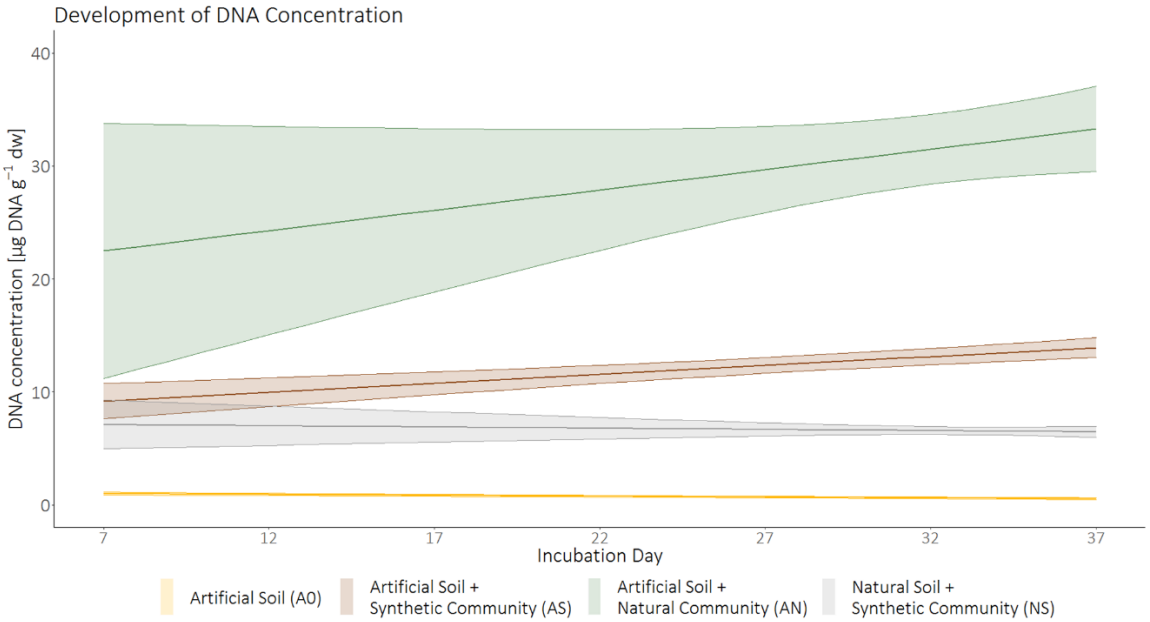


Suppl. Figure 1 Development of the daily CO_2 exchange rate over time. Data given as mean (line) ± 1 standard error (shaded area). Linear relationship over time assumed. Statistical comparison of Artificial Soil (abiotic control) with "Synthetic Soil" (model ecosystem) was done with Wilcoxon test. For details see Suppl. Tab. 1



Suppl. Figure 2 Development of the daily NH_4 concentration over time. Data given as mean (line) ± 1 standard error (shaded area). Linear relationship over time assumed. Statistical comparison of Artificial Soil

(abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. For details see Suppl. Tab. 1



Suppl. Figure 3 Development of the daily DNA concentration over time.
Data given as mean (line) ± 1 standard error (shaded area). Linear relationship over time assumed. Statistical comparison of Artificial Soil (abiotic control) with Synthetic Soil (model ecosystem) was done with Wilcoxon test. For details see Suppl. Tab. 1

Suppl. Table 1 All results from the pairwise comparisons of soil parameters. Artificial Soil (abiotic control) and Synthetic Soil (model ecosystem) in week one and week five after mixing were analyzed with Wilcoxon tests, changes within experimental units were analyzed with paired Wilcoxon tests.
Artificial Soil (A0) **W1** vs. **W5**: n = 10 (n = 8 for NH_4^+)
Synthetic Soil (AS) **W1** vs. **W5**: n = 18 (n = 14 for NH_4^+)
Artificial Soil (A0) vs. Synthetic Soil (AS) **W1**: n = 14 (n = 11 for NH_4^+)
Artificial Soil (A0) vs. Synthetic Soil (AS) **W5**: n = 14.

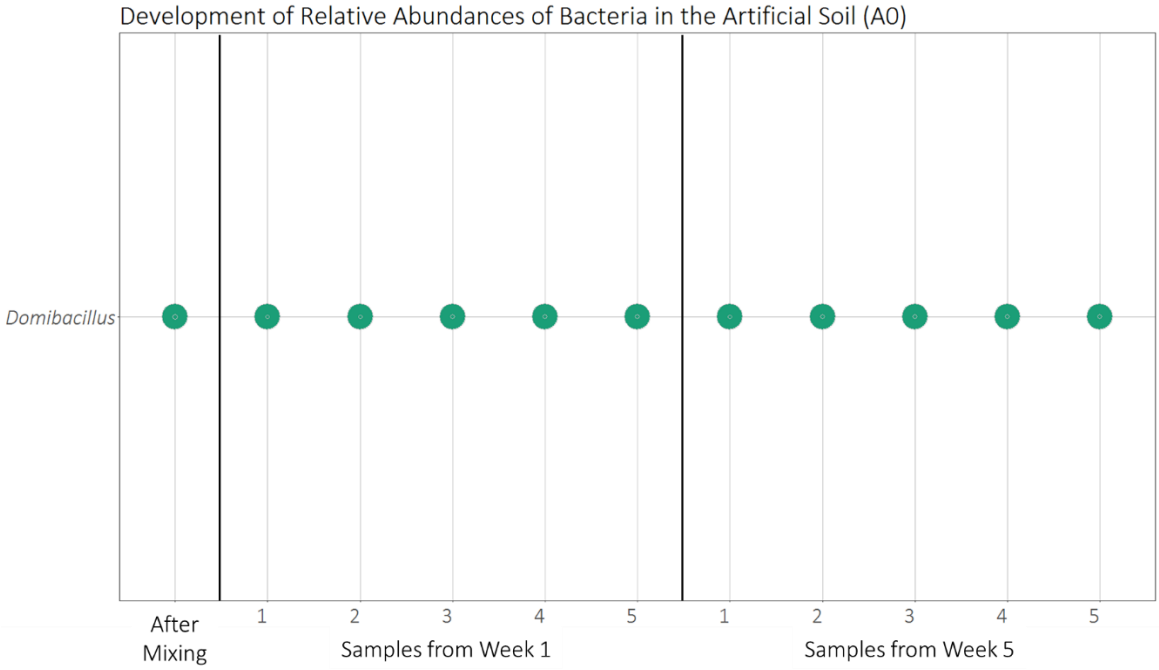
		Artificial Soil (A0) **W1** vs. **W5**	Synthetic Soil (AS) **W1** vs. **W5**	Artificial Soil (A0) vs. Synthetic Soil (AS) **W1**	Artificial Soil (A0) vs. Synthetic Soil (AS) **W5**
pH	p-value	0.060	0.009	0.350	0.003
	r	-	0.89	-	0.80
CO_2	p-value	0.063	0.004	0.001	0.001
	r	-	0.89	0.80	0.80
DNA	p-value	0.125	0.039	0.001	0.001
	r	-	0.69	0.80	0.80
C_{total}	p-value	1	0.074	0.240	0.519
	r	-	-	-	-
N_{total}	p-value	0.813	1	0.147	0.519
	r	-	-	-	-
DOC	p-value	0.063	0.129	0.147	0.007
	r	-	-	-	0.70
TDN	p-value	0.063	0.004	0.001	0.001
	r	-	0.89	0.80	0.80
NH_4^+	p-value	0.125	0.016	0.649	0.001
	r	-	0.89	-	0.80

W = Week after mixing;
r = effect size: 0.10 - 0.29 small effect; 0.30 - 0.49 moderate effect; >0.5 large effect.

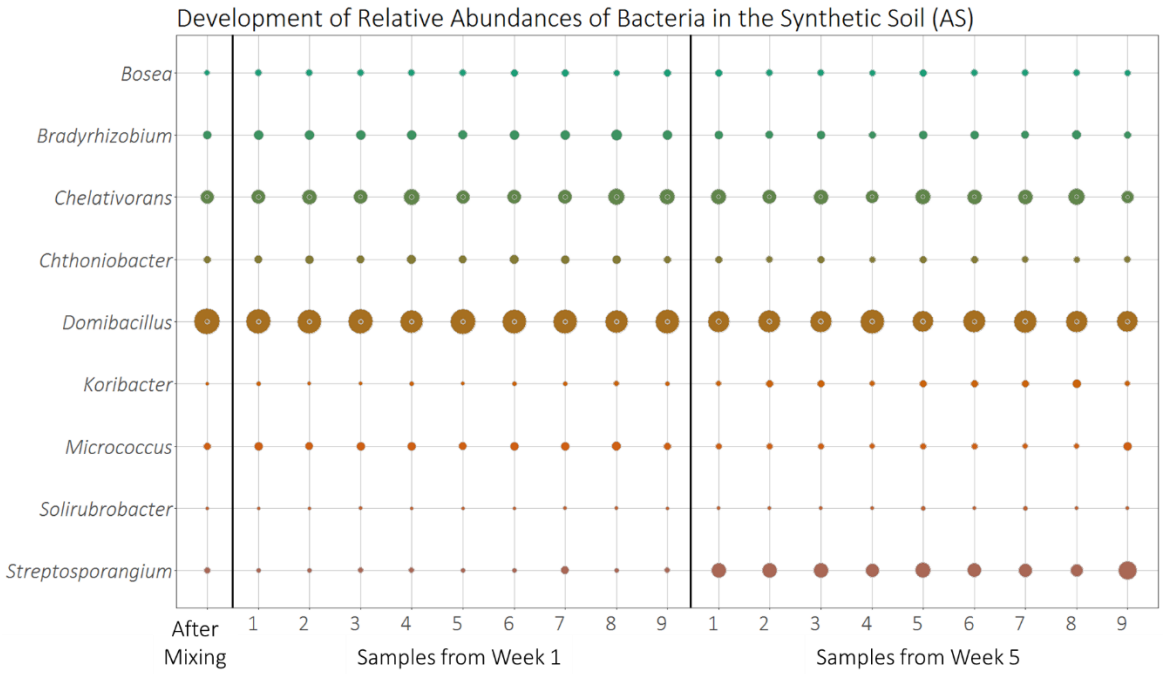
Suppl. Table 2 All results from the pairwise comparisons of enzymatic activities. Artificial Soil (abiotic control) and Synthetic Soil (model ecosystem) in week five of incubation were analyzed with Wilcoxon tests.
Artificial Soil vs. Synthetic Soil **W5**: n = 14.

	Cellobiosidase		β-Glucosidase		Chitinase		Xylosidase	
	p-value	r	p-value	r	p-value	r	p-value	r
Artificial Soil (A0) vs. Synthetic Soil (AS) **W5**	0.001	0.80	0.001	0.80	0.001	0.80	0.001	0.80

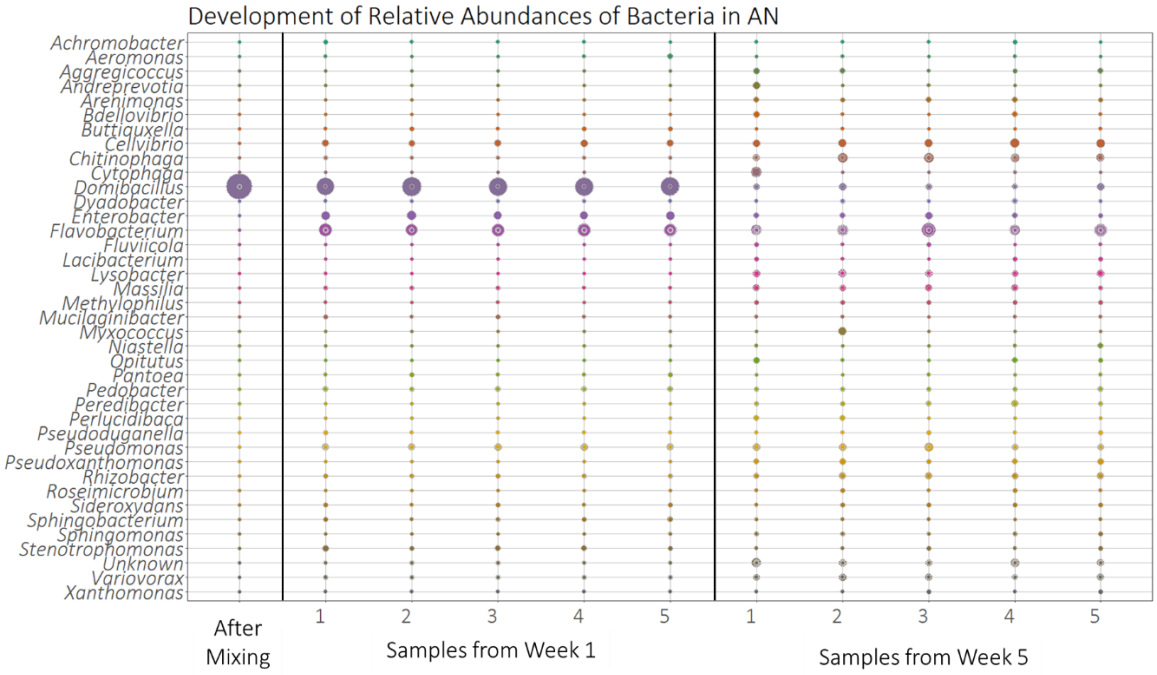
W = Week after mixing;
r = effect size: 0.10 - 0.29 small effect; 0.30 - 0.49 moderate effect; >0.5 large effect.



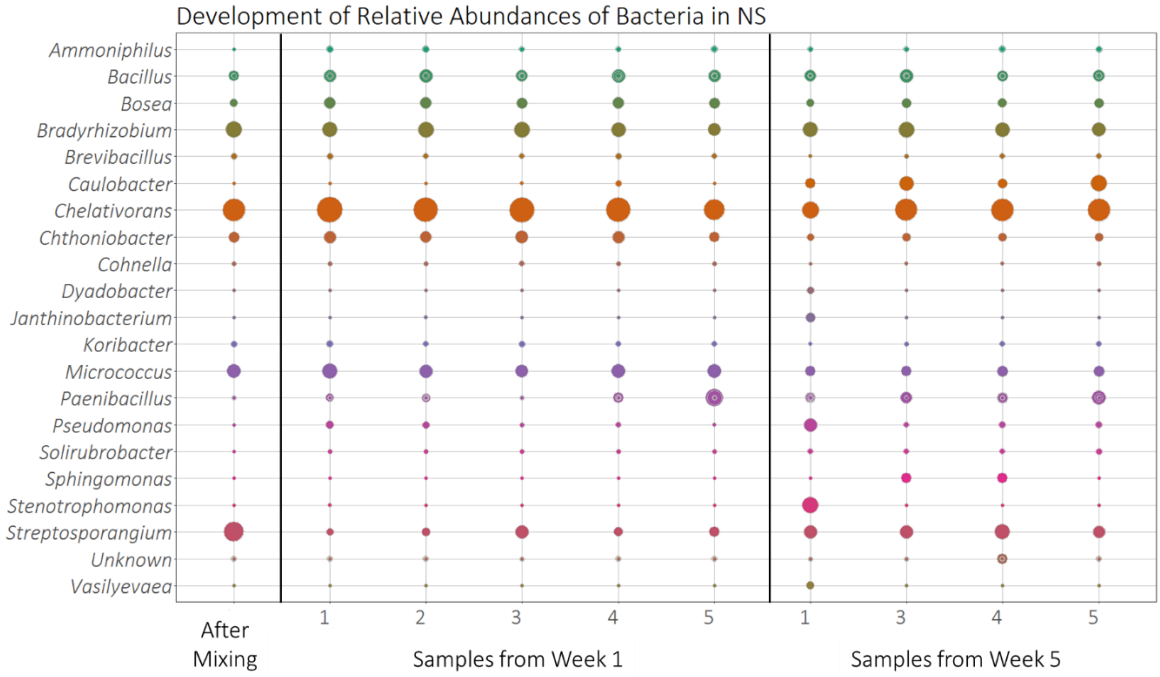
Suppl. Figure 4 Development of the relative abundances of bacteria in the Artificial Soil (abiotic control). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of 16S rRNA genes. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.



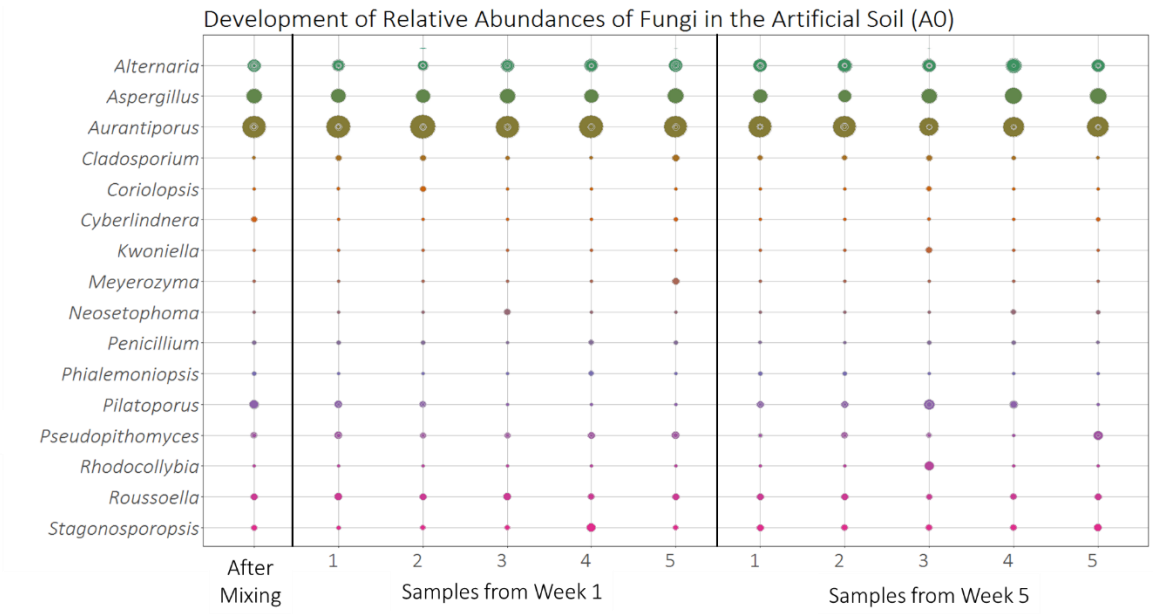
Suppl. Figure 5 Development of the relative abundances of bacteria in the Synthetic Soil (model ecosystem). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of 16S rRNA genes. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 19.



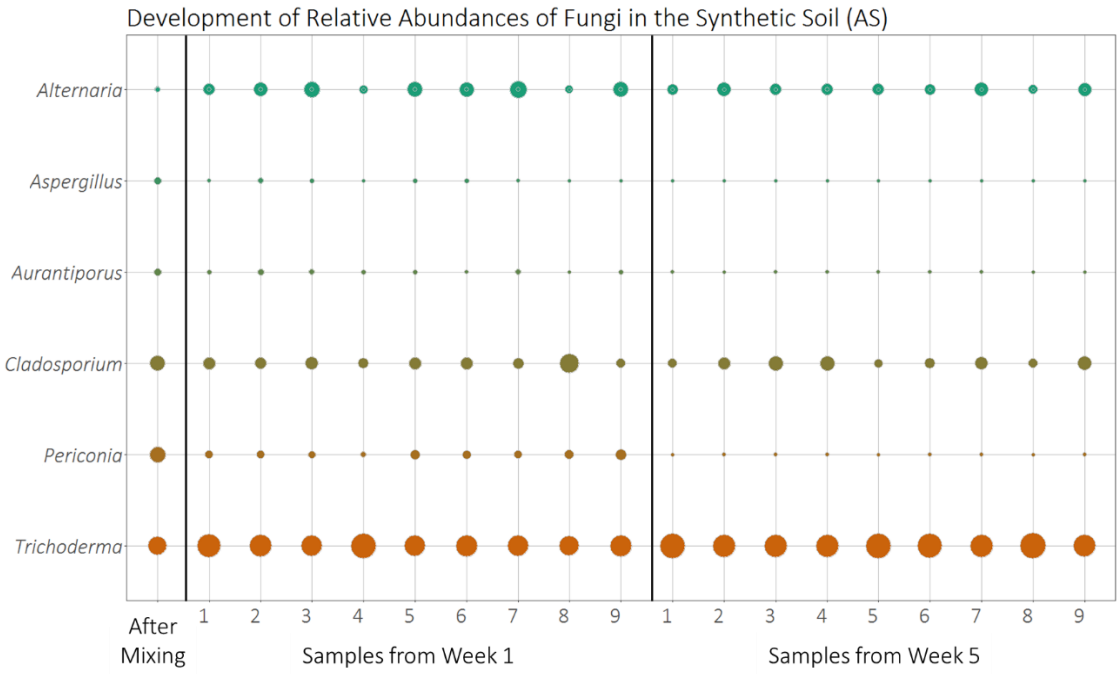
Suppl. Figure 6 Development of the relative abundances of bacteria in the artificial soil incubated with a NatCom (AN). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of 16S rRNA genes. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.



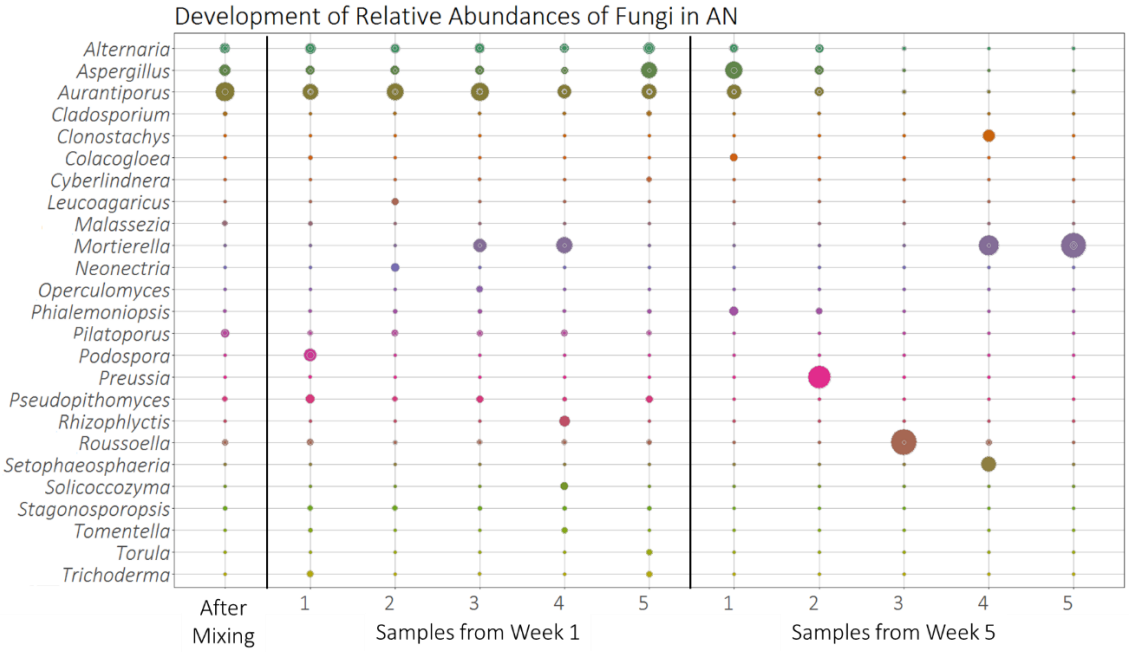
Suppl. Figure 7 Development of the relative abundances of bacteria in a natural soil incubated with the SynCom (NS). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of 16S rRNA genes. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.



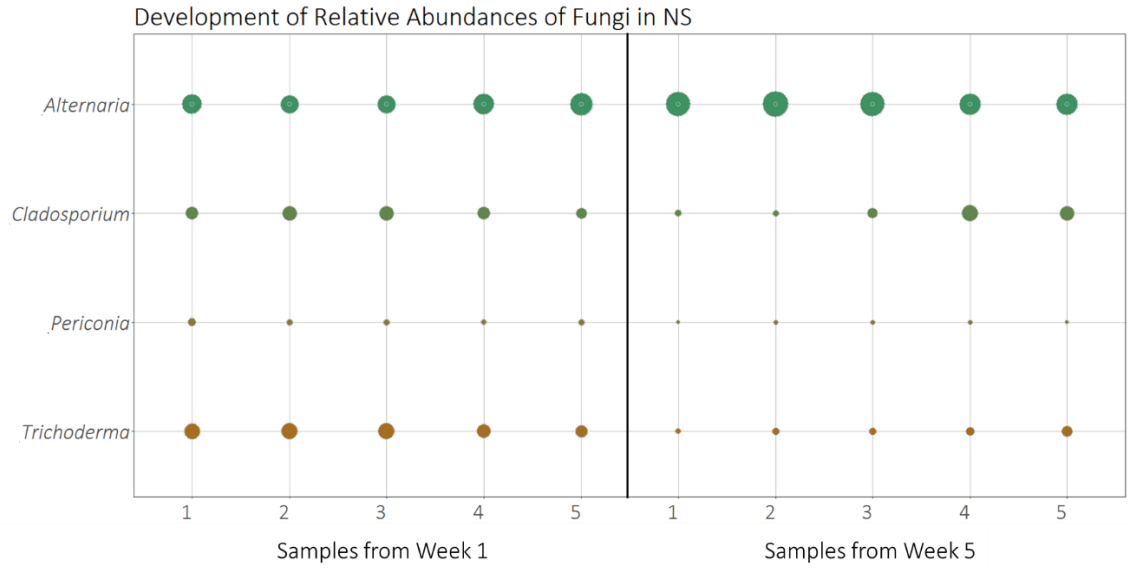
Suppl. Figure 8 Development of the relative abundances of fungi in the Artificial Soil (abiotic control). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of the ITS1 region. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.



Suppl. Figure 9 Development of the relative abundances of fungi in the Synthetic Soil (model ecosystem). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of the ITS1 region. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 19.



Suppl. Figure 10 Development of the relative abundances of fungi in the artificial soil incubated with a NatCom (AN). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of the ITS1 region. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.



Suppl. Figure 11 Development of the relative abundances of fungi in in a natural soil incubated with the SynCom (NS). ASVs (amplicon sequencing variants) were identified via multiplexed amplicon sequencing of the ITS1 region. Samples were taken immediately after mixing of the soil components, one week, and five weeks later respectively. ASVs (on a genus level) with at least 300 reads from the 25 most abundant phyla are shown. The circle size reflects the relative abundance [%] of an ASV, 1% is the minimum threshold. n = 11.

Suppl. Table 3 Single inorganic soil components of the artificial soil before and after treatments in 50 mL tubes to lower component's pH. Oxides remained untreated with acid. **in bold**: components with high pH when not acid treated. Measurement of pH performed in SN or in component's slurry; measurement in slurry: after adding 5 mL MQ water to 1 g of the component, proper mixing for 2 minutes, and 30 minutes of resting time.

Fraction	Fraction size [mm]	Sample	untr.	γ-irr.	γ-irr. + HCl; 1x washed	SN HCl; 3rd washing	γ-irr. + HCl; 3x washed	SN HCl + drying	γ-irr. + HCl + dried; 4x washed	SN HCl+ drying + 8thwashing	γ-irr. + HCl + dried; 8x washed
Very coarse sand	2 - 1	Quartz sand	-	4.9	2.7	3.8	4.7	4.8	4.8	-	-
Coarse sand	1 - 0.25	Quartz sand	-	5	2.7	3.9	4.6	4.7	4.9	-	-
Fine sand	0.25 - 0.125	Na-K Feldspar	-	6.6	2.8	3.9	4.1	4.7	4.8	-	-
Very fine sand	0.125 - 0.063	Al-Fe Silicate	**6.9**	**7**	2.3	2.6	3.2	3.3	3.8	-	-
Silt	0.063 >	Feldspar dust (K)	**7.9**	**8**	3.5	4.2	4.6	4.9	5.3	7.1	5.6
Clay	0.002 >	Montmorillonit (Na-Al-silicate)	**8.3**	**8.5**	4.8	4.8	5.1	6.3	6.2	6.7	6.5
Fe-oxide		Goethite	5.9	5.3	-	-	-	-	-	-	-
Al-oxide		Boehmite	6.2	7	-	-	-	-	-	-	-

untr. = untreated, as delivered from the companies;
γ-irr. = γ-irradiated;
HCl = incubation with 0.2M HCl for 15 minutes followed by washing steps with MQ water and centrifugation; SN = supernatant in the tube;

Suppl. Table 4 pH development of the assembled components inside 50 mL tubes; the component's proportions are equal to the later Artificial Soil. **Tube 1** contained all mineral components. They were γ-irradiated, incubated with 0.2M HCl for 15 minutes, 4x washed with MQ water; oxides and plant litter (both autoclaved and γ-irradiated) were additionally added. In the other tubes, one component was not HCl-treated: in **tube 2** the smallest sand fraction (0.063 - 0.125 mm); in **tube 3** Feldspar, in **tube 4** Montmorillonit. **Tube 5** contained all mineral components and plant litter, all were γ-irradiated before, exception: the smallest sand fraction (0.063 - 0.125 mm) was additionally HCl-treated and 4x washed. In **tube 6** Feldspar dust was HCl-treated, in **tube 7** Montmorillonit, and in **tube 8** Feldspar dust and Montmorillonit. **Tube 9** contained the same combination as tube 8 but the acid-treated components were washed 8 times. After the components were mixed and each tube contained approximately 5 g soil, they were let to rest for 15 minutes followed by a pH determination (measurement in slurry after adding 5 mL MQ water to 1 g of the component, proper mixing by hand for 2 minutes, and 30 minutes of resting time). The test soils were kept at 4°C for two days and pH was determined again to investigate its development over time; **numbers in bold**: tube **1a** = mix from tube 1, after the second measurement additionally treated with 50 mM $NaHSO_4$ (3.3 g of mixture + 24.75 mL $NaHSO_4$); tube **1b** = content of tube 1a additionally washed 5 times. After the first pH measurement, 15 mL 50 mM $NaHSO_4$ were added to 2 g of the mixture from tube 9 (now tube **9a**); when 5 more washing steps were applied, pH was determined again (now tube **9b**) ***numbers in bold and italic***: mixture's pH is slightly acidic and / or close to the desired pH 5.

Sample	all components + HCl **Tube 1**	very fine sand 0.125 - 0.063 - HCl **Tube 2**	Feldspar dust - HCl **Tube 3**	Montmorillonit - HCl **Tube 4**	all components + HCl + 50 mM $NaHSO_4$; 5x washed **Tube 1a**	all components + HCl + 50 mM $NaHSO_4$; 10x washed **Tube 1b**
ca. 5 g artificial soil	***5.2***	6.4	5.8	7.5	**3.7**	**4.1**
after 48 hours in the tubes	***5.1***	6.2	6.1	7.8	-	-

	very fine sand 0.125 - 0.063 + HCl + dried; 4x washed **Tube 5**	Feldspar dust + HCl + dried; 4x washed **Tube 6**	Montmorillonit + HCl + dried; 4x washed **Tube 7**	Feldspar dust and Montmorillonit + HCl + dried; 4x washed **Tube 8**	Feldspar dust and Montmorillonit + HCl + dried; 8x washed **Tube 9**	Feldspar dust and Montmorillonit + HCl + dried; 8x washed + 50 mM; 5x washed **Tube 9a**	Feldspar dust and Montmorillonit + HCl + dried; 8x washed + 50 mM; 10x washed **Tube 9b**
ca. 5 g artificial soil	7.5	7.9	7	***6.6***	***6.4***	**3.7**	**4.2**

Suppl. Table 5 Recipes for liquid and solid media as provided by German Collection of Microorganisms and Cell Cultures (DSMZ). Recipes concern the candidate strains of the synthetic community (SynCom), the cultivation of the Kefir community, *E. coli*, and *C. puteana*.

Medium 1266

liquid

Ingredient	Amount
MES (2-Morpholino-ethanesulfonic acid)	1.95 g
$MgSO_4$ x $7H_2O$ 20mM	10 ml
$CaCl_2$ x $2H_2O$ 30mM	10 ml
$(NH_4)_2HPO_4$ 20mM	10 ml
Selenite tungstate sol.*	1 ml
Trace element sol. **	1 ml
Distilled water	960 ml
NaOH / KOH	
Vitamin sol. ***	3 ml
Glucose 0.2M (filter sterilized)	10 ml
pH 5.5	

solid

MES	3.9 g
$MgSO_4$ x $7H_2O$ 20mM	20 ml
$CaCl_2$ x $2H_2O$ 30mM	20 ml
$(NH_4)_2HPO_4$ 20mM	20 ml
Selenite tungstate sol. *	2 ml
Trace element sol. **	2 ml
Distilled water	920 ml
NaOH / KOH	
Vitamin sol. ***	6 ml
Glucose 0.2M (filter sterilized)	40 ml
Agar 3% washed and autoclaved	1000 ml
pH 5.5	

(for *C. flavus; K. versatilis; S. usitatus*)

Selenite tungstate sol. *

Ingredient	Amount
Na_2SeO_3 x $5H_2O$	0.003 g
Na_2WO_4 x $2H_2O$	0.004 g
NaOH	0.5 g
Distilled water	1000 ml

Trace element sol. **

$CoCl_2$ x $6H_2O$	190 mg
$CuCl_2$ x $2H_2O$	2 mg
FeCl2 x $4H_2O$	1.5 g
H_3BO_3	6 mg
HCl (25% 7.7M)	10 ml
$MnCl_2$ x $4H_2O$	100 mg
Na_2MoO_4 x $2H_2O$	36 mg
$NiCl_2$ x $6H_2O$	24 mg
$ZnCl_2$	70 mg
Distilled water	990 ml

Vitamin sol. ***

4-aminobenzoate	13 mg
Biotin	3 mg
D,L-6,8-thioctic acid	10 mg
Folic acid	4 mg
HemiCa D-(+)-pantothenate	17 mg
Nicotinic acid	33 mg
Pyridoxamine-HCl	50 mg
Riboflavin	10 mg
Thiamine-HCl x $2H_2O$	33 mg
Vitamin B12	17 mg
Distilled water	1000 ml

Medium 220 (for *P. xenovorans*)

Ingredient	Amount
Peptone Casein	15 g
Peptone Soymeal	5 g
NaCl	5 g
Distilled water	1000 ml
Agar	15 g
pH 7.3	

Medium 65 (for *S. roseum*)

Ingredient	Amount
Glucose	4 g
Yeast extract	4 g
Malt extract	10 g
$CaCO_3$	2 g
Distilled water	1000 ml
Agar	12 g
pH 7.2	

Medium 129 (for *T. flavofuscum*)

Ingredient	Amount
Potato infusion*	4 g
Glucose	20 g
Distilled water	1000 ml
Agar	15 g

Potato infusion*

Potatoes	200 g
Water	1000 ml

Medium 90 (for *A. bulbosa*; *P. cyanescens*)

Ingredient	Amount
Malt extract	30 g
Soya peptone	3 g
Distilled water	1000 ml
Agar	15 g
pH 5.6	

LB Medium (for *E. coli*)

Ingredient	Amount
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Distilled water	1000 ml
Agar	15 g
pH 7	

Malt extract (for *C. puteana*)

Ingredient	Amount
Malt extract	20 g
Dextrose	20 g
Peptone	6 g
Distilled water	1000 ml
Agar	15 g
pH 5.5	

Medium 1509 (for *L. luteola*)

Ingredient	Amount
Nutrient broth	6 g
NaCl	1.5 g
Na-Pyruvat	1 g
Distilled water	1000 ml
Agar	14 g
pH 7	

Medium 830

(for *C. multitrophicus*; *F. ginsengisoli*; *S. soli; S. spitsbergense*)

Ingredient	Amount
Yeast extract	0.5 g
Proteose peptone (Difco no.3)	0.5 g
Casamino acids	0.5 g
Glucose	0.5 g
Soluble starch	0.5 g
Na-pyruvate	0.3 g
K_2HPO_4	0.3 g
$MgSO_4 \times 7H_2O$	0.05 g
Distilled water	1000 ml
Agar	15 g
pH 7.2	

Medium 1426 (for *L. pratensis*)

Ingredient	Amount
Peptone	0.5 g
Yeast extract	0.25 g
Glucose	0.1 g
HEPES	2.38 g
SSE*	500 ml
Distilled water	500 ml
Trace element sol. (see Medium 1266)	1 ml
Vitamin sol.**	1 ml
pH 7	

SSE (double conc.) *

$CaCl_2 \times 2H_2O$	0.2938 g
$Ca(NO_3)_2 \times 4H_2O$	0.236 g
$CaSO_4 \times 2H_2O$	0.8606 g
$FeSO_4 \times 7H_2O$	0.0111 g
K_2SO4	0.087 g
KH_2PO_4 (100mM solution)	0.5 ml
$MgCl_2 \times 6H_2O$	0.2036 g
$MgSO_4 \times 7H_2O$	0.739 g
$NaNO_3$	0.424 g
NH_4Cl	0.1069 g
$(NH_4)_2SO_4$	0.1983 g
Distilled water	1000 ml

Vitamin sol.**

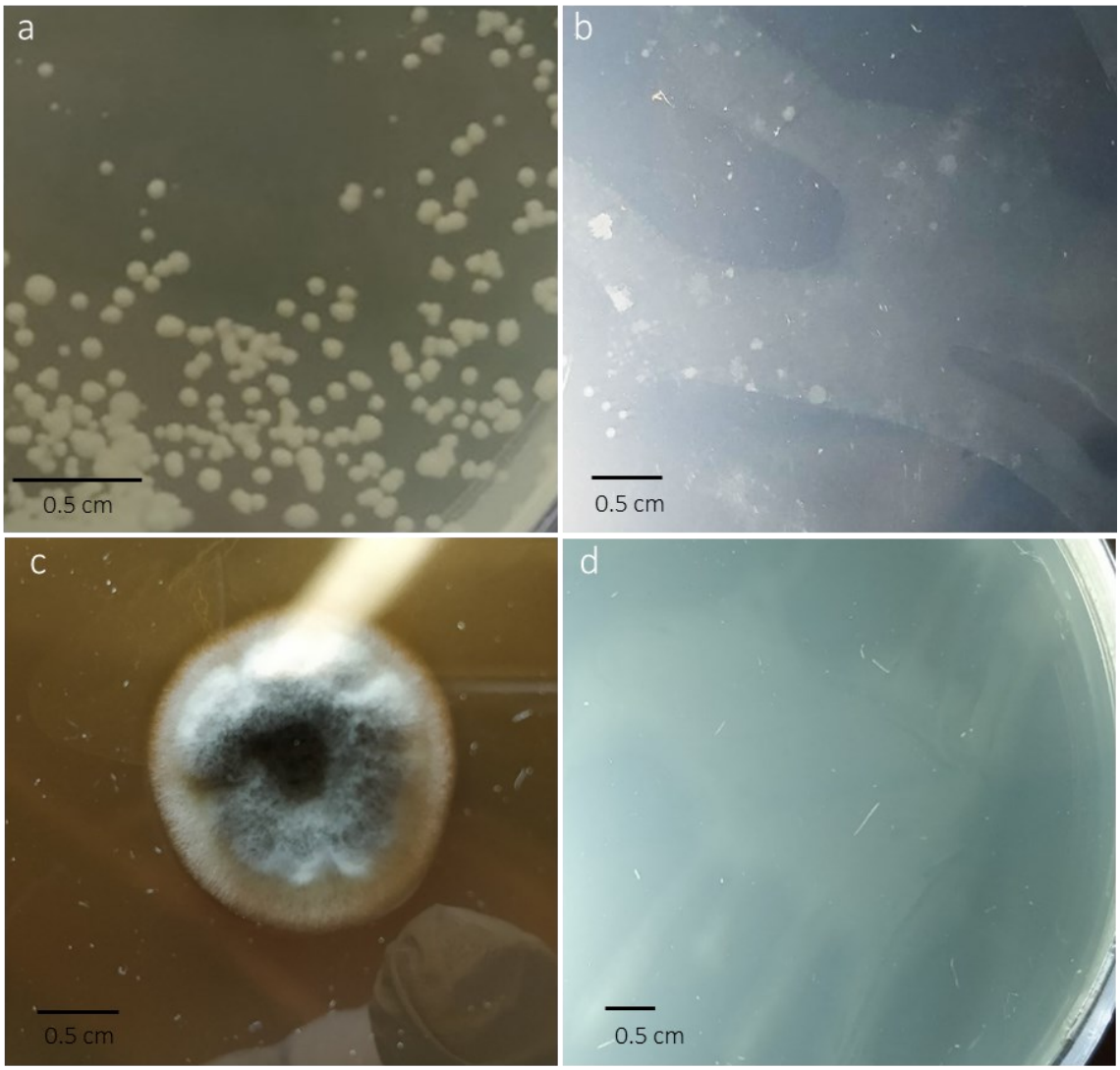
Biotin	2 mg
D-Ca pantothenate	5 mg
Folic acid	2 mg
Lipoic acid	5 mg
Nicotinic acid	5 mg
p-Aminobenzoic acid	5 mg
Pyridoxine-HCl	10 mg
Riboflavin	5 mg
Thiamine-HCl $\times 2H_2O$	5 mg
Vitamin B12	0.1 mg
Distilled water	1000 ml

Medium 1 (for *P. alginolyticus*)

Ingredient	Amount
Peptone	5 g
Meat extract	3 g
Distilled water	1000 ml
Agar	15 g
pH 7	

Water Kefir (for the Kefir community)

Ingredient	Amount
Kefir crystals	3 tbsp
Brown sugar	80 g
Dried fruits	30 g
Lemon	half
Water	1000 ml



Suppl. Figure 12 Results from plate incubation (plating) of Artificial Soil. a: Artificial Soil from sampling in week one after 8 days of incubation on LB medium, cell growth was detected, very similar colonies were also found when plating the other replicates of this experimental group. b: Artificial Soil from sampling in week five after 18 days of incubation on R2A medium, minimal cell growth was detected; very similar colonies were also found when plating the other replicates of this experimental group. c: Artificial Soil from sampling in week one after 8 days of incubation on malt extract medium, fungal cell growth was detected. d: the same replicate of the Artificial Soil from sampling in week five after 18 days of incubation on the same medium as in c, no cell growth was detected.

Summary

Soils are structurally, chemically, and biologically extremely diverse. Bacteria and fungi govern carbon (C) and nutrient turnover on a µm scale, but influence larger scales, since these processes also manifest themselves at the ecosystem level. Microorganisms are the key for understanding soil functioning, but even after more than hundred years of microbiology it remains challenging to link soil processes to the causative organisms. The inaccessibility of the scale, the unexplored biodiversity, the properties emerging from interactions of microorganisms, and more prevent fundamental questions of soil science from being investigated until today.

The aim of this thesis was to build a Synthetic Model Ecosystem, an artificial soil with a reduced biological complexity, which should resemble the functional properties of a temperate soil. While in previous studies, either artificially assembled soils were combined with a microbial inoculum (extracted from soil) of unknown biological composition, or synthetic microbial communities or consortia were grown in artificial matrices, such as petri dishes or microfluidic devices, we here combined the two approaches and constructed a basic soil ecosystem from an artificial soil and a synthetic community, to a biotically and abiotically fully defined soil model ecosystem.

Seven different sterilized primary and secondary minerals of various size-classes were used as a basis of the artificial soil, with the intention to mimic mineral properties of a Cambisol and a sandy loam texture. More specifically we used quartz sand, Na-K-Feldspar, Al-Fe-Silicate, K-Feldspar dust, Montmorillonite, Goethite, and Boehmite. The organic fraction of the artificial soil consisted of five organic components. As source for particulate (solid) organic matter (POM) we sterilized different fractions of plant and microbial origin. More specifically, we used plant litter (three different size classes between 0.063 and 1 mm), *Escherichia coli*, *Coniophora puteana*, and a Kefir community (consisting of yeasts and *Lactobacilli*). The microbial cells were sterilized, broken up, DNAse treated and washed to remove all soluble components, so that mainly cell wall fragments were left. The sand fraction of the artificial soil was impregnated with dissolved organic matter (DOM) derived by extracting plant litter with sterilized water. This assembled artificial soil contained approximately 1% carbon, 0.1% nitrogen, and had an initial pH value of 6.5.

A reduced, minimal synthetic community (SynCom) for the soil was designed *in-silico*. Briefly, from a pool of genome-sequenced soil microorganisms, which were available from the German Collection of Microorganisms and Cell Cultures (DSMZ), strains were chosen according to their orthologous groups of proteins, which together cover a high proportion of essential soil functions (pathways). Eight different bacteria (*Chthoniobacter flavus, Chelativorans multitrophicus, Koribacter versatilis, Labilithrix luteola, Paenibacillus alginolyticus, Solirubrobacter soli, Spirosoma spitsbergense, Streptosporangium roseum)* and four different fungi (*Armillaria bulbosa, Periconia macrospinosa, Psilocybe cyanescens, Trichoderma flavofuscum*) were cultivated in axenic cultures, and approximately 0.4 g fresh biomass from each culture were combined to a synthetic community, before being added to 500 g artificial soil and carefully mixed under sterile conditions inside the air filtered chambers of an isolator. This Synthetic Soil was then incubated in glass jars in the sterile chamber of the isolator to prevent contaminations by environmental microorganisms. We monitored the Synthetic Soil for five weeks to allow the development of a stable microbial community, capable of fulfilling a spectrum of ecosystem processes, characteristic for the functionality of a soil.

In a proof-of-concept experiment, we evaluated the development of the Synthetic Soil after one week (week 1) and after five weeks (week 5) and compared it to the initial soil, directly after the individual components were mixed together (week 0). We assessed the soil pH, the concentrations of total carbon and nitrogen (C_{total}, N_{total}), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), ammonium (NH_4^+), and DNA, the respiration as CO_2 released from the soil and the activity of four extracellular hydrolytic enzymes. For tracking the relative abundances of the single organisms and thus of the community composition, we analyzed the 16S rRNA (for bacteria) and the ITS1 (for fungi) gene abundance by amplicon-sequencing; this also allowed us to evaluate possible contaminations of the soil. The experiment was designed to compare the Synthetic Soil (the artificial soil + synthetic community; AS) to the (sterile) Artificial Soil (artificial soil, no microbial community; A0) as a control. We also combined the artificial soil with a microbial inoculum from a natural soil (artificial soil + natural community; AN), which should allow to evaluate the general suitability of the artificial soil to support microbial growth, and a triple sterilized natural soil

with the synthetic community (natural soil + synthetic community; NS), to assess the potential of the added bacterial and fungal strains to develop as a community.

After five weeks of incubation, we were able to re-identify most of the strains added in AS, as well as in NS, except for *Spirosoma spitsbergense* and the two Basidiomycota. The two missing Basidiomycota were likely not detected because of the primer used for the PCR, but the fate of the missing bacterial strain is so far unclear. However, our results clearly demonstrated that a microbial community developed in AS, indicated by changes of the relative abundances of the added strains. Additionally, active growth of microorganism in AS but not in A0 could be shown by a clear increase in DNA content in all experimental units to which the synthetic community had been added. The organisms were actively decomposing the organic matter added, as indicated by a depletion of DOC and an increase in NH_4^+ (microbial mineralization of organic N) over time. The fact that microorganisms were actively metabolizing the organic matter in the Synthetic Soil was supported by stable respiration rates and the expression of extracellular enzymes involved in degradation of the added plant and microbial litter. Our findings were strengthened by the similarity to the assessed parameters in AN, in which the same artificial soil was inoculated with a community extracted from the natural soil. The Artificial Soil without a microbial community (A0) did not show signs of biological activity, except of a low CO_2-exchange rate, which likely was of abiotic origin.

In summary, the construction of the Synthetic Soil system in this study was a successful and promising first step on the way to a fully functional Synthetic Model Ecosystem, which should allow addressing fundamental questions of soil science in the future.

Zusammenfassung

Böden sind enorm divers in ihrer Struktur, Chemie und Biologie. Bakterien und Pilze steuern den Kohlenstoff- (C) und Nährstoffumsatz auf der µm-Ebene, beeinflussen jedoch auch übergeordnete Größenordnungen, da sich diese Prozesse selbst auf Ökosysteme auswirken. Mikroorganismen sind der Schlüssel zum Verständnis von Bodenfunktionen, doch auch nach hundert Jahren mikrobiologischer Forschung bleibt es eine Herausforderung, Bodenprozesse mit den ursächlich beteiligten Organismen in Verbindung zu bringen. Die Unzugänglichkeit bedingt durch die Größenskala, die

unerforschte Biodiversität, emergente Eigenschaften, die aus mikrobiellen Interaktionen entstehen und mehr, haben es bis heute verhindert, dass fundamentale Fragen aus der Bodenbiologie erforscht werden konnten.

Das Ziel dieser Masterarbeit war es, ein Synthetisches Modellökosystem zu schaffen, einen künstlichen Boden reduzierter biologischer Komplexität, der in seinen funktionellen Eigenschaften einem temperaten Boden ähnelt. Während in vorangegangenen Studien entweder künstlich zusammengesetzte Böden mit einem Inokulat unbekannter biologischer Kombination (extrahiert aus Boden) kombiniert wurden, oder synthetische mikrobielle Gemeinschaften oder Konsortien in einer künstlichen Matrix, wie Petrischalen oder Mikrofluidiksystemen herangezogen wurden, kombinieren wir hier diese beiden Ansätze und haben die Basis für ein Bodenökosystem geschaffen. Aus einem künstlichen Boden und einer synthetischen Gemeinschaft wurde ein biotisch und abiotisch gänzlich definiertes Modellökosystem für Boden erzeugt.

Als Basis für den künstlichen Boden wurden sieben verschiedene, sterilisierte, primäre und sekundäre Minerale unterschiedlicher Korngrößen verwendet, um die Eigenschaften eines Cambisol (Braunerde) mit einer sandigen, lehmigen Struktur abzubilden. Im Detail haben wir Quartzsand, Na-K-Feldspat, Al-Fe-Silikat, K-Feldspatstaub, Montmorillonit, Goethit und Boehmit verwendet. Der organische Anteil des künstlichen Bodens bestand aus fünf Komponenten. Als Quelle partikulären (festen) organischen Materials (POM) haben wir verschiedene Anteile pflanzlichen und mikrobiellen Ursprungs sterilisiert. Konkret haben wir Pflanzenschnitt (drei verschiedene Größenkategorien zwischen 0.063 und 1 mm), *Escherichia coli*, *Coniophora puteana* und eine Kefirgemeinschaft (bestehend aus Hefen und Lactobazillen) verwendet. Die mikrobiellen Zellen wurden sterilisiert, aufgebrochen, mit DNAse behandelt und gewaschen, um lösliche Komponenten zu entfernen, damit hauptsächlich Zellwandbestandteile zurückbleiben. Die Sandfraktion des künstlichen Bodens wurde mit gelöstem, organischem Material (DOC) imprägniert, welches zuvor aus der Extraktion von Pflanzenschnitt mit sterilisiertem Wasser gewonnen wurde. Der so zusammengebaute künstliche Boden hatte einen Kohlenstoffgehalt von etwa 1%, einen Stickstoffgehalt von 0.1% und einen anfänglichen pH-Wert von 6.5.

Für den Boden wurde eine reduzierte, minimale, synthetische Gemeinschaft (SynCom) *in-silico* entworfen. Zusammengefasst, wurden aus einer Gesamtheit genom-sequenzierter

Mikroorganismen aus dem Boden, die über die Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) erhältlich sind, Organismen ausgewählt, basierend auf den orthologen Gruppen ihrer Proteine, die in ihrer Gesamtheit einen Großteil essenzieller Bodenfunktionen (Stoffwechselwege) überspannen. Acht verschiedene Bakterien (*Chthoniobacter flavus, Chelativorans multitrophicus, Koribacter versatilis, Labilithrix luteola, Paenibacillus alginolyticus, Solirubrobacter soli, Spirosoma spitsbergense, Streptosporangium roseum)* und vier verschiedene Pilze (*Armillaria bulbosa, Periconia macrospinosa, Psilocybe cyanescens, Trichoderma flavofuscum*) wurden in Reinkulturen herangezogen. Ungefähr 0.4 g frische Biomasse jeder Kultur wurden zur synthetischen Gemeinschaft kombiniert und etwa 500 g künstlichem Boden zugesetzt. In den luftgefilterten Kammern eines Isolators, unter sterilen Bedingungen wurden beide Teile vorsichtig vermischt. Dieser Synthetische Boden wurde dann in Gläsern in der sterilen Kammer des Isolators inkubiert, um eine Kontamination mit Mikroorganismen aus der Umwelt zu verhindern. Wir haben den Synthetischen Boden über die Dauer von fünf Wochen beobachtet, um die Entwicklung einer stabilen mikrobiellen Gemeinschaft zu ermöglichen, die fähig ist, ein Spektrum von Ökosystemprozessen zu erfüllen, die charakteristisch für die Funktionalität von Boden sind.

In einem „proof-of-concept"-Experiment, haben wir die Entwicklung des Synthetischen Bodens nach einer Woche (Woche 1) und nach fünf Wochen (Woche 5) erhoben und mit dem ursprünglichen Boden verglichen, wie er direkt nach der Vermischung aller Einzelkomponenten (Woche 0) beschaffen war. Wir haben sowohl den pH-Wert, die Gesamtkonzentrationen von Kohlenstoff, Stickstoff, gelöstem organischen Kohlenstoff (DOC), gelöstem Stickstoff (TDN), Ammonium (NH_4^+) und DNA bestimmt, als auch die Atmung in Form von CO_2, freigesetzt aus dem Boden und die Aktivität von vier extrazellulären, hydrolytischen Enzymen. Um die relativen Abundanzen der einzelnen Organismen und damit die Zusammensetzung der Gemeinschaft beurteilen zu können, haben wir die Häufigkeit der 16S rRNA (für Bakterien) und der ITS1 (für Pilze) Gene mittels Amplikonsequenzierung erhoben. Dadurch war es uns außerdem möglich eine potenzielle Kontamination festzustellen. Das Experiment war so gestaltet, dass der Synthetische Boden (künstlicher Boden + synthetische Gemeinschaft; AS) mit dem (sterilen) Künstlichen Boden (künstlicher Boden, keine mikrobielle Gemeinschaft; A0) als Kontrolle, verglichen wurde.

Wir haben außerdem den künstlichen Boden mit einem Inokulat aus Mikroorganismen eines natürlichen Bodens (künstlicher Boden + natürliche Gemeinschaft; AN) kombiniert, wodurch es uns möglich war, die generelle Eignung des künstlichen Bodens zu beurteilen, mikrobielles Wachstum zu ermöglichen. Zusätzlich wurde ein dreimal sterilisierter, natürlicher Boden mit der synthetischen Gemeinschaft inokuliert (natürlicher Boden + synthetische Gemeinschaft; NS), um die Fähigkeit der zugesetzten Bakterien und Pilze zu beurteilen, sich gemeinschaftlich zu entwickeln.

Nach einer Inkubationszeit von fünf Wochen war es uns möglich, die meisten Arten, die AS und NS zugesetzt wurden, erneut zu identifizieren, mit der Ausnahme von *Spirosoma spitsbergense* und den beiden Basidiomycota. Die beiden fehlenden Basidiomycota wurden wahrscheinlich aufgrund des für die PCR verwendeten Primers nicht wiedergefunden, doch das Schicksal des fehlenden Bakteriums ist vorerst unklar. Unsere Ergebnisse haben jedoch klar gezeigt, dass sich eine mikrobielle Gemeinschaft in AS entwickelt hat, angezeigt durch Veränderungen in den relativen Häufigkeiten der zugesetzten Organismen. Zusätzlich konnte aktives Wachstum in AS im Gegensatz zu A0 gezeigt werden, aufgrund eines klaren Konzentrationsanstieges des DNA-Gehalts in allen Gruppen, in denen die SynCom zugesetzt wurde. Die Organismen haben aktiv das zugesetzte, organische Material verarbeitet, nahegelegt durch eine Erschöpfung von DOC und einen Anstieg von NH_4^+ (mikrobielle Mineralisierung organischen Stickstoffs) über die Zeit. Die Tatsache, dass Mikroorganismen des Synthetischen Bodens aktiv organisches Material metabolisiert haben, wurde durch stabile Respirationsraten und die Bildung extrazellulärer Enzyme, die in den Abbau zugesetzter pflanzlicher und mikrobieller Bestandteile involviert sind, bekräftigt. Unsere Ergebnisse wurden außerdem durch die Ähnlichkeit zu den erhobenen Parametern aus AN gestärkt, wo derselbe künstliche Boden mit einer Gemeinschaft, gewonnen aus natürlichem Boden, kombiniert wurde. Der künstliche Boden ohne mikrobielle Gemeinschaft (A0) zeigte keine Anzeichen biologischer Aktivität, außer eines geringen Ausmaßes an freigesetztem CO_2, welches vermutlich abiotischen Ursprungs war.

Zusammengefasst bedeutet die Herstellung des Synthetischen Bodensystems dieser Studie einen erfolgreichen und vielversprechenden ersten Schritt auf dem Weg zu einem voll funktionalen Synthetischen Modellökosystem, das es in Zukunft ermöglichen soll, fundamentale Fragen der Bodenwissenschaften zu behandeln.

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