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Invisible Spheres of Urban Nature

The Role of Microbial Ecology for Landscape Architecture

Florian Opitz
Landscape Architecture for Global Sustainability (M-GLA)

Contents

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Preface

Background of this thesis

A workshop on microbial ecology for landscape architecture students? That was something I and certainly my fellow students had not expected at the beginning of the autumn semester 2022 of the GLA master's program at NMBU. But Jake Robinson, a British microbial ecologist who had been invited by Jorg Sieweke to Norway for the start of the semester, was soon able to turn my initial surprise into great fascination. Because microorganisms are far more than microscopic creatures that cause diseases. They are the basis of all life on earth and are central for the health of the human immune, metabolic, and nervous system. And besides their role in human health, they enable important ecosystem functions, are essential for soil formation and enable the degradation of pollutants through bioremediation in water and soil. All these processes are topics that landscape architecture deals with. The task of last year's studio course was therefore to develop strategies for incorporating microorganisms and their diverse functions into planning.

It is end of November 2022, and I am evaluating an experiment that I conducted as part of the studio project. The idea behind it was to engage with microorganisms in practice to physically experience some of the processes that we studied during this semester. And since microorganisms are not observable with the human eye, the growth of plants in differently treated soil was intended to provide information about the interactions between microbial activity and their health. While harvesting the alfalfa plants that I had sown in a greenhouse 6 weeks earlier, I noticed knot-like nodules on the roots. After a short research it turned out that these are bacteria that form a symbiosis with certain plants and can fix nitrogen from the air to make it plant-available. At that moment I also realised something else: without noticing it, a symbiosis between rhizobium bacteria and alfalfa had ensured that the plants were able to grow healthily over the last few weeks. And this process was certainly not the only one that remained hidden from my eyes and thus did not exist in my thoughts.
This realisation contributed to my decision to find out more about such invisible processes to be able to address them in landscape architectural planning. To meet challenges such as climate change, biodiversity loss and human health threats, it is of great importance that landscape architects in their role as generalists include the services and potentials of microorganisms in their work. In this way microbial ecology can be part of an inclusive planning process that links different disciplines such as natural sciences, humanities, or arts within projects. In my master's thesis, my intention is to change the usual point of view to explore the

field of microbial ecology that has received little attention in landscape architecture so far. This is a contribution in broadening and updating the way of planning in landscape architecture by making the invisible spheres of microorganisms visible and therefore tangible.

Thesis structure

The **first chapter** contains the theoretical framework of this thesis with an introduction to the world of microorganisms and the state of knowledge in microbial ecology on human health. Afterwards, approaches from the perspective of microbial ecology to promote microbial diversity in an urban context are discussed as well as developments in landscape architecture that overlap with them. Then I formulate my research question based on this scientific framework: Does urban spontaneous vegetation promote microbial activity in the soil and what conclusions can be drawn from this for landscape architectural planning?
In the **second chapter**, I explore Pfeiffer's circular chromatography soil test (PCC), an experimental method to visualise the components of a soil sample that also allows conclusions about microbial activity. PCC is not used as a substitute for more complex scientific analysis, but as a visual approach to engage with soil and its biology from a new perspective and with relatively basic resources.
The **third chapter** describes the learning curve during the conduction of the PCC experiments as well as the soil sampling in places with different vegetation during my field trip to Berlin and Munich. The results of the PCC tests are then analysed and interpreted.
In the **fourth chapter**, I discuss the results of the interpretation, addressing findings on microbial activity of the different sampling sites and the suitability of the PCC method for landscape architecture. Finally, I conclude what the results of my literature review and experiments mean for landscape architectural planning and the further research questions that arise from this thesis.

1 Introduction

The role of microorganisms for human and environmental health in landscape architecture

1.1 The role of microorganisms on earth

Key functions of microorganisms

Microorganisms are the very basis of all life on earth. They have populated the planet in various forms for around 3.8 billion years and can be found in all ecosystems worldwide (Cavicchioli et al., 2019). Even in places with extreme environmental conditions, characterized by e.g. heat, cold, high pressure, salinity, or acidity, they can survive and thrive (Shu & Huang, 2022). Microorganisms play a key role in enabling earth's biogeochemical cycles (Cavicchioli et al., 2019). They fix and release carbon through photosynthesis and decomposition (carbon cycle), transform nitrogen to plant available ammonia and nitrate (nitrogen cycle) and are important in sulphur and phosphorus cycles (Tortora et al., 2010). They are the basis of food chains and are central to the health of ecosystems with their animals and plant species. Thus, they ensure the existence of all higher life forms in a functioning biosphere (Cavicchioli et al., 2019; Tortora et al., 2010). Humans are not exempt from this either and benefit from microorganisms that colonise the human body and enable for example organ functions like our digestion. We also depend on microorganisms for the synthesis of vitamins, for adaptation and evolutionary processes as well as protection against pathogens or pollutants (Flandroy et al., 2018; Tortora et al., 2010).
But what are microorganisms actually?

1.2 Defining microorganisms

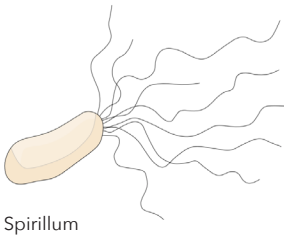
What counts as a microorganism?

The term "microorganism" describes different groups of organisms that are not visible to the human eye due to their small size and can only be observed with the help of a microscope (Sanz, 2011; Stanier et al., 1977; Tortora et al., 2010). The resolution limit of the human eye is about 0.2 mm, which is why common definitions set the maximum size of microorganisms at 0.1 mm or smaller (Sanz, 2011). As an example, Cavicchioli et al. (2019) define microorganisms in their consensus

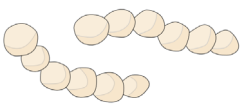
statement as organisms smaller than 0.05 mm that occur in unicellular, multicellular, aggregate or viral form.
Microorganisms include **bacteria** and **archaea**, which are single-celled organisms without a cell nucleus, so-called prokaryotes. Microorganisms such as **protozoa**, microscopic **algae** and **fungi** (yeasts and moulds), on the other hand, are eukaryotes, which means they have a cell nucleus (Tortora et al., 2010). While protozoa have only one cell, microscopic algae and fungi occur as unicellular and multicellular organisms (Sanz, 2011; Smith, 2018; Tortora et al., 2010).
Viruses, however, are not clearly classifiable: although they possess genetic material, they do not have their own cell structure, which is widely considered to be a prerequisite for life. Moreover, they are inactive outside their host cell (Smith, 2018; Tortora et al., 2010). Just like viruses, also **prions**, disease-causing proteins, cannot be clearly labelled as living organisms (Smith, 2018). Nevertheless, viruses and prions are included in the category of microorganisms in most definitions (Microbiology Society, n.d.; Sanz, 2011; Smith, 2018; Tortora et al., 2010).
Another exception are so-called **arthropods**, which include microscopic animals such as mites, tardigrades or nematodes that are barely or not visible to the human eye. Although arthropods, as eukaryotes, are definitely living organisms and are often very small, they are not counted as microorganisms (Tortora et al., 2010).

Bacteria

Bacteria are single-celled organisms that do not carry their genetic material in a cell nucleus and are therefore called prokaryotes. They reproduce by cell division and occur in different forms. With the help of chemical signals, bacteria can communicate with each other (quorum sensing) and are able to form into groups depending on their genus or species. Most bacteria feed on organic material, but some can also process inorganic substances or carry out photosynthesis. Contrary to popular belief, very few bacteria are responsible for disease. Instead, they enable the health of animals, plants and ultimately all ecosystems worldwide (Tortora et al., 2010). Bacterial processes also influence the climate due to their role in biogeochemical cycles (Cavicchioli et al., 2019) and are important for agricultural production fixation of nitrogen and insect pest control. In addition, bacteria make modern medicine possible, for example through the production of antibiotics and contribute in the bioremediation of pollutants in wastewater or soil (Tortora et al., 2010). *Graphic: Opitz, 2023.*



Spirillum
6 - 15 µm

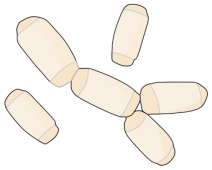


Streptococci
0,5 - 2 µm

Archaea

Archaea have procaryotic cells like bacteria, but they vary from bacteria and eukaryotic cells because of differences in their cell wall and genetic material, so that they form their own domain. Archaea are extremophiles, which means that they live under extreme environmental conditions

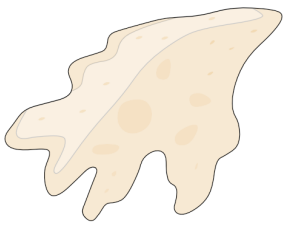
such as high salt concentrations, high temperatures or in habitats with extremely low pH values. They also colonise the human body but are not known to cause diseases. Archaea can metabolise various chemicals and are the only organisms that produce methane. They play an important role in sewage treatment and their enzymes, so-called extremozymes, are irreplaceable for biotechnology, such as polymerase chain reaction (PCR) (Cabrera & Blamey, 2018; Tortora et al., 2010). *Graphic: Opitz, 2023.*



Euryarchaeota
5 µm

Protozoa

Protozoa are single-celled eukaryotic organisms, which means that they have a cell nucleus that contains their genetic material. They come in many different forms and reproduce asexually by cell division or by sexual reproduction. Protozoa live in water and soil, where they feed on bacteria and microscopic nutrients. They also occur as parasites and draw on the nutrients of their host. Protozoa guide their food to them useing extensions of various kinds and through their movements they are able to navigate their surroundings. Some protozoa species are responsible for water-borne diseases such as malaria, but the majority of protozoa do not cause diseases in humans (Tortora et al., 2010). Instead, they have important functions in the ecosystem food cycles, regulating bacterial populations through predation, digesting and returning them to the soil as nutrients, participating in the process of wastewater treatment and are used to control pests in agriculture (Efstratiou, 2018; Tortora et al., 2010). *Graphic: Opitz, 2023.*



Amoeba proteus
220-760 µm

Algae

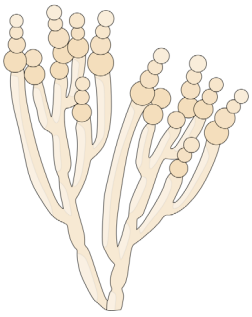
Algae occur in a wide variety of shapes and sizes and therefore exist as micro- but also macro-organisms. Microscopic algae are mostly eukaryotic unicellular organisms that reproduce asexually and sexually. They occur in fresh and salt water, in the soil or in community with plants and fungi. The chlorophyll pigment in the cells gives most algae their green hue and enables the production of glucose for nutrition and oxygen through photosynthesis (Tortora et al., 2010). Algae such as marine plankton play a crucial role in global biogeochemical cycles, as they bind about half of global CO2 and produce about 50 per cent of the world's oxygen through photosynthesis (Cavicchioli et al., 2019). Furthermore, algae are the basis for aquatic food chains, are important in human food production and have symbiotic functions in animals. Furthermore, algae are indicators of water pollution, as high nutrient concentrations lead to so-called algal blooms (Tortora et al., 2010).
In association with fungi, algae can form **lichens**, a mutualistic relationship in which the fungal hyphae enclose the algae and protect them from dehydration. The fungi in turn benefits from the photosynthetic products of the algae (Tortora et al., 2010). *Graphic: Opitz, 2023.*

Chlorella
2-10 µm

Fungi are eukaryotes and, like algae, occur in a wide variety of shapes and sizes and are therefore not uniformly classified as microorganisms. Microscopic fungi include **yeasts**, oval unicellular organisms that reproduce primarily by asexual cell division. **Moulds**, on the other hand, form filamentous, multicellular structures, so-called hyphae, which can grow into the visible spectrum to form a mycelium. An example of this is mould on bread and fruit or fungal fruiting bodys (e.g. mushrooms). Moulds reproduce asexually by fragmentation of their hyphae or sexually by the development of spores. All fungi rely on nutrients in their environment for nourishment and cannot photosynthesise (Tortora et al., 2010). Pathogenic fungi cause a variety of human and plant diseases that will continue to increase in the future in the process of climate change (Gadre et al., 2022; Tortora et al., 2010). However, fungi play important roles in global food webs due to their ability to decompose organic material into its elements, they form symbiotic relationships with almost all plants, so-called mycorrhizae, and are used by humans to produce food, medicines and drugs (Tortora et al., 2010). *Graphic: Opitz, 2023.*

Fungi

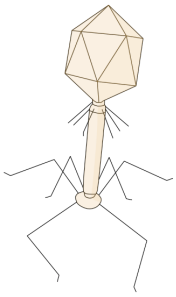
Yeasts
3-4 µm



Mould hyphae, ⌀ 4-6 µm

Viruses, as described in the beginning, represent a unique type among the groups of microorganisms, as they are not classified as living organisms according to most definitions. They are smaller than all other forms of microorganisms and possess only either DNA or RNA, which is enclosed in a protein coat. Because of this, they can only reproduce by infecting host cells, and are sometimes described as temporally alive at this stage. Viruses are widely known as pathogens due to their parasitic behaviour (Tortora et al., 2010). However, it is often overlooked that they are an important factor for global geochemical cycles, driving the evolution of microorganisms through gene transfer and mineralising organic compounds through lysis (Gao et al., 2022). *Graphic: Opitz, 2023.*

Viruses



Bacteriophage
24 - 200 nm

1.3 A human perspective on microorganisms through time

The discovery of an invisible world

Microbiology and microbial ecology are relatively young sciences. The reason for this is simply the small size of microorganisms, which only became visible and therefore explorable with the invention of the microscope in the 17th century. But the existence of microorganisms was already believed much earlier. For example, Kuhad et al. (2021) describe that small, invisible creatures already played a role in traditional Indian knowledge of Jainism thousands of years before Christ. They were mentioned by Indian sages ("Rishis") as "Krimis" in religious texts ("Vedas") and were attributed both good and negative effects on health

Ancient knowledge about microorganisms

(Kuhad et al., 2021). Also Marcus Terentius Varro, a Roman scholar, spoke and warned as early as the first century B.C. about certain tiny creatures, invisible to the eye, that are found in swamps and can cause disease (Cato & Varro, 1935). Centuries later, the Italian scientist Girolamo Fracastoro (1483-1553) described that typhus was transmitted through the air by small, spore-like particles (Pesapane et al., 2015).

First observations of microorganisms

But it was not until the Dutch merchant and amateur scientist Antoni van Leeuwenhoek built microscopes that were able to actually give insights into the world of microorganisms. His constructions allowed a magnification of 300 times with the help of a small spherical lens. Leeuwenhoek documented the results of his research on the "animalcules" he discovered in texts and drawings to the Royal Society of London between 1673 and 1723 (Lane, 2015; Tortora et al., 2010). In 1675, for example, he described "living creatures" observed in stored rainwater (Leeuwenhoek, 1677).

The Germ Theory of Diseases

But it still took some time from the first visualisation of microorganisms to gaining knowledge about their effects and functions in the environment and on humans. A first milestone was Jenner's vaccination experiment in 1798 with cowpox, which produced an immunity of the injected person against smallpox. At that time, however, scientists were not yet aware of how exactly this immunity was produced.
It was not until Luis Pasteur's explanation of fermentation in 1857 and his invention of pasteurisation (1864), meaning the elimination of microorganisms through heating, that it was proven that microbial activity was correlated with the spoilage of food and that microorganisms might also cause diseases (Tortora et al., 2010). This created the basis for a number of research. In 1861, Robert Koch published the **Germ Theory of Diseases,** and proved by means of different experiments that certain microorganisms can be assigned to certain diseases. The intensive research on vaccines that began at this time was based on these findings in order to create immunisation against diseases caused by viruses or bacteria (Tortora et al., 2010).

The beginnings of microbial ecology

At the same time, the foundations for microbial ecology were established by Sergei Winogradsky. The researcher studied bacterial processes in the environment and succeeded in isolating pure cultures of nitrifying bacteria in 1888. In the course of this he proved that they convert ammonia to nitrite and nitrite to nitrate and thus explained the sulphur and nitrogen cycles in nature (Dworkin & Gutnick, 2012).

Discovery of antibiotics

A few years later, the discovery of penicillin by Fleming in 1928 initiated the **"Antibiotics Era"**. Fleming accidentally discovered a substance, produced by a mould, which restricted the growth of bacteria on old

contaminated petri dishes. Antibiotic substances like penicillin have been produced by microorganisms for million of years to protect themselves against other microbes. This discovery marked another milestone in research and expanded medicine beyond the possibilities of vaccination towards the targeted use of metabolites of microorganisms as antibiotics against bacterial diseases (Tortora et al., 2010).

Human-made crises and the loss of the "Old Friends“

Misuse of antibiotics leads to antimicrobial resistance

The "Golden Age of Microbiology" from 1857 to 1911 (Tortora et al., 2010) marked a time of enormous scientific progress, the birth of the sciences of microbiology and microbial ecology, and a revolution in medicine. People’s worldview on the existence of microorganisms was also fundamentally changed in a relatively short period of time, considering that diseases in humans or agriculture were previously widely interpreted in religious terms (Tortora et al., 2010). But with the development of antibiotics, antimicrobial resistance (AMR) began to emerge. Due to the use, overuse and misuse of antibiotics in medicine, agriculture and animal breeding, more and more microorganisms (especially bacteria, viruses and fungi) developed resistances to antibiotics as a result of evolutionary processes (Samreen et al., 2021).
Today, the WHO ranks AMR among the top 10 threats for global health and warns that without AMR control, even mild and previously easily treatable diseases may soon become untreatable (UNEP, 2023; WHO, 2022). The UNEP report also points out that the environment plays a key role in the spread of AMR (UNEP, 2023). Resistant microorganisms and their resistant genes enter the soil, water or air through hospital wastewater, agricultural waste or sewage treatment plants (Samreen et al., 2021). Thus, the environment acts as a repository, source of new resistance and disseminator of AMR and therefore also plays an important role in addressing this problem (Samreen et al., 2021; UNEP, 2023). Human-induced climate change and the resulting rise in temperatures further exacerbate the risk of infection with resistant microorganisms, and it can be assumed that extreme weather events as well as land use and the associated loss of biodiversity positively influence the emergence and spread of AMR (UNEP, 2023).

Lack of interaction with microorganisms can restrict immune system functions

Just as AMR is closely linked to anthropogenic crises such as climate change, biodiversity and nature loss (UNEP, 2023), there is extensive evidence that non-communicable diseases (NCDs) are due to changing human interaction with their environment. The **"Hygiene Hypothesis"** was first formulated in 1989 (Strachan, 2000) and suggests that the excessive use of detergents in the 1980s was preventing exposure to microorganisms that cause crowd infections (such as measles), which were thought to have an immune-training role. Rook refined

and corrected the Hygiene Hypothesis in 2004: His **"Old Friend's Hypothesis"** explains that the advancing urbanisation in general since the beginning of the 19th century decreased the contact with diverse microorganisms ("Old Friends"). Humans have co-evolved with a variety of microorganisms from the environment in evolutionary history, and they cause important regulations in the human immune system. A decreasing exposure to them played a significant role in the rapid increase in autoimmune diseases and other chronic inflammatory diseases in the western wealthy countries. Of particular importance for human health is the gut microbiota, which has a major influence on immune regulations and is additionally altered or impaired by modern nutrition and antibiotics (Rook, 2012). Furthermore, there is increasing evidence from animal research that the gut microbiota also regulates the development and function of the metabolic and nervous system along the so-called "gut-brain axis" of mammals, which include also humans (Morais et al., 2021).

Excursus: microbiota vs. microbiome

The term **microbiota** describes the entirety of all living microorganisms (Bacteria, Archaea, Protozoa, Fungi, and Algae) that colonise a specific area, for example, the human gut or the rhizosphere of plants (Berg et al., 2020). The term **microbiome**, on the other hand, describes not only the entirety of all living microorganisms in this specific area, but also includes all "materials" produced by microorganisms, such as structural elements (e.g. proteins, DNA), metabolic products (e.g. signalling molecules), as well as the DNA of dead microorganisms or structures not classified as living, such as phages and viruses (Berg et al., 2020). Since the term is based on the definition of the "biome", the conditions of the surrounding environment are also included in the microbiome, which are influenced by the host (Berg et al., 2020; Marchesi & Ravel, 2015). Whipps and his colleagues, who coined the term microbiome in 1988, describe all the mentioned "materials" together with the conditions of the surrounding environment as the "theatre of activity" of all living microorganisms in a given area (Berg et al., 2020).

Scientific studies supporting the "Old Friend's Hypothesis"

The "Old Friend's Hypothesis" goes back to Blackly's research in the 1870s, in which he describes that aristocrats and people who live in cities are much more likely to get hay fever than farmers (Blackley, 1873; Rook, 2012).

A study from 2016 comparing the susceptibility to asthma between the Amish and Hutterite ethnic groups in the USA tends in a similar direction: both ethnic groups are genetically very similar and practice agriculture, but the Amish continue to use traditional farming methods while the Hutterites practice industrial agriculture. The study reports that the traditionally working Amish have a much lower rate of asthma

incidences and high innate immunity compared to the Hutterites. The authors reason that this is due to the much greater diversity of microorganisms that the Amish are exposed to in their daily lives, which activate their innate immune response to asthma (Stein et al., 2016).
The actual effect of urbanisation on the presence of diverse communities of microorganisms, which are important for immune system regulations, was evaluated by Finnish scientists in 2018. They examined door mats at house entrances in rural and urban areas in Finland and concluded that the diversity of gut-associated bacteria carried into houses decreased the more densely built-up the environment was. At the same time, the number of potentially pathogenic microorganisms increased with higher levels of urbanisation (Parajuli et al., 2018).
To find out if biodiversity interventions could counteract this effect, Roslund et al. (2020) carried out studies in schoolyards which they manipulated with forest floor materials and their microorganisms. They found out that such interventions can improve a child's microbiome and with this its immunoregulation.

The Symbiocene - Acknowledging microbial functions for human and environmental health

A new perspective on coexistence

Looking back at the development of human knowledge in relation to microorganisms makes it possible to overview and grasp phases and tendencies and allows to embed the current state of science in a context. Scientific progress has always been closely linked to social circumstances and new discoveries revolutionised medicine. By the middle of the 20th century, for example, infectious diseases, which had been the greatest health threat in previous centuries, now accounted only for less than 1% of deaths in England (Smith et al., 2012).
Today, non-communicable diseases and antibiotic resistance are among the greatest health threats in a world affected by the impacts of climate change and the loss of ecosystems and their biodiversity (IPCC, 2023; Marco, 2021; WHO, 2022). Marco (2021) describes in her publication that together with the emergence of these issues, science has also evolved in recent decades and attention has moved from microorganisms as disease-causing "germs" (e.g. Germ Theory of Diseases) to their positive properties and functions for human health, animals, plants and the environment in general. One of the guiding principles for this development was certainly the description and definition of the microbiome by Whipps and his colleagues in 1988 (Berg et al., 2020). In the early 1990s, Lynn Margulis coined the term **"holobiont"** and used it to illustrate that humans or plants are not solitary organisms but interact as hosts with a wide variety of microorganisms with which there is a close evolutionary history. Together, they make up

a holobiont (Bruno et al., 2022; Margulis & Fester, 1991). It is important to understand that holobionts such as humans, animals or plants are not isolated systems between host and microorganisms, but that they are in constant exchange with the microbiota of their surroundings. Therefore, their health is inherently linked to the health of other living organisms and the environment as a whole (environment-microbiota-health axis) (Flandroy et al., 2018). To express this advancing paradigm shift, Glenn Albrecht introduced the term "Symbiocene" as a way out of the "Anthropocene" to emphasise the interconnectedness of all life on earth and to create an alternative vision of the future based on coexistence (Albrecht, 2019).

Microbiome-focused projects

In order to gain a more comprehensive insight into microbial biodiversity and its interactions with other organisms on this planet, various microbiome-focused projects have been launched in recent decades: The **Earth Microbiome Project** (EMP) was started in 2010 and aims to create a "global catalogue of the uncultured microbial diversity of this planet" (Gilbert et al., 2014, p. 1). With regard to indoor environments, the **Hospital Microbiome Project** deals with the emergence and transmission of hospital-acquired infections in order to generate solutions (Smith et al., 2013). Under the title **MetaSUB** (Metagenomics & Metadesign of Subways & Urban Biomes) a variety of projects are gathered that address the mapping of metagenomes in cities and metros, detect sites of antimicrobial resistance and explore new biosynthetic gene clusters (BCGs) for drug discovery (Mason et al., 2016; MetaSUB, n.d.). The aim of the initiative is to make the generated data available to city planners, public health officials, and designers, to support forward-looking and solution-oriented urban development (MetaSUB, n.d.). Only recently, the **Society for the Protection of Underground Networks** (SPUN) was initiated with the aim of mapping the biodiversity of mycorrhizal networks worldwide in order to identify and protect underground ecosystems and especially biodiverse areas. The initiative started with sampling field trips in Chile, Italy and also in the Netherlands, where the focus was on the green roofs of Utrecht and how they can help support fungi biodiversity in cities (SPUN, 2022).

Summary of section 1.3

This section explains the historical development of microbial sciences and the progress in medicine, but also the emergence of antimicrobial resistance (AMR) and non-communicable diseases (NCDs) as novel and serious health threats. The reviewed sources identify the overuse and misuse of antibiotics and conditions that allow antibiotic substances and resistant genes to enter, develop and spread in the environment as the main cause of AMR. The main reason for NCDs has been identified as the increasing urbanisation in western wealthy countries since the

beginning of the 19th century, leading to a decrease in contact between people and diverse environmental microbiota. This is supported by growing evidence in recent decades about the key functions of microorganisms in the development and regulation of the human immune, metabolic, and nervous systems, which have very similar functions in animals and plants. These scientific findings have enabled a new understanding of coexistence on Earth and shifted the focus from microorganisms as "germs" to them as important co-habitants of all living organisms whose microbiota is in constant exchange with microorganisms in their surroundings. A number of recent microbiome-focused research projects are addressing this topic aswell.

1.4 Connecting microbial ecology to landscape architecture

Introduction

Up to this point in my thesis, I have not specifically mentioned landscape architecture. However, the reader will already recognise clear links to the central role that landscape architecture can play in promoting microbial diversity for human health in cities. In addition, microorganisms enable important ecosystem functions and are therefore central for restoration ecology, degradation of pollutants in soil and water, for soil formation and the health and growth of plants.
In the context of section 1.3, this section describes approaches to today's challenges to human health from the perspective of microbial ecology. In doing so, the focus lies explicitly on approaches that fall within the subject area of landscape architecture, even though this is not always clearly mentioned in the literature. Following that, different development pathways from the perspective of landscape architecture are described that are linked or can be linked to microbial ecology.

Microbial ecology research perspectives connected to landscape architecture

Flandroy et al. (2018) on "microbe-associated health-promoting practices"

In order to address the scientific findings described in the previous chapter and to identify solutions for the health challenges of our time, the literature review by Flandroy et al. (2018) summarises so-called **"microbe-associated health-promoting practices"** that explicitly address the planning disciplines. At the heart of promoting good health lies the human contact with nature in urban spaces, especially for young children. This includes access to open spaces such as **parks, squares, gardens**, and **urban farms**, as well as **green walls** and **roofs**. Beyond that, however, no more concrete suggestions for planning are mentioned, but the need for further research on the "environment-microbiota-health axis" to be able to formulate good practice guidelines and to

integrate them into urban planning policies. According to the scientists, this generally requires more knowledge about how green infrastructure in cities positively affects the human microbiome and how this then translates concretely into positive health effects. On the other hand, there is a lack of detailed research about how disturbances such as the overbuilding of open spaces influence the relationship between humans and microorganisms and thus also their health in a negative way (Flandroy et al., 2018).

In order to successfully fill these knowledge gaps, the researchers mention that despite scientific specialisation, it is essential to remain open to the bigger picture to be able to place one's own topic in the larger context (Flandroy et al., 2018). This statement is particularly interesting as it emphasises the value that generalist disciplines such as landscape architecture have, that stands at the intersection of natural sciences, humanities, and arts, among others. Landscape architecture could thus be important in maintaining the "overview" and in linking microorganism-centred research and planning to develop concrete measures on how to promote biodiverse microbiomes in the city.

Bruno et al. (2022) on "urban regeneration"

Bruno et al. (2022) also emphasise the importance of transdisciplinary collaboration and the role of science in fostering collaborations to enable **"urban regeneration"**. One key to "urban regeneration" is bio-informed design, which means that planning decisions are based on the expertise of public health, microbiology, and ecology. In addition, the authors underline the role of innovations in technology to obtain data on urban ecosystems to investigate "air and water purification, soil regeneration, seed dispersal" and "microbial co-occurrence" to be able to respond to changes (Bruno et al., 2022, p. 3). In addition to such technical approaches, social components such as access to high-quality green areas for everyone also play an important role in promoting human health in the process of "urban regeneration".

Robinson et al. on "Microbiome-inspired Green Infrastructure"

Unlike most of the microbial ecology research publications I have encountered during my research for this thesis, the papers by Jake Robinson et al. very directly address landscape architecture and the potential to consider and think about microorganisms in landscape architecture. In addition to various scientific publications, Robinson also publishes articles on his website blog, including the article "What do microbes have to do with landscape architecture?". Here he describes the role of microorganisms for human and plant health, stormwater management or bioremediation, all of which are part of the task spectrum of landscape architects (Robinson, 2022). Most recently, Robinson published his book "Invisible Friends" to communicate the role of microorganisms for humans and the environment to a wider

audience also outside the scientific world (Robinson, 2023). In 2018, Robinson et al. (2018) introduced the term **"Microbiome-Inspired Green Infrastructure"** (MIGI). This term describes all planning and management efforts that promote microbial diversity and interaction with health-promoting microbiomes in an urban context. In order to achieve this, Watkins et al. (2020) emphasise the importance of collaboration between disciplines such as urban design, public health, environmental microbiology, and microbial ecology. To ensure that collaboration is as low-threshold as possible, the authors suggest integrating collaboration guidelines into the "plan of work" (in the UK) or the "Leistungsphasen" (in Germany) of building projects. This means that comments in the respective phase of the planning and construction of a project indicate where collaboration between microbiologists/microbial ecologists and landscape architects or other participants in the construction process is recommended. These collaboration guidelines include, for example, the preparation of an ecological assessment at the beginning of a project, advice on plant selection or possible microbial inoculations, surveillance during construction and subsequent monitoring and advice on maintenance. This is in order to realise the full potential of microbial diversity in the context of the respective project, to ensure its continuity as "Microbiome-Inspired Green Infrastructure" and to learn from it for future MIGI projects (Watkins et al., 2020). To get an overview of what "Microbiome-Inspired Green Infrastructure" (MIGI) all can be, I describe concrete and tangible examples below.

MIGI for example refers to the creation of **"foraging-friendly green spaces"**. From an evolutionary perspective, food gathering has always brought humans into contact with diverse microorganisms. This way of interacting with the environment is rare in urban spaces today and would enhance contact with the environmental microbiota. However, it should be noted that this could also increase contact with pollution and therefore needs specific considerations in planning (Robinson et al., 2018). In this context, I would like to mention the development of so-called "edible cities", a movement that goes back to an initiative by Pam Warhurst and Mary Clear in Todmorden, UK (Incredible Edible Todmorden) (Incredible Edible Network, n.d.). Since then, the idea has been taken up all over the world, for example in Germany, where there are a number of "edible cities" such as the Essbare Stadt Andernach. In Andernach, a wide range of edible plants have been planted and managed on the city's green spaces and are accessible to the general public (Stadtverwaltung Andernach, n.d.).

Other examples of MIGI are green spaces in a variety of dimensions from **rain gardens** to **urban parks** (Robinson & Jorgensen, 2020). "Natural green barriers" such as **hedges** and **tree lines** can act as living walls to protect people and microbiomes from different kinds of pollution

(Robinson et al., 2018). **Plants** have an impact on microbial communities and can be used to influence the soil microbiome or to affect pathogen occurrence. Microbial diversity subsequently also has a positive effect on the health and function of plants. Particularly complex plantings or the use of wild plants (e.g. wildflower verges) can also increase microbial diversity (Robinson et al., 2018; Robinson & Jorgensen, 2020). A study on airborne bacteria provided further evidence that complex and tall vegetation such as scrubs caused greater aerobiome diversity and reduced the emergence of pathogenic microorganisms compared to grassland. In this context, **vertical stratification** of vegetation plays a role. Green roofs and walls for example can help to diversify the aerobiome upwards (Robinson & Jorgensen, 2020; Robinson et al., 2021a).
Robinson et al. attribute particular importance to the ecological **restoration and rewilding** of urban spaces and thus of their microbiomes. They refer especially to the "Microbiome Rewilding Hypothesis" (Mills et al., 2017), which I will describe afterwards. In parallel to human-induced rewilding, Robinson and Jorgensen (2020) also discuss **"urban wildscapes"**, referencing the publication "Wild urban woodlands: new perspectives for urban forestry" by Kowarik and Körner (2005). Urban wildscapes are places such as unused open spaces or old industrial sites that are not subject to any typical function (in German referred to as "Brache") and are characterised by ruderal vegetation. Unlike active rewilding, they develop independently and without direct human intervention and can be found in almost every urban area. "Brachen" provide space for the process of natural succession in the city, which not only has already researched potentials such as climate change adaptation and support of general biodiversity but could also specifically have an impact on rewilding and diversifying the local microbiome. In this way, "Brachen" would have the "benefit of enhancing the urban microbiome with limited human input" (Robinson & Jorgensen, 2020, p. 344). However, in order to better assess the actual value of "Brachen" for the microbiome, the authors point out that there is a need for further research on whether and how exactly the natural succession on these areas affects the local microbiome, whether there are interactions between the microbiome of "Brachen" and adjacent areas, and how this ultimately influences human health (Robinson & Jorgensen, 2020).

Mills et al. (2017) on the "Microbiome Rewilding Hypothesis"

The **"Microbiome Rewilding Hypothesis"** is based on the scientific evidence that living in an industrialised and urbanised environment has a negative impact on the human immune system. The reason for this is the lack of contact with a diverse environmental microbiota. In order to regenerate the urban environment and thus achieve positive health effects, the hypothesis calls for the "restoration of biodiverse habitat in urban green spaces" to "rewild the environmental microbiome"

(Mills et al., 2017, p.2). The key contributors to the regeneration of the environmental microbiome are plants, which, like humans, have a close evolutionary history with microorganisms and form associations with them. Consequently, plants are able to regulate the activity of microorganisms in their rhizosphere and thus can be used as engineers to specifically address the soil microbiome. As an example, Gellie et al. (2017), describe that the restoration of former pastureland with native plants significantly changed the soil microbiota after eight years, so that its microbial composition was similar to that of adjacent preserved natural areas (Gellie et al., 2017).
Although the Microbiome Rewilding Hypothesis can already be interpretated as a useful basis for calling for landscape architectural action to rewild microbiomes in urban areas through habitat restoration, there are still clear knowledge gaps: Can habitat restoration alone truly regenerate natural environmental microbiomes? How does biodiverse planting in detail influence the occurrence of microbiomes that have positive health effects for humans? And are the desired microbial groups more likely to be found in the soil, on leaves or in the air? And finally, what is the best way for humans to interact with them and what level of exposure has a positive impact on health (Mills et al., 2017)?

Summary of microbial ecology research perspectives connected to landscape architecture

The previous section presents solutions from the perspective of microbial ecology research to the health challenges for humans and the environment today. At the centre stands the need for promoting biodiverse green spaces in the city to enable interactions between humans and the environmental microbiome.
Corresponding interventions include the provision of urban green space in different dimensions and complexity as well as the active restoration of biodiverse habitats in the city for the rewilding of microbiomes. In addition, spontaneously growing ruderal vegetation (urban wilderness) could also have a positive impact on local microbiomes and their surroundings. Central to the positive health effects of such interventions is the promotion of interaction between humans and environmental microbiota. Interdisciplinary collaboration and knowledge transfer is crucial for the application of existing knowledge of microbial ecology in practice.

Landscape architectural developments connected to microbial ecology

Potentials vs. reality

The reviewed scientific findings show the central role landscape architects can play in promoting the diversity of urban microbiomes with their planning decisions and in facilitating the interaction between health-promoting microorganisms and people. Moreover, microbiome-inspired planning generally has a positive impact on the functions of

urban ecosystems. However, it is certainly fair to say that knowledge about the multifaceted roles of microorganisms in the environment has not yet entered the landscape architecture mainstream.

Limited societal knowledge

A reason for this is probably the generally limited societal knowledge about microorganisms as well as reports in the media, which often only represent the negative aspects of microorganisms (Timmis et al., 2019). And despite the value of microbial diversity for ecosystem functions up to their major influence on element cycles worldwide, they receive remarkably little attention in the context of climate change discussions outside of microbial sciences and in current development (ASM, 2022; Cavicchioli et al., 2019; Han et al., 2023). According to Timmis et al. (2019), microorganisms remain intangible and difficult to grasp for the general public and therefore play a subordinate role in human thinking. For example, a survey of Slovenian citizens aged 14 and older revealed clear misunderstandings in the distinction between the terms virus, bacterium and microorganism. And almost half of all respondents stated that microorganisms are not addressed sufficiently in school education (Špernjak et al., 2023).

Lacking accessibility to knowledge

Although microorganisms have been visible to scientists since the 17th century, the fact that microorganisms remain invisible in normal everyday life of humans is certainly a factor in their often disregarded role. In an article published in Landscape Architecture Magazine in 2017, landscape architect Rick Quinn describes how ecology and ecosystem functions are part of the curriculum of landscape architects but are often left behind in practice due to conventional structures, lack of knowledge of the involved stakeholders and financial priorities. He also emphasises that understanding the functions of microorganisms like mycorrhiza, means that a landscape architect must also have a thorough knowledge of soil science, entomology, mycology, and botany. In Quinn's view, a more holistic way of thinking could be possible for landscape architects if disciplines such as soil sciences made their research more accessible and comprehensible to those outside the field. However, he also emphasises the role of landscape architects in taking the initiative and consulting other disciplines (Rubin, 2017).

Knowledge gaps

Another reason could be that research on microbiomes in relation with human and environmental health is still relatively young. Consequently, there are many knowledge gaps and the need for further and detailed research.

Development tendencies integrating microorganisms

Nonetheless, there are several examples of developments in the field of landscape architecture in which microorganisms are directly involved that should be mentioned.

These include, for example, the inoculation of urban green, especially trees, with microorganisms such as mycorrhizal substrate or soil bacteria (GEFA Fabritz, n.d.; PHC, n.d.). This method, which is already an established practice in agriculture and horticulture, has gained more and more popularity in the field of landscape architecture in recent years: when I visited the GaLaBau trade fair 2018 in Nuremberg for example, I noticed several suppliers advertising products with microorganisms as adjuvants for plant health. The German company GEFA Fabritz for instance offers endo- and ectomycorrhiza inoculants for deciduous and coniferous trees, which are intended to support the tree in the event of drought, nutrient deficiencies, and pollutant stress (GEFA Fabritz, n.d.).
For the remediation of the Rheinauensee in Bonn, Germany, the municipality relied on the use of so-called "Effective Microorganisms" (EM). These are marketed in Germany by the company EMIKO and are supposed to be able to not only improve aquatic environments but also, for example, the soil. However, the author of an article in the journal "Garten + Landschaft" about this intervention criticises the very limited scientific basis for the actual impact of EM, as it strongly generalises the complex functions of microorganisms (Schlichenmayer, 2021).
An article in the Landscape Architecture magazine focuses on the preservation of old trees in Austin, Texas. To assess the health of the soil, the landscape architects used the so-called "microBIOMETER", a relatively inexpensive and easy-to-use tool to obtain information on soil biology, quantity of microbial populations and bacteria-to-fungi ration. The data provides them with a better understanding of the processes in the soil and deficiencies can be specifically targeted (microBIOMETER, n.d.; Mortice, 2021).

Urban ecology and the link to microbiome rewilding

At this point, I would like to mention again the Microbiome Rewilding Hypothesis (Mills et al., 2017) and Robinson and Jorgensen (2020) observations about "urban wildscapes" and their potentials for urban microbiome diversity. Because the ecological significance of wilderness and nature in the city is also emphasised by urban ecology (in German "Stadtökologie"), which is closely linked to landscape architecture.
Urban ecology is concerned with "urban ecosystems and urban landscapes with their mutual relationships as well as the relationships of these systems to the inhabitants, their actions and planning" (Endlicher, 2012, p. 11) (own translation). Herbert Sukopp is considered the pioneer of urban ecology in Germany and of the "Berlin School of Urban Ecology", which evolved from a unique circumstance: as a result of the Second World War, Berlin was characterised by a large number of ruins and wastelands ("Brachen") that were reclaimed by vegetation through natural succession over time. Sukopp has been observing this process since the 1970s (Endlicher, 2012; Kowarik, 2021). His work includes the

mapping of the flora of different land use types in Berlin, the human influence on the occurrence of plants, the study of non-native species and his central contribution to urban nature conservation (Kowarik, 2021).

The value of spontaneous vegetation for urban biodiversity was also described by the Dutchman Louis LeRoy already in 1978 in his book "Natur Ausschalten, Natur Einschalten". LeRoy emphasised that it is much more sensible to work with the spontaneously growing vegetation of a place than to remove it and replace it with new plantings (Kühn, 2006; Le Roy, 1978). This way of integrating nature into the urban context was a very different approach and Le Roy's work had a great influence on the ecological development in landscape architecture of his time (Kühn, 2006).

At the end of the 1980s, the "International Building Exhibition Emscher Park" (IBA Emscher Park) in the Ruhr region in German provided new impulses also from an ecological perspective for the structural change of this former industrial region. Part of the projects within the framework of the IBA was, for example, the "Landscape Park Duisburg-Nord", which integrates itself into the industrial relicts of a former steel plant. The park also incorporates and stages the species-rich ruderal vegetation that had developed there after the industry closed down. This and other projects of the IBA Emscher Park represent a considerable step in the development of societal acceptance and interest for this type of vegetation.

In 1992, Ingo Kowarik introduced the concept of "nature of the fourth kind", also widely known as "fourth nature", to describe this kind of urban industrial vegetation. Unlike "nature of the third kind" (e.g. parks or gardens), "fourth nature" is not deliberately created but spontaneously emerges on sites with strong anthropogenic influence in the past such as former industrial sites or railway areas. Often, these sites have unique soil, water and air conditions due to their former use and their location in urban areas. This results in the occurrence of site-specific vegetation that provides niches for numerous species with their own requirements. As a result, cities are often richer in species than their hinterland (Kowarik, 1992).

Another important step in the development of general acceptance and appreciation of "fourth nature" was the opening of the "Natur Park Südgelände" in Berlin as part of the World Expo 2000. The park covers the area of the former Tempelhof marshalling yard and allows visitors to experience successional nature in connection with relicts of the former use such as railway infrastructure, a water tower and an old steam locomotive. The entire area is designated as a landscape conservation area and partly as a nature reserve and is maintained in accordance with nature conservation requirements, for example by grazing to keep

the meadows open (Kowarik & Langer, 2005; Natur Park Südgelände, n.d.). The inclusion of existing "fourth nature" in the design of parks on former railway sites can also be observed, for example, in the "Park am Gleisdreieck", the "Park auf dem Nordbahnhof" (both in Berlin) or along the green corridor at the central railway areas in Munich.

Peter del Tredici, an American botanist and lecturer at Harvard University, takes up this development of urban ecology, which was mainly influenced by German and European research until the 1990s. In his article "Spontaneous Urban Vegetation: Reflections of Change in a Globalized World", published in 2010, he summarises urban ecology research in a very comprehensible way: he emphasises the unique quality of spontaneous urban vegetation, which has adapted to the prevailing environmental conditions and provides important ecosystem services and natural attenuation. Therefore, this form of vegetation has a great advantage, as it is much more stress-tolerant and adapted to anthropogenic disturbance than species that would have originally occurred in the respective place. For this reason, del Tredici emphasises the role of ecologists and landscape architects, among others, to address spontaneous vegetation in the city in depth because of its ecological functions, as well as to value its social significance as a reflection of human influence on its environment (Del Tredici, 2010).

The meadows and woodlands of the nature conservation area of the Natur Park Südgelände. *Photo: Opitz, 2023.*

The described development of urban ecology shows the value of "fourth nature" for biodiversity, natural processes such as natural attenuation, as well as its social relevance in the city. The goal of urban ecology to promote urban nature has a surprising number of parallels and overlaps with research in microbial ecology, which emphasises the health value of microbiome rewilding for people and the environment. Despite the fact that both disciplins share the same goal of promoting urban nature, according to my literature review, apart from the examples mentioned in section 1.4 of this introductory chapter, **there is no literature that specifically links the urban ecology movement in landscape architecture with microbial ecology and its objectives for urban microbiome rewilding**. From the perspective of microbial ecology, as described above, there are first attempts to translate its research on promoting the diversity of urban microbiomes from the realm of science into practice. In this context, knowledge transfer and collaboration with planning and design disciplines such as landscape architecture are central, as the publications by Robinson et al. highlight.

1.5 Conclusion of introductional chapter

Key findings from this introductional chapter

Identifying this missing link is one of the most important results of my literature review for this thesis. After a summary of all the key findings from this introductory chapter below I will present my research questions that build on them and introduce the practical part of this thesis.

- The existence of a diverse environmental microbiota is central to the regulation of the human immune system and in general to environmental health.
- Increasing urbanisation limits people's contact with diverse environmental microbiota. This is a major factor in the significant increase in non-communicable diseases (NCDs) in the last century in western wealthy countries.
- The development of antimicrobial resistance is a result of the overuse of antibiotics and the destruction of the environment in the Anthropocene.
- From the perspective of microbial ecology research, promoting accessible urban nature with diverse microbiomes is central for human health and ecosystem functions.
- To achieve this, interdisciplinary collaboration and knowledge exchange between science and planning disciplines such as landscape architecture is essential.

- Approaches on how to create urban nature with diverse microbiomes are suggested e.g. by the Microbiome Rewilding Hypothesis (Mills et al., 2017) and the observations by Robinson and Jorgensen (2020) about the possible potential of "fourth nature" for urban microbiome diversity.
- The reviewed literature points out the need for further research on the following topics, among others: What specific actions promote diverse urban microbiomes? Does the spontaneous occurrence of „fourth nature“ promotes microbiome rewilding? How do people come into contact with health-promoting environmental microbiota? How exactly do they affect health?
- In landscape architecture, microbial ecology plays a relatively marginal role.
- The urban ecology movement in landscape architecture appreciates the value of fourth nature for biodiversity and ecosystem services as well as its cultural importance. But until now, the links between the promotion of "fourth nature" and microbiome rewilding for human and environmental health have not been explored from an landscape architectural perspective.
- If it really turns out that the occurrence of "fourth nature" also contributes to microbiome rewilding, which in turn has a positive effect on the human immune system, this would be a strong argument on a whole new level for the promotion, planning and acceptance of spontaneously developing urban vegetation.
- Linking the discipline of landscape architecture/urban ecology with microbial ecology is the basis for making diverse urban microbiomes that are the basis for human and ecosystem functions a reality in our citys.

Research questions

In order to address the identified research gaps and to connect the urban ecological developments with regard to "fourth nature" from a landscape architectural perspective with microbial ecology, I formulated the following research questions for the practical part of this thesis:

- Does urban spontaneous vegetation ("fourth nature") promote microbial activity in the soil?
- What conclusions can be drawn from the result of this research question for landscape architectural planning?

To narrow down the research question, I explicitly focus on soil. Soil interacts directly with the vegetation and is a central place for microbial activity in the environment. In order to obtain relevant soil samples,

I coducted a field trip to Berlin and Munich within the framework of this thesis to collect soil from places with "fourth nature". Also, I took reference soil samples from sites with vegetation contrasting "fourth nature" (e.g. lawns, urban forests). As a method of analysing the soil samples, I chose Pfeiffer's circular chromatography soil test (PCC). Why I chose this method and how it works is described in detail in chapter 2. With the help of PCC, soil components such as soil organic matter can be identified, which is closely related to microbial activity. In this way, I am able to draw conclusions about the microbial activity in the soil samples of "fourth nature" areas. These results are then compared with the ones from the reference areas. As a final step, this makes it possible to formulate an approximation of whether urban spontaneous vegetation ("fourth nature") promotes microbial activity in the soil and what that means for landscape architectural planning. In addition, the implementation of PCC allows to gain experience about the suitability of this method as a way of enganging with the soil for landscape architects.

"Railway wildernis" in Berlin's Natur Park Südgelände. Is "fourth nature" able to contribute to the rewilding of the urban microbiome and thus generate positive effects for human health and ecosystem functions? *Photo: Opitz, 2023.*

2 Methods

A method to make the invisible visible - Pfeiffer's circular chromatography soil test

2.1 Introduction to different ways of seeing

Discovering the microbial world around us with our senses

Visibility through size

United under the collective term of microorganisms, many exceptions can be found, for example with regard to the factor size and visibility. The bacterial species *Epulopiscium fishelsoni*, discovered in the Red Sea in 1985, forms a symbiosis in the intestine of the brown surgeonfish and grows up to 0.6 mm or larger in the course of a day (Bresler et al., 1998). This corresponds roughly to the size of a grain of salt and thus the bacterium is visible to the human eye in some of its stages. Other examples of unusually large and therefore visible bacteria are marine sulphur bacteria: In 1997, scientists discovered remarkably large bacteria (*Thiomargarita namibiensis*) with singular cells up to 0.75 mm in size in sediments off the coast of Namibia (Schulz et al., 1999). Recently, Volland et al. (2022) reported another bacterium of the genus *Thiomargarita* that even grows up to one centimetre in length in the water of mangrove forests in Guadeloupe, making it the largest bacterium known today (*Thiomargarita magnifica*).

Visibility through colour

Apart from these examples, most bacteria remain invisible to humans as individuals because of their microscopic size. However, indications of metabolic processes carried out by microorganisms are also transferred to the visible spectrum. One example of this is colouration, which can be observed in nature but also in an isolated context, for example in the so-called Winogradsky columns. As part of the studio project last semester we conducted an experiment with Winogradsky columns under the guidance of the microbial ecologist Jake Robinson. Transparent containers filled with aquatic sediments from the university campus, water, sulphate sources such as boiled eggs as well as carbon sources such as paper functioned as small ecosystems of their own, in which the metabolic processes of the bacteria present were observable: Aerobic processes on the surface to anaerobic processes at the bottom of the container became visible through the different colours of the

microorganisms involved and the colour of their metabolic products.
A prominent example in nature of colours as an indicator of microbial activity are hot springs such as the "Grand Prismatic Spring" in Yellow Stone National Park. The coloured zones of the spring are created by the accumulation of pigmented bacteria such as cyanobacteria. These bacteria perform photosynthesis, producing chlorophyll, which is responsible for the spring's green hues. The carotenoids, on the other hand, which protect the bacteria from the strong sunlight, appear in hues ranging from yellow to orange and red (Brock, 1994).
Further examples for the inference of microbial activity based on colour are so-called "algal blooms", which can be observed due to their green or red colour ("red tides") (see next paragraph). Besides coloured microorganisms, there are also bacteria that produce light. Luminous bacteria are mainly found in marine ecosystems and, due to their lux genes, become visible in the dark in nature or in the laboratory (Dunlap & Urbanczyk, 2013).

Visibility through ecosystem impact

Other processes involving microorganisms are noteworthy because of their scale and impact. Even though the participating organisms are microscopic, their impact on the ecosystem is still observable. The American Society for Microbiology published a report in 2020 that addressed the Deepwater Horizon catastrophe in the Gulf of Mexico in 2010. In retrospect, microorganisms that can degrade hydrocarbons played a central role in the bioremediation of the oil spill (ASM, 2020). In response to the disaster, a diverse population of oil degrading bacteria developed to an unprecedented extent in just a few weeks. This "illustrates the diverse and large metabolic potential of the Gulf's natural microbial population" (Kleindienst et al., 2016, p. 413).
So-called "dead zones" are another example of microbial activity affecting entire ecosystems and becoming visible in this way. The excessive use of fertiliser in agriculture causes nutrients to enter river ecosystems. The abundance of nutrients in the Mississippi estuary into the Gulf of Mexico, for example, promotes the excessive growth of phytoplankton, resulting in so-called algal blooms with toxic and oxygen depleting properties. These blooms are visible due to their green or red colouring and can even be observed from space. When the microscopic algae die and are broken down, this again consumes oxygen in the water. The resulting low oxygen content, also known as hypoxia, leads to marine mass mortality and, in the example of the Gulf of Mexico, results in the largest global "dead zone", which is uninhabitable for most marine life (Joyce, 2000; Stauffer et al., 2019).

Visibility through accumulation

However, the phenomena described in the previous two paragraphs are not only visible due to the factor colour or luminescence. The most

important factor is rather the large number of organisms involved, which only become visible through their accumulation. In the studio project last semester, we also worked on the cultivation of microorganisms. For this purpose, we transferred scrapings from various surfaces such as plant leaves or handrails into a petri dish with agar culture medium (agar plate). After an incubation period of several days, we were able to observe many different bacterial cultures that had grown into visible colonies due to the ideal conditions.

One of the most prominent examples in nature of microorganisms becoming visible due to accumulation are so-called biofilms. These are aggregations formed by microorganisms such as bacteria, protozoa, algae, or fungi with all their metabolic products. Biofilms occur on a wide variety of materials primarily in aquatic environments. Biofilms can be reservoirs and propagation sites for antimicrobial resistance, but apart from their negative connotation, they have many important environmental functions: They are of particular importance for the degradation of pollution in water or soil. Due to their composition consisting of different microbial groups, they are often able to degrade a wide variety of pollutants, either individually or collectively (Yadav, 2017).

Visibility through scent

Finally, microorganims also produce various scents that that can be perceived by humans. Bacteria of the genus Streptomyces, for example, are the most common bacteria in our soils. They depend on aerobic conditions and can metabolise a variety of organic matter in the soil by producing enzymes. Streptomyces also produce geosmin, a gaseous compound that humans and some animals can sense at very low concentrations. It gives the soil its earthy, musty smell, which is typical for forest soils or can be noticed while gardening. The production of geosmin has an important ecological function for Streptomyces, as soil organisms such as springtails are attracted by this specific odour and support the dispersal of bacterial spores for their reproduction (Becher et al., 2020).

Methods that expand the human senses

Basic methods

With the help of the human senses, we can already perceive indications about the invisible life around us. But to gain deeper insights into the world of microorganisms, methods and techniques are needed that enhance the human senses. There are many different possibilities of doing this, ranging from do-it-yourself methods to advanced laboratory techniques. To learn more about microbial activity in soil on a basic level, there are methods like the cotton soil test or tea bag index. Both rely on the measurement of decomposition of organic matter through microbial activity. For the cotton test, a piece of cotton fabric is buried

in the soil for a few months. Before and after this, the fabric is weighed and the difference in weight indicates the amount of decomposition through biological activity. The cotton test is particularly suitable for measuring soil life in general, including micro- and macroorganisms.
In contrast to the cotton test, the tea bag index allows a more precise approximation of microbial activity, as the nylon net of the tea bags prevents larger organisms from accessing the organic material. As with the cotton test, the organic material is weighed before and after burying. Since the development of the tea bag index in 2010, tests have been carried out at about 2000 locations worldwide, and the results can be viewed online in an interactive map. Anybody can upload their collected data and there is a considerable amount of scientific literature available on the method (Tea Bag Index, n.d.).
Another method to learn more about soil life is through ecoacoustics. With the help of specific microphones and sound software, sounds produced by organisms in the soil can be recorded and amplified. A recent study compared degraded and restored forest plots and concluded that ecoacoustics is suitable for assessing soil biodiversity (Robinson et al., 2023).
The so-called "microBiometer" is advertised as a compromise between do-it-yourself methods and more advanced techniques. According to the producer, it offers the possibility to generate information about soil biology, quantity of microbial populations and bacteria-to-fungi ratio (microBIOMETER, n.d.).

Complex methods

However, to obtain reliable data that goes beyond rough approximations of microbial activity in the soil, more complex analytical methods are applied. Due to their complexity, they are usually not available for general private use. To bridge this gap, there is an entire sector that offers soil analyses as a service for agriculture and horticulture. Depending on what information is required about the microorganisms in a given soil sample, a variety of laboratory techniques exist at different levels of complexity. Culture-dependent methods, for example, are based on the propagation of microorganisms on petri dishes. However, it should be noted that the majority of species cannot be cultivated. Amplicon sequencing, on the other hand, allows the identification of all the bacterial community compositions and their diversity in a sample. With the help of shotgun metagenomics for eukaryotic and prokaryotic cells, every gene in a sample can be identified. Metagenomics can be divided into different sub-areas: Metatranscriptomics is used for detailed analysis of the functional role of the genes in the ecosystem, metabolomics provides information about microbial cellular activity and metaproteomics enables the understanding of microbes at the molecular level.

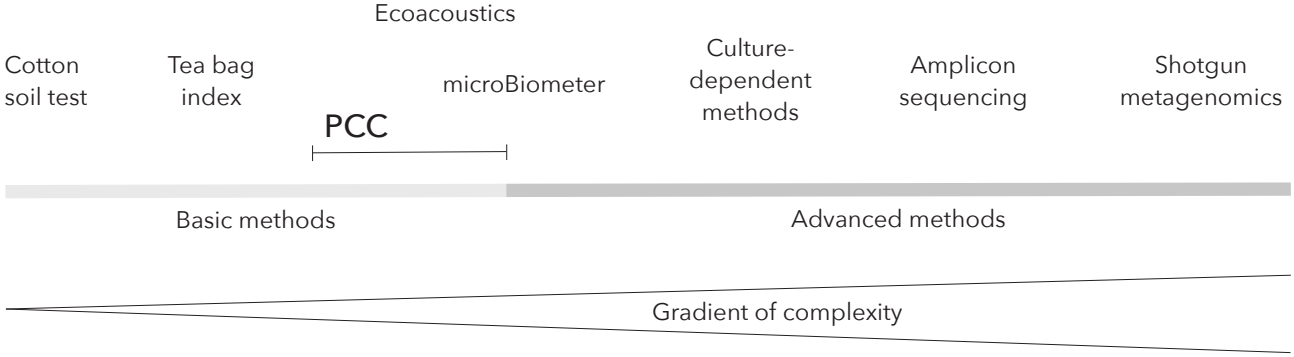
The method used in this thesis

For the analysis of soil microbial activity in this thesis I chose a very experimental and visual method. Pfeiffer's circular chromatography soil test (PCC) allows conclusions about the components of a soil sample such as soil organic matter, which is an indicator for microbial activity (Gregorich et al., 1994; Mooshammer et al., 2022). Factors for selecting this method for this thesis were...

- … the practicability of the method as a basic method with the right amount of complexity to work with it in the framework of this thesis with limited resources.
- … the uniqueness of the results, which unlike other methods are not number-based but rely on the interpretation of an overall picture.
- … that the visual quality of PCC follows the tradition of landscape architecture to perceive landscape with all the human senses. I am basing this on the landscape definition of the European Landscape Convention (ELC) (Council of Europe, 2000).
- … the established application of PCC in practice in an agricultural and permacultural context.
- … the possibility to investigate the suitability of PCC as a method for landscape architecture.
- … that based on my research this is probably the first time PCC is used in a landscape architectural context to investigate microbial activity in urban soil of „fourth nature" areas.

This chapter provides an overview of the method and the state of scientific knowledge, explains the experimental procedure, and presents different ways of interpretating. This is the basis for chapter 3, in which I describe the PCC experiments in the context of this thesis and analyse and interpret their results.

Estimation of complexity of presented methods



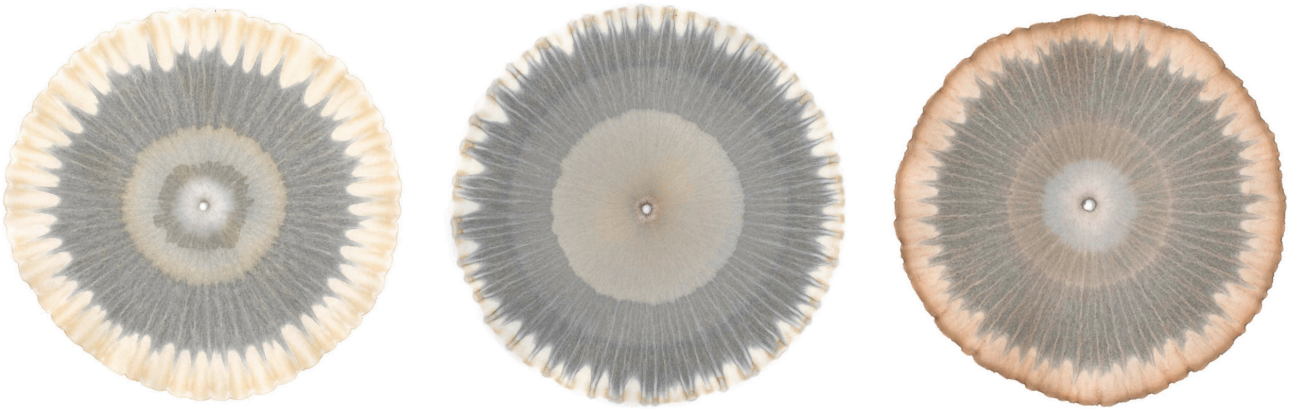
2.2 Introduction and framing of Pfeiffer's circular chromatography soil test (PCC)

Chromatography

Chromatography as a method to separate components of a mixture is of great importance for biochemistry, molecular biology and physiology (Kössel & Bonk, 1999). It is the third most commonly used method in laboratories, along with methods for weighing and determining pH value (Lorke, 2010). The IUPAC (1997, p. 92) describes chromatography as "a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction". The mobile phase can either be a liquid (liquid chromatography) or a gas (gas chromatography) moving through a flat or columnar stationary phase (Kössel & Bonk, 1999). The chromatography method goes back to the botanist Mikhail S. Tsvet (1872-1919), who describes it first in 1906 and whose observations relate to the separation of chlorophyll pigments (Altova & Hargittai, 2022).

Pfeiffer's circular chromatography (PCC)

Pfeiffer's circular chromatography (PCC) is a paper chromatography method with a flat stationary phase (filter paper) and a liquid mobile phase. It was developed by Ehrenfried Pfeiffer (1899-1961) to analyse the quality of soils, composts, and crops. The background for the development of PCC was Pfeiffer's ambition to achieve an overall picture of the soil or compost quality instead of testing only for individual soil components such as nutrients. This includes also findings about "differences in the formation of humus in soils, as well as in compost differences which cannot be determined by chemical analysis" (Pfeiffer, 1984, p. 40).



Example of chromatograms with different concentric and radial features, colours and zones in different sizes.
Photos & Chromatograms: Opitz, 2023.

In the PCC test, the mobile phase consists of a digest solution of the sample to be tested. The solution is absorbed from the centre of the filter paper and migrates through the filter paper due to capillary forces. In the process, the different fractions of the solution settle at different distances from the centre. In this way, the sample is separated into its various components. They then react with silver nitrate, a photoreactive substance with which the filter paper was treated beforehand. The resulting shapes, patterns and colours make it possible to interpret the composition of the sample (Pfeiffer, 1984).
The PCC method was adopted by Siegfried and Uta Lübke, who had been researching on composting on their farm in Austria since the mid-1960s and used PCC to assess the quality of soil and compost. Through their work, the Lübkes perfected "Controlled Microbial Composting" (CMC) (Diver, 2004). In addition to Pfeiffer's original publication, there is another monograph in German that describes chromatography for soil assessment and addresses farmers and gardeners (Voitl & Guggenberger, 1986).

Field of application

Unlike Tsvet's method, PCC does not solely aim at the separation of components of a mixture, but at the creation of an entire picture, which, based on its shapes, patterns, and colours allows conclusions to be drawn about the quality and components of the sample (picture-forming method) (Kokornaczyk et al., 2017). Because of its accessibility and simplicity without expensive equipment, circular chromatography is used worldwide, especially in the context of biodynamic agriculture and agroecology by farmers and community groups (Bezerra et al., 2019; Carneiro & Campos, 2021; Ford et al., 2021; Kokornaczyk et al., 2017). Bezerra et al. (2019), for example, used PCC to study soil changes due to agroecological conversion of family farming practices in Brazil. Furthermore, Zuazagoitia & Villarroel (2016) point out the importance of studying soil organic matter using chromatography techniques in secondary education. And also at university level PCC is used: the Chair of Being alive at ETH Zürich for example organised a field trip in which landscape architecture students tried out different field methods that are simple to conduct, including soil chromatography, to get a better understanding of the site (Breit et al., 2022).

Recent scientific publications about PCC

There are three recent publications in scientific journals that have addressed Pfeiffer's circular chromatography in detail. In all three studies, the soil samples were analysed through circular chromatography tests but also evaluated using standard chemical soil analysis methods. Then the results of the chemical analysis were correlated with the chromatogram patterns. In this way it was possible to relate specific soil components to chromatogram characteristics like shape, pattern,

or colour. This helps to verify the suitability of PCC to draw reliable conclusions about the composition of soil samples (Ford et al., 2021; Graciano et al., 2020; Kokornaczyk et al., 2017):

Kokornaczyk et al. (2017) key information

Kokornaczyk et al. (2017) confirm that PCC may be suitable for general assessments of soil condition, especially when the aim is to compare the fertility of soils and not to obtain the exact amount of soil components. Their study indicates a correlation between pronounced radial features of the chromatograms and intensive coloration of the samples with high contents of organic matter, total nitrogen, phosphorus, bromine and low soil compaction (signs for good soil quality). High compactness and low contents of organic matter, total nitrogen, phosphorus and bromine (signs for poor soil quality) on the other hand creates noticeable concentric features and blurred colours. In this study, a total of 16 soil samples in different managed agriculture fields from a farm in Spello in the Perugia region of Italy were analysed.

Graciano et al. (2020) key information

Graciano et al. (2020) also confirm the suitability of PCC for testing soil quality. The results of the study demonstrate correlations between chromatogram patterns and certain physical, chemical and biological soil properties. The main findings show that the central zone of the chromatograms correlates with total organic content as well as structure of the soil. The median zone refers to soil microbial biomass carbon and the enzymes contained in the soil can be observed in the outer zone of the chromatograms. They further emphasise that this method is well-suited to monitor soil changes and that it can make farmers more self-reliant and independent, as it is inexpensive and easy to carry out. In this study, a total of 12 soil samples from different management systems in Bandeirantes in the Paraná State in Brazil were analysed.

Ford et al. (2021) key information

The study by Ford et al. (2021), however, is in contrast to the findings of Graciano et al. (2020) and Kokornaczyk et al. (2017), as their study does not confirm that fertile soil (high organic content and microbial activity) is associated with pronounced radial features and intensive coloration. Also, their study does not indicate that concentric features indicate poor soil quality. These differences may be related to the fact that the other studies mentioned evaluated significantly fewer samples, that there are differences between different regions and continents, or that certain soil properties such as pH value influence the expression of the patterns (Ford et al., 2021). An important finding of the study by Ford et al. (2021) is that they found only weak relationships between chromatogram features and soil properties. However, there was a remarkable correlation between microbial activity and the total radius as well as the central zone of the chromatograms. The scientists conclude

that a large total radius and a large central zone may indicate a high microbial activity in the respective region of the study. In this study, a total of 343 soil samples from different land uses in the South Coast region of Western Australia were analysed.

More publications about PCC and soil quality testing

In addition to these three recent and detailed studies, there are other publications addressing PCC and soil quality. Khemani et al. (2008) addressed the interpretation of chromatograms in terms of their shape, size and colour in order to draw conclusions about the mineral content of the soil. Follador (2015) published an article on the holistic interpretation of chromatograms as part of a publication by the "The Nature Institute", that proposes a more holistic science. Saavedra et al. (2018) analysed 15 soil samples taken from three different production systems for their size, shape, colours, harmony, and integration of the patterns. In this way, they drew conclusions about the soil structure, nitrogen content, organic matter, and microbial and enzymatic activity. For their interpretation, Saavedra et al. (2018) referred to the criteria of the publication of Restrepo and Pineiro (2011) in Spanish, where the authors provide an insight into the history and methods of circular chromatography and the analysis of chromatograms on more than 200 pages with the use of many pictures and graphics.

PCC and compost/ food products testing

Besides studies dealing with soil quality, there are other scientific papers related to PCC and the determination of compost quality and rotting degree (Haßold-Piezunka, 2003) as well as the analysis of the quality of agricultural products (Geier & Seitz, 2006; Knorr, 1982; Mäder et al., 2007). Fritz et al. (2011; 2020) also used three picture-forming methods, including circular chromatography, to analyse the quality of wheat grain in Switzerland and the quality of grape juice in Germany.

2.3 PCC as a method for landscape architecture

Introducing PCC to landscape architecture

Pfeiffer's circular chromatography soil test (PCC) was developed in an agricultural context and, according to the studies mentioned before, the application of this method is still mainly limited to agriculture, horticulture and community gardening. To my knowledge, the field trip by students at ETH Zurich (Breit et al., 2022) was the first attempt to apply soil chromatography as a field method in the context of landscape architecture. Experiences like these together with this thesis should contribute to exploring the possible potential of PCC for teaching and for the practice within the discipline of landscape architecture. Even though scientific discussions about the reliability of Pfeiffer's circular chromatography differ, in my view this method offers great potential for landscape architecture to engage in an experimental way with the soil:

- The extent to which PCC allows precise statements to be made about soil quality must be evaluated by further studies. However, the existing literature suggests that **general statements** about soil condition are possible. In addition, there are clear indications of correlations between chromatogram patterns and **microbial activity** in the tested soil. In this way, the microbial spheres of the soil that are invisible to humans can be made visible.

- PCC is a relatively **simple, low-tech, and inexpensive method** that can complement other field methods to get a better understanding of the soil at a given site.

- PCC might be a helpful tool to **observe soil changes** over time and it can be combined with other monitoring methods. This could provide information about, for example, the effect of a landscape architecture intervention on a given site before, during and especially after completion.

- Since PCC is primarily used in an agricultural context there is a need for further studies about the use of this method for landscape architects or other disciplines working with e.g. **urban soils and urban soil microbiomes**.

- PCC represents an **expressive, visual method** to establish a relationship with the soil. This follows the tradition of landscape architecture, to perceive landscape with all the human senses.

- PCC offers a **complement** to conventional, complex and expensive soil analyses, whose results are purely number based. In this way, PCC also stimulates debates about whether the world around us can really be explained purely rationally and based on numbers, and how science can be more open to holistic approaches.

- Because PCC is a very visual, accessible method it can act as a **bridge** between soil sciences, landscape architecture and art. PCC can create curiosity and interest in people who find conventional science and its conventions of representing and knowledge-sharing too technical and theoretical. Furthermore, it can serve as part of introducing soil sciences at school and university level in a fun and engaging way.

2.4 Experimental procedure

Introduction

In the following, the different steps of the circular chromatography soil test are described. The procedure is based on Pfeiffer's original method and complemented by the experimental design protocol of Ford et al. (2019; 2021), Kokornaczyk et al. (2017) and Restrepo & Pineiro (2011).
The following description refers to one singular soil sample and one singular chromatogram as the result. If several samples are to be tested at the same time, leading to the generation of multiple chromatograms, the quantities must be adjusted accordingly. Kokornaczyk et al. (2017) recommend to carry out the experiment always in triplicate per soil sample and to repeat the experiment three times on different days, so that in the end a total of 9 chromatograms per sample are made. This is to obtain a reliable result since there can be differences in the chromatogram patterns produced on different days (Kokornaczyk et al., 2017).
In addition, it is important to standardise all experimental steps as best as possible, especially when several people are working together. This includes, for example, taking the soil samples with the same method and at the same time and carrying out all further steps simultaneously and in the same way. In this manner, variables such as temperature, light, contamination, etc. affect all chromatograms the same.

Further notes

- Careful! Sodium hydroxide and silver nitrate can cause severe skin burn and eye damage. Use laboratory gloves and protection glasses when working with these chemicals!

- The experiment process inside the laboratory should be carried out at room temperature and under normal light conditions without direct sunlight.

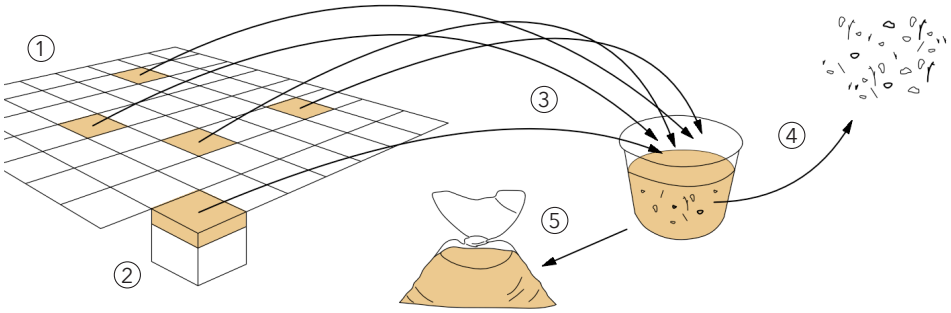
- Only when working with silver nitrate it should be as dark as possible. The filter papers impregnated with silver nitrate should dry in a dark place.

- It is beneficial that the room where the experiment is taking place is as clean as possible without a lot of dust in the air. Also, it could be beneficial to touch the filter paper only with laboratory gloves and only on the edges to avoid fingerprints.

Soil Sampling

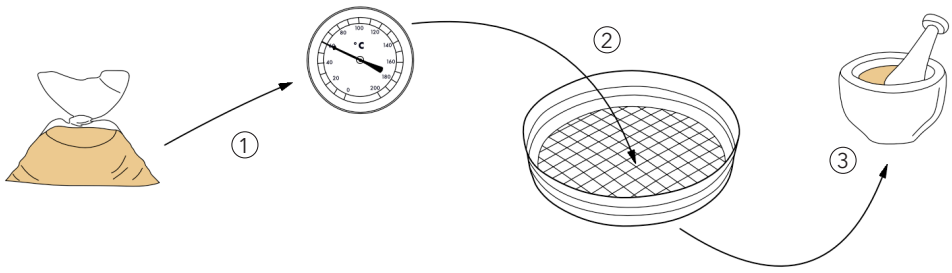
Collect soil sample

Divide the site into a grid structure and decide for 5 or more soil sample locations with the help of a randomising method (1). Collect the samples from a depth of ca. 0-10 cm with a clean stainless-steel auger (2). Make sure to collect at least 10 cm deep connected cores in the same amount from each location. Alternatively, you can also use a spade to collect the soil. Mix the samples in a container to obtain a composite sample (3) and remove grass and rocks (4). Weigh 250 g of the sample into a plastic bag (5) to take to the laboratory and label the bag to distinguish the samples afterwards (Ford et al., 2019; Ford et al., 2021; Kokornaczyk et al., 2017). Keep the soil out of the sunlight and in a cool place until the next step. *Graphic: Opitz, 2023.*



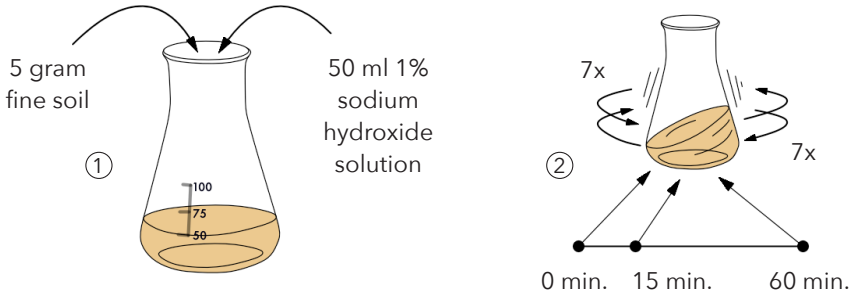
Dry, sieve and grind sample

Dry the sample in an oven at 60 °C or in a warm place outside of direct sun (1) - all other steps should be carried out at room temperature. Sieve the soil to remove components that are bigger than 2mm (2) and grind a part of the sample with mortar and pestle to receive 5 g of fine soil (3) (Ford et al., 2021; Kokornaczyk et al., 2017). *Graphic: Opitz, 2023.*



Mix sample with NaOH solution

Put the soil sample into a flask and add 50 ml of 1% sodium hydroxide solution (1 g NaOH in 100 ml distilled water) (1). Agitate the solution by swivelling the flask in the beginning, after 15 minutes and 1 hour (2). Let the solution settle after the last agitation for 5 hours before using it (Pfeiffer, 1984). To standardise the agitation, Restrepo and Pineiro (2011) and Kokornaczyk et al. (2017) propose to swivel the flask alternately 7 times to the left and 7 times to the right, for a total of 3 cycles per side.
Graphic: Opitz, 2023.

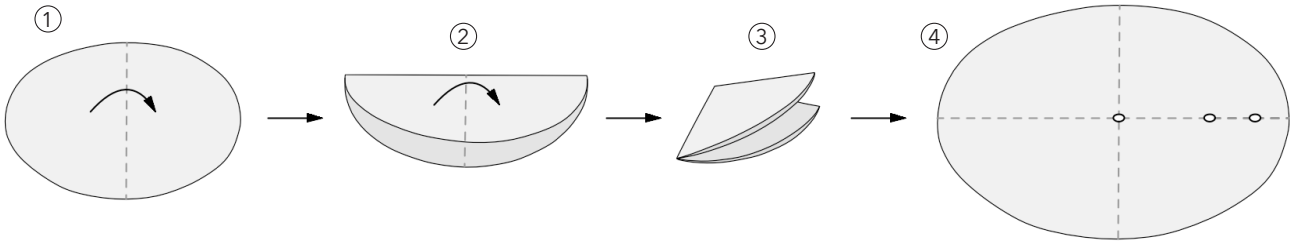


Filter paper preparation

Make a template

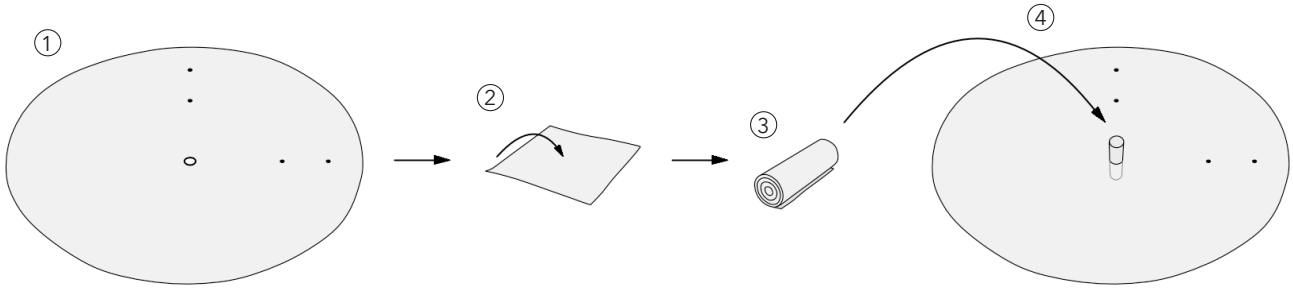
Prepare a round paper template with a diameter of 150 mm. Fold the template in half (1) and in half again (2) so that it is exactly a quarter of the original circle (3). Then unfold the paper again and puncture a hole through the centre of the circle where the two fold lines cross. Now place a ruler along one of the four fold lines so that the 0 cm mark aligns with the hole in the centre. Mark the template at 4 and 6 cm away from the centre of the paper and puncture a hole at each mark through the template (4) (Ford et al., 2021; Kokornaczyk et al., 2017; Pfeiffer, 1984).

Graphic: Opitz, 2023.



Modify filter paper

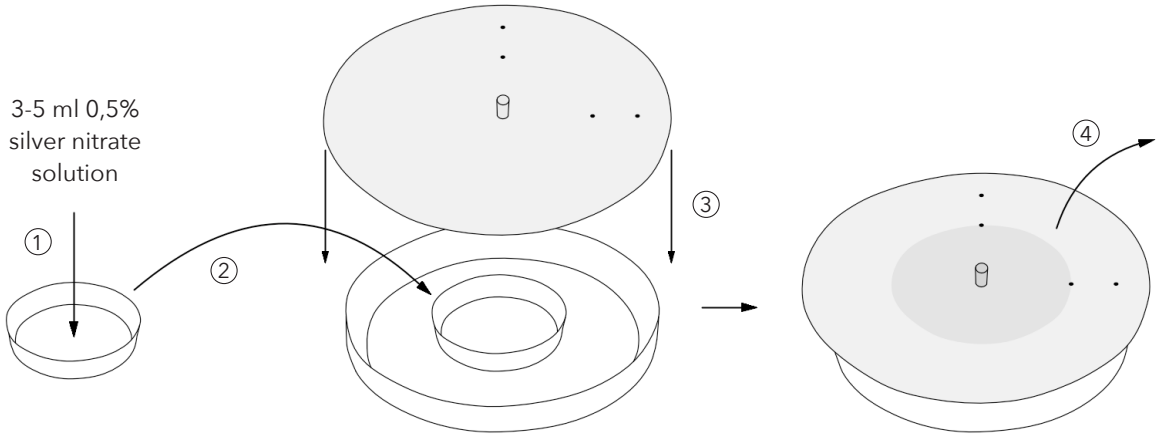
Place the template on a Whatman #1 filter (150 mm diameter) and mark the underlaying filter paper through the three holes (centre, 4 and 6 cm). Since the paper might absorb the solution slightly unevenly, repeat the 4 and 6 cm mark somewhere else on the paper. Puncture a ca. 2mm wide hole through the centre (1). Cut a 20 x 20 mm piece from another filter paper (2) and roll it up to form a wick (3). Put the wick through the hole in the centre of the prepared filter paper and make sure that the wick touches the edges of the hole evenly (4) (Ford et al., 2021; Kokornaczyk et al., 2017; Pfeiffer, 1984). *Graphic: Opitz, 2023.*



Impregnate filter paper with AgNO3

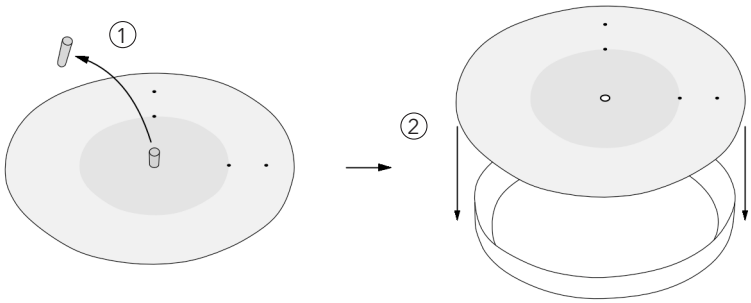
Make sure the room is as dark as possible. Fill a small petri dish with 3 to 5 ml of 0,5 % silver nitrate solution (0,5 g of AgNO3 in 100 ml distilled water) (1) and place it in the middle of a larger petri dish (ca. 60 mm diameter) (2). Place the filter paper with inserted wick on top of the large petri dish, so that the centre of the paper aligns with the centre of the large and small petri dish. Make sure that the wick touches the ground of the small petri dish to absorb the solution (3). Remove the filter paper when the soaked-up solution reaches 1-2 mm before the 4 cm mark (4) (Ford et al., 2021; Kokornaczyk et al., 2017; Pfeiffer, 1984).

Graphic: Opitz, 2023.



Dry filter paper

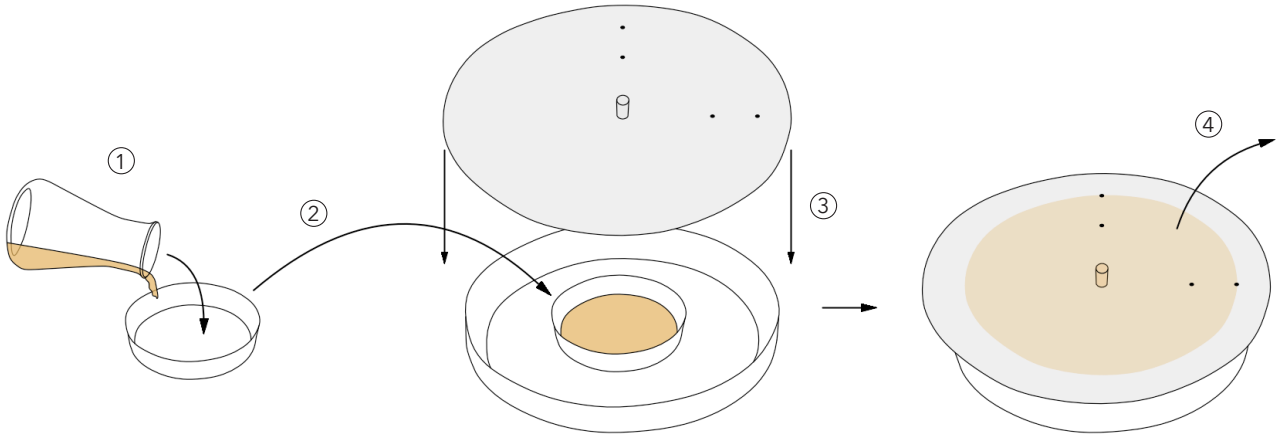
Remove the wick carefully to not damage the hole (1) and leave the paper on another petri dish for drying in the dark (2). When the paper is dry, it should be stored in a dark and dry place like a cardboard box. The impregnated paper should be used after 3 to 5 hours before the AgNO3 starts to react. When the impregnated paper has signs of discolouration or didn't dry evenly it shouldn't be used for the experiment (Pfeiffer, 1984). *Graphic: Opitz, 2023.*



Chromatogram making

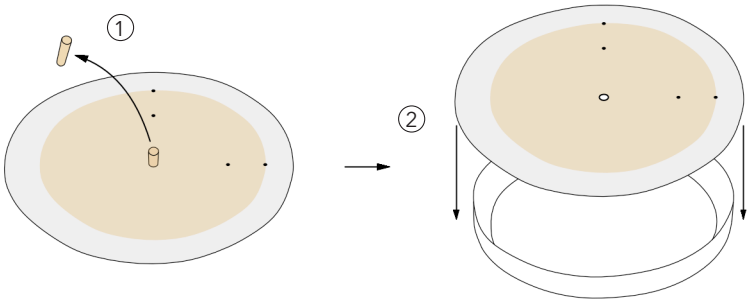
Soak filter paper with soil digest solution

Decant 5 ml of the soil digestion solution (NaOH solution with soil) into a small petri dish so that no soil particles enter (1). Place the dish into the middle of the large petri dish (2). Label the filter paper (impregnated with AgNO3) according to the soil sample that is to be analysed. Inserted a new wick and place it on top of the large petri dish, so that the centre of the paper aligns with the centre of the large and small petri dish. Make sure that the wick touches the ground of the small petri dish to absorb the solution (3). Remove the filter paper when the soaked-up solution reaches 1-2 mm before the 6 cm mark (4) (Pfeiffer, 1984).
If possible, perform this step in a closed fume hood with humidifier inside (Ford et al., 2021) or a similar setup like Pfeiffer (1984) describes it in his publication. *Graphic: Opitz, 2023.*



Drying and development of chromatogram

Remove the wick (2) and place the filter paper on a petri dish to dry (2). Let the dry chromatogram develop in diffuse light but not direct sunlight, e.g. by hanging it on a north-facing window for 7 to 10 days (Ford et al., 2021; Pfeiffer, 1984). *Graphic: Opitz, 2023.*



Preserve chromatogram

Scan the fully developed chromatograms in colour and store them in the dark. Place a clean white sheet of paper between the chromatograms to avoid reactions between them (Kokornaczyk et al., 2017; Pfeiffer, 1984).

Material list for the experimental procedure

This list gives an overview about the materials that are needed for making one chromatogram as described in the text above:

Products

- Sodium hydroxide (NaOH)
- Silver nitrate (AgNO3)
- Distilled water
- Filter paper Whatman #1 (150 mm diameter)
- 5 gram of fine soil sample (dried, sieved and ground)

Tableware

- 2 Erlenmeyer flasks
- 3 small dishes to put chemicals/soil in for weighing
- 2 small petri dishes (ca. 20-30 mm diameter)
- 1 large petri dish (ca. 60 mm diameter)

Tools

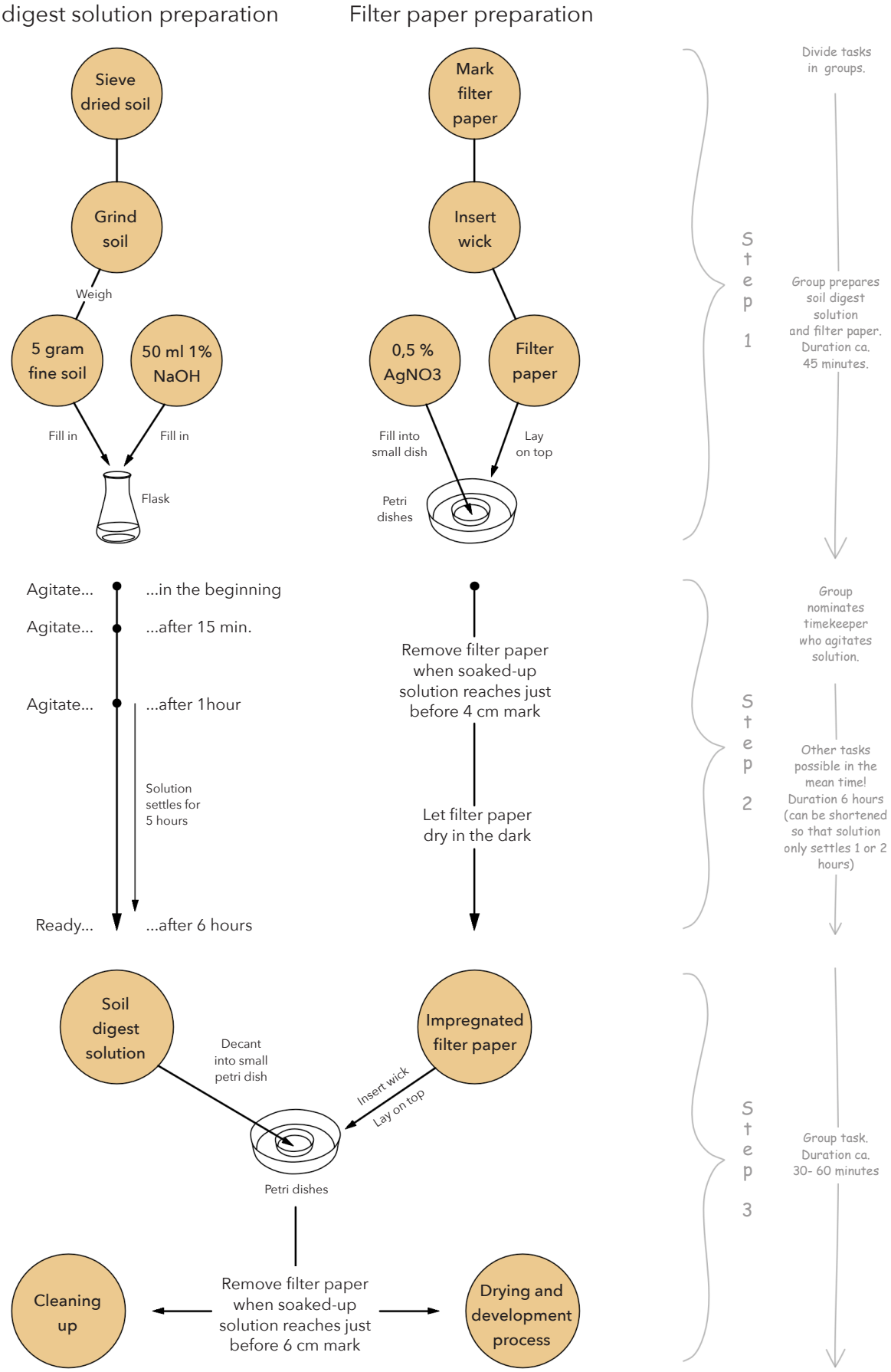
- Sieve 2 mm
- Mortar and pestle
- Sensitive scale
- Measuring cup
- 3 small spoons
- Filter paper template
- Ruler
- Pencil
- Scissor
- Cutter knife
- 1 Nail

Additional items

- Laboratory gloves
- Closable cardboard box, at least 40 x 40 cm in size
- Paper towels to clean up spills

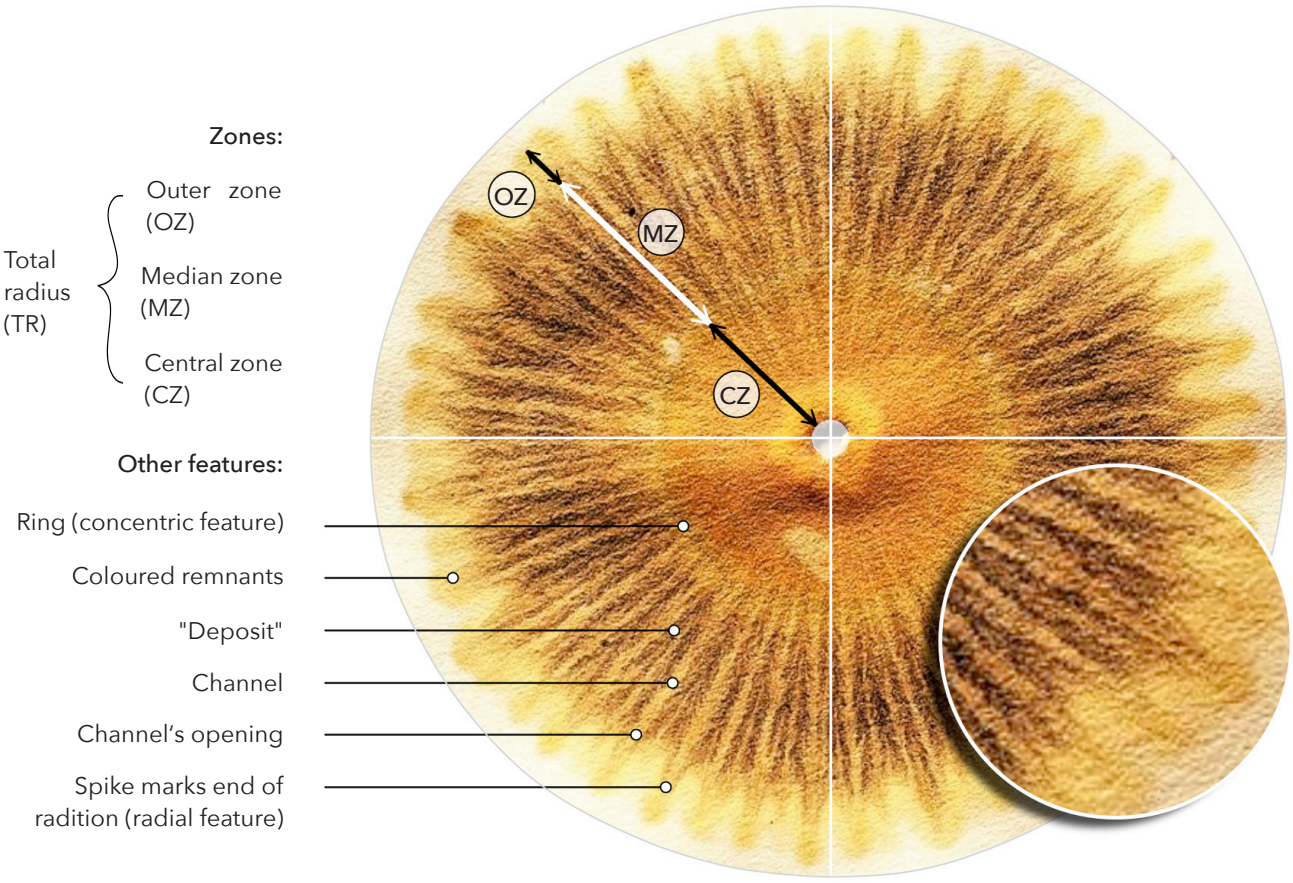
Proposal for experimental procedure and time plan

Concept & Graphic: Opitz, 2023.



2.4 Analysis and interpretation

The original publication by Pfeiffer (1984) not only describes the background and experimental procedure of circular chromatography but also offers a rough insight into his developed approaches for the analysis and interpretation of the chromatograms. At first, Pfeiffer defines different features of the chromatograms, such as zones, rings, peaks, and colours. Then, exemplary soil samples are described based on these features and conclusions are drawn to some extend about the soil quality. In addition to chromatography, Pfeiffer also examines the samples for their pH value, organic matter, and nutrient content. Pfeiffer's publication provides basic orientation for the interpretation. But in relation to the very detailed experiment description Pfeiffer's interpretation approach remains rather vague and unclear. Because of this I added information from a table of Ford et al. (2021) with further insights about what the chromatogram features mean for interpretation. Furthermore, I summarised the results of the paper by Kokornaczyk et al. (2017) that defines negative and positive corralations between chromatogram features and soil components.



Chromatogram features based on Pfeiffer (1984), Kokornaczyk et. al. (2017) and Ford et al. (2021). *Graphic: Opitz, 2023. Underlaying photo: Livia Bischof Pian, 2017.*

Pfeiffer (1984) - Correlations between chemical soil analysis and chromatogram patterns

Chromatography does not replace the chemical analysis of the soil, but it offers a way to analyse the soil as a chromatographic image that provides clues about its qualitative and biological properties based on its colour and pattern (Pfeiffer, 1984). Pfeiffer emphasises the importance of working with "standard" chromatograms that serve as orientation for interpretation. They depict soils whose properties (e.g., nutrients, humus content, structure) are known and therefore reveal correlations between them and the chromatogram featurs. If chromatograms of unfamiliar soils are produced and compared with these standards, similarities and deviations can be determined, which ultimately gives information about the soil components of the unfamiliar soils (Pfeiffer, 1984).

Zones

Zones: Pfeiffer distinguishes between three zones in the chromatograms, which he calls outer zone, middle zone, and central zone. When observing the zones, attention should be paid to the number (a), width (b) and colour (c) of the zones as well as to their "regular or irregular formation and shading" (Pfeiffer, 1984, p. 43).

- Number (a): Typically, three zones are found in a chromatogram. The outer and middle zones are mainly related to the organic material of the soil sample. The central zone provides information on whether mineralisation processes are taking place in the soil or not (Pfeiffer, 1984).

- Width (b): The width of the zones reflects the quantity of components in the soil sample that are responsible for the formation of the respective zone (for example, organic material in the outer and middle zone) (Pfeiffer, 1984).

- Colour (c): Uniform light to medium brown shades indicate good humus formation and distribution in the soil. Acidic humus substances can be identified through dark brown enclosures. A strong mineralisation of the soil, in which organic compounds are broken down, is signalled by purple radiations (Pfeiffer, 1984).

Concentric features

Concentric features: In addition to the zones, Pfeiffer also pays attention to the formation of rings between the middle and outer zones and at the outer edge of the outer zone (Pfeiffer, 1984).

Radial features

Radial features: Finally, the number, colour, and shape of the radiations is evaluated. Purple radiations in the inner zone indicate that mineralisation is taking place (Pfeiffer, 1984).

Exemplary chromatograms

C1: Very fertile soil, posessing "a natural, stable humus, (and) an ideal, friable structure". Medium brown outer zone with brown, dark "spots", harmonious radial features, and a dark brown inner zone without purple colouring (Pfeiffer, 1984, p. 45).

C2: "Infertile" soil with "structural problems" and little microflora. "Lack of form and faint brown coloration of the edge and middle zone" indicates the lack of a good humus structure. The relatively large inner zone gives little indication of humus in the soil (Pfeiffer, 1984, p. 47).

C3: Soil from moist and rather acidic site. Outer zone doesn't indicate "colloidal stable humus formation" (Pfeiffer, 1984, p. 48).

C4: Fertile, well-aerated soil with high organic matter and high nitrogen content (Pfeiffer, 1984).

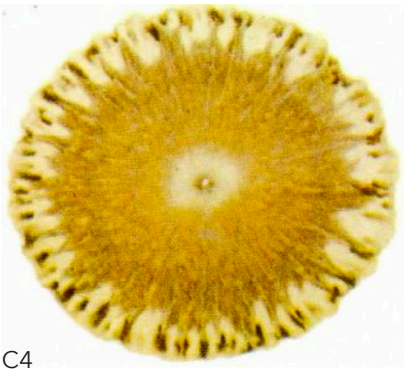
C5: Soil "with a reasonably good humus formation". The outer zone, gives information about the "degree of humus formation". The middle zone and spikes indicate the moisture of the site (Pfeiffer, 1984, p. 48).

C6: Soil from same area than C5. The soil is fertile, well aerated and without waterlogging. Pfeiffer points to the outer and middle zone, which has a stronger brown-yellow colour than C5, but he does not interpret this observation further (Pfeiffer, 1984).


C1


C2


C3


C4


C5


C6

Examples of Chromatograms depicting soils with very different characteristics by Pfeiffer (1984). *Photos: Pfeiffer, 1984.*

Table 1. Summary of chromatogram featurs and what soil components they represent based on Pfeiffer (1984) and Ford et al. (2021)

Feature	Pfeiffer (1984)	Ford et al. (2021)
Central zone (CZ)	CZ provides information about whether mineralisation processes are taking place in the soil or not. Purple radiations indicate that mineralisation is taking place. The width of the zone reflects the quantity of soil components that are responsible for its formation.	"Patterns in the central zone inform about the presence of minerals. These are the heaviest contents of the digest to move into the filter paper and are thus move the least distance from the centre of the filter paper" (p. 6).
Median zone (MZ)	MZ is related to the organic material of the soil sample. The width of the zone reflects the quantity of soil components that are responsible for its formation.	"Structure indicates the presence of proteins, organic carbon and organic matter (minerals and humus)" (p. 6).
Outer zone (OZ)	OZ is related to the organic material of the soil sample. The width of the zone reflects the quantity of soil components that are responsible for its formation.	"Clouds at the ends of spikes indicate available nutrients. Bacterial enzyme activity displayed in this zone" (p. 6).
Channel development		"Greater number of channels suggests increased organic matter and nutrients. Channels extending across zones indicate integration of soil components" (p. 6).
Spike number		"Greater number of spikes suggests increased organic matter and nutrients. Well-developed spikes are thought to represent healthy soil" (p. 6).
Colour	Uniform light to medium brown shades indicate good humus formation and distribution in the soil. Acidic humus substances can be identified through dark brown enclosures.	"Warm colours (gold, red, yellow, orange, cream) and/or high colour intensity indicate healthy soil. Colder colours (grey, dark brown, or blueish) suggest soils with less microbial activity" (p. 6).
Concentric rings		"Strong rings indicate possible excess of soluble minerals" (p. 6).

Table 2. Kokornaczyk et al. (2017) - Correlations between soil components and chromatogram patterns

This chart presents a synthesis of the texts and tables from the paper by Kokornaczyk et al. (2017) showing their most important results concerning the analysis of chromatograms. *Synthesis & Grafik: Opitz, 2023.*

This row shows the different soil components that were analysed

The pattern zones were evaluated by measuring them with a ruler

The pattern characteristics were visually evaluated *

This row shows the differnt PCC patterns that were evaluated

High contents of these soil components are as sign of rather high soil quality, low contents are a sign of rather poor soil quality

Sandy soil promotes low soil compactness

Silty soil and clay promotes high soil compactness

	Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spikes	Colour intens.	Con. rings
Organic matter	N. C.	N. C.	N/A	P. C.	P. C.	P. C.	P. C.	N. C.
Total nitrogen	N. C.	N. C.	N/A	P. C.	P. C.	P. C.	P. C.	N. C.
Phospho-rus	N. C.	N. C.	N/A	P. C.	P. C.	P. C.	P. C.	N. C.
Bromine	N. C.	N. C.	N/A	P. C.	P. C.	P. C.	P. C.	N. C.
Sand	N. C.	N. C.	N/A	P. C.	P. C.	P. C.	P. C.	N. C.
pH	P. C.	P. C.	N/A	N. C.	N. C.	N. C.	N. C.	P. C.
Silt	P. C.	P. C.	N/A	N. C.	N. C.	N. C.	N. C.	P. C.
Clay	P. C.	P. C.	N/A	N. C.	N. C.	N. C.	N. C.	P. C.

CZ = Central zone. MZ = Median zone. OZ = Outer zone. Colour intens. = Colour intensity. Con. rings = Concentric rings. N/A = No data available. P. C. = Positive correlation. N. C. = Negative correlation.

Concentric pattern feature. It correlates positvely with other concentric pattern features and neagtively with radial pattern features.

Radial pattern feature. Correlates positvely with other radial pattern features and neagtively with concentric pattern features.

CZ radius and OZ breadth show **strongest correlations with soil components**.

Positive correlation. When the one variable rises, the other variable rises too. Example: The higher the organic matter content in the soil, the higher the colour intensity of the chromatogram.

Negative correlation. When the one variable rises, the other variable falls. Example: The higher the organic matter content in the soil, the higher the colour intensity of the chromatogram.

* The visual evaluation for pattern characteristics of Kokornaczyk et al. (2017) works with a point system: **Channels** (1 point if absent, 5 points if full developped), **Spikes** (1 point if absent, 5 points if full developped), **Colour intensity** (1 point when colours are blurred, 5 points when intense), **Concentric rings** (1 point for every contentric ring). The definition of how these patterns are defined are presented on the previous page.

Evaluation scheme for the analysis and interpretation of the chromatograms in this thesis

Additional pattern categorie

The approach and categories of Pfeifer (1984), Kokornaczyk et al. (2017) and Ford et al. (2021) provided guidance for the development of this evaluation scheme. Additionally, in order to be more specific about the range of patterns of the chromatograms in this thesis, the categorie "Spike-channel ratio" was defined. It was introduced in order to be able to consider different spike shapes. During the evaluation I noticed that sometimes not only one channel leads into one spike, but several channels into the same spike, so that this influences its shape.

Step 1 - General visual interpretation

Look at each chromatogram and notice its colours and patterns. Try to categorise what you see according to the categories in table 1. Use the information in this table to then draw conclusions about the possible soil components that are represented by the chromatogram features.

Step 2 - Analytical interpretation

To confirm the results from the general visual interpretation measure and visually assess the chromatograms as described in the following and note the respective results into its own table. Since the chromatograms are always in triplicate, take the average of the results.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
mm	mm	mm	mm	1-5 pts.	no.	1:1 - 1:no.	Text	1-5 pts.	no.

CZ = Central zone. MZ = Median zone. OZ = Outer zone. S.-c. ratio = Spike-channel ratio. Colour intens. = Colour intensity. Con. rings = Concentric rings.

Measuring and assessing the different features

Total radius: Measure the distance from the edge of the hole in the centre to the outer edge of the chromatogram.

Central zone (CZ) radius: Measure the distance from the edge of the hole in the centre to the outer edge of the central zone.

Middle zone (MZ) breadth: Measure the distance from the outer edge of the central zone to the outer edge of the middle zone (ends at the bases of the spikes).

Outer zone (OZ) breadth: Measure the distance from the outer edge of the middle zone (ends at the bases of the spikes) to the outer edge of the outer zone.

Channels: Visually assess the appearance of the channels. Give 1

point if no channels can be seen and up to 5 points the more they are developed.

Spike number: Count the number of fully developed, clearly distinguishable spikes.

Spike-channel ratio: Notice if the spikes are related to only one channel at a time or if several channels lead into one spike. Note down the minimum and maximum spike-channel ratio.

Colour range: Describe the range of colours occuring in the entire chromatogram.

Colour intensity: Visually assess the appearance of the coloursin the entire chromatogram. Give 1 point if the colours are rather faint and blurreda and up to 5 points the more intense they are.

Concentric rings: Visually assess the chromatogram if there are strong colour differences within a zone or between zones so that visible rings are formed. Only consider them if they are very well noticeable. Every ring gives one point.

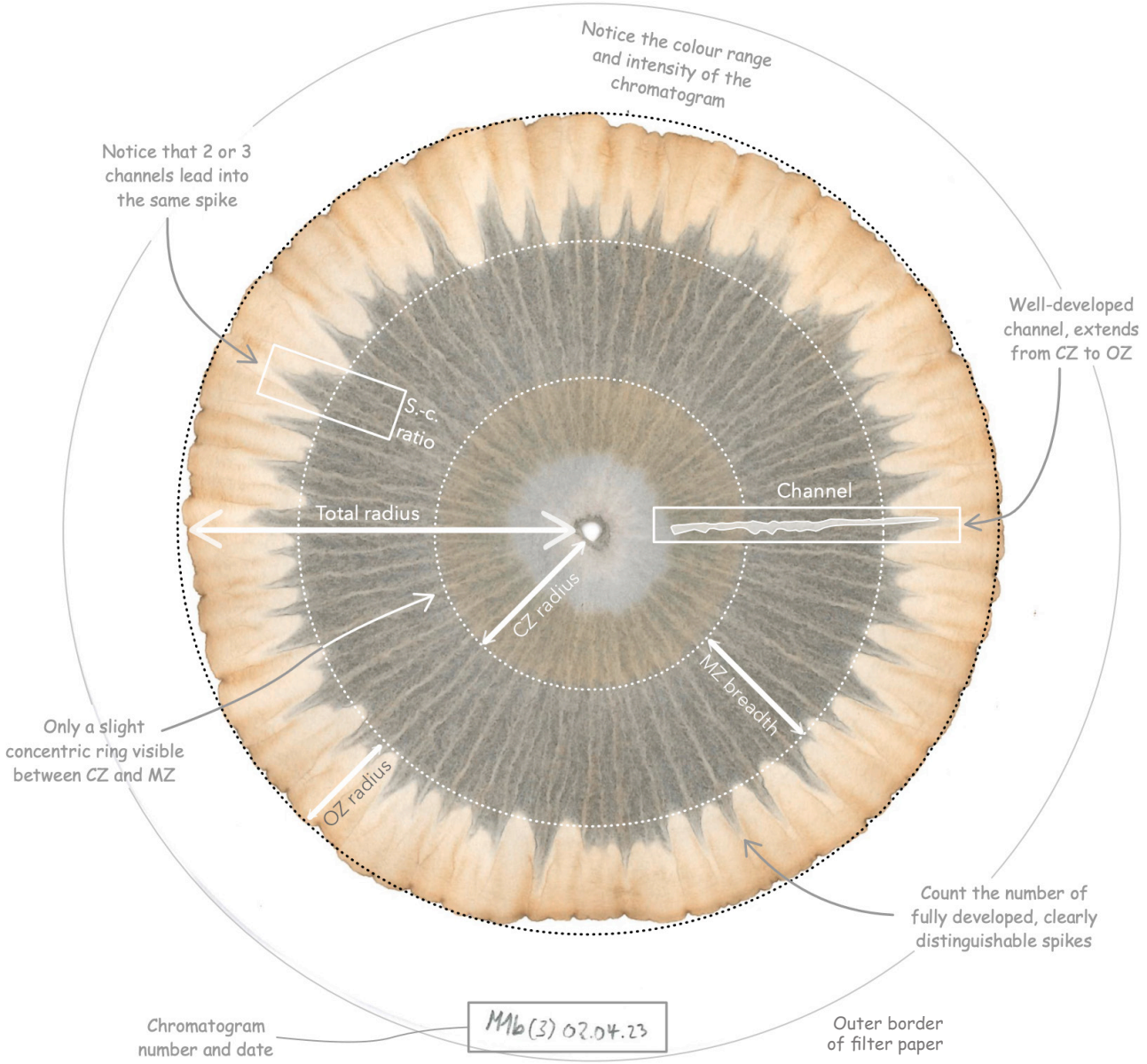
Now look at the values in each chromatogram feature category to identify the high and low value. The range between these two values is divided into three "stages" (low, middle, high stage). Following that, all values of the respective category are assigned to one of these three stages on the basis of their numerical values. In this way it is possible to say whether the respective chromatogram has a rather low, middle or high value in comparison to the other chromatograms related to the respective category. An example: the total radius of all chromatograms was measured. One chromatogram has the highest value with 60 mm, the chromatogram with the lowest value is 54 mm. The range between 54 mm and 60 mm is now divided into three "stages": 54 to 56 mm is the low stage, 56 to 58 mm represents the middle stage and 58 to 60 mm is the high stage. Now each chromatogram is assigned to a stage according to its total radius value.

Lastly, the chromatograms are compared with Table 2 on the basis of the numerical values of their features, which were assigned to different stages. This gives information about which soil component the respective feature points to and the amount of this component in the soil sample. Due to the division into stages, it is also possible to compare the chromatograms with each other. An example: the total radius of a chromatogram has a negative correlation with organic matter according to Table 2. This means that the larger the total radius, the lower the organic matter content in the soil sample. If the total radius of an examined chromatogram is large compared to the

other chromatograms and it thus belongs to the high stage, this is an indication of a rather low content of organic matter in this soil sample compared to the other samples.

Further notes

- The chromatograms of the soil samples from Berlin and Munich, which are interpreted in the framework of this thesis, are first considered separately. Thus, there are separate low, middle and high stages for both locations.
- In order to be able to compare the chromatograms of the soil samples from Berlin and Munich, low, middle and high stages were additionally determined on the basis of the values of all chromatograms.
- When interpreting the chromatogram features using Table 2 to verify the results of the visual interpretation, only information about the organic matter content of the soil samples is taken into account. Organic matter influences the microbial activity in the soil, which is the main aim to be evaluated by the experiment.
- As it was very difficult to evaluate the concentric rings of the chromatograms using numbers, this characteristic is mainly taken into account in the general visual interpretation.
- Formulations such as "high" or "low" refer to that in comparison with the other chromatograms of this thesis the given parameter is rather high or low.
- Steps 1 and 2 of this interpretation scheme should help to compare and verify the results of the two different interpretation approaches.



How to measure and assess the different chromatogram features on the example of chromatogram M1b.
Photo & Graphic: Opitz, 2023.

3 Results

Application of Pfeiffer's circular chromatography soil test in practice

3.1 Documentation of experiments - A learning curve

First experiment

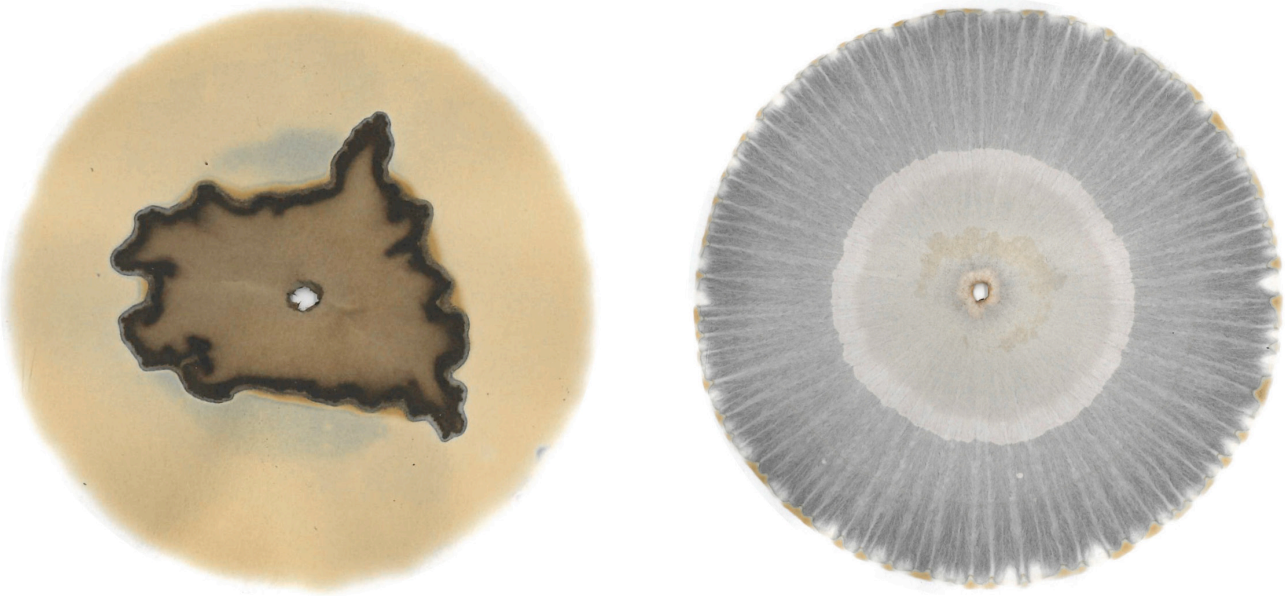
Context

I conducted my first chromatography experiment after reading about the subject for several weeks at the beginning of thesis semester. This was also to prepare for a workshop, where I wanted to apply the PCC method together with other students. In advance of this first trial, I had arranged for all the necessary materials and substances with the support of the university's soil science department and was allowed to use a room in the newly established Sustainability Hub at NMBU as a "laboratory". In order to be able to observe the whole process as closely as possible, I decided to produce only two chromatograms. For making the soil digest solution I took the soil from my experiment last semester: the first one was a rain garden soil which is also used for the construction of the rain gardens on campus and which has a very high mineral and a very low humus content. As a contrast, for the second sample I used soil from the Marka forest nearby Oslo with a very high humus content. During the experimental procedure, I followed the protocol (chapter 2) as strictly as possible, the only change being the length of time that the soil was to soak in the sodium hydroxide liquid. Since the time would be limited during the upcoming workshop, I wanted to test this factor out in advance. So I decided to let the soil soak for only two hours instead of the six hours in the original protocol.

Observations

The chromatograms turned out very differently. The chromatogram reflecting the rain garden soil developed as I expected after reading literature about PCC. The chromatogram reflecting the forest soil on the other hand remained very small with an unusual shape with only one constant brown area and a thick, bulged dark brown edge. The yellow colouring around the edge is due to the silver nitrate with which the filter paper is impregnated beforehand and that reacts with the light.

The time for the soil to soak seems to be an important factor and has two main functions from my view: firstly, all the substances in the soil can dissolve in the liquid during this time. Secondly, this period allows the soil to settle at the bottom of the Erlenmeyer flask. Because of the high organic matter content in the forest soil in contrast to the rain garden soil in my experiment, many particles had not yet settled and entered the petri dish while decanting the liquid. My assumption is that organic matter particles in the soil digest solution cause the pores in the paper of the wick and the filter paper to clog and, above a certain amount of accumulation this prevents the liquid from further spreading into the paper. This observation supports the statements of Kokornaczyk et al. (2017), who indicated a negative correlation between the total radius of a chromatogram and the organic matter content in the corresponding soil sample. I assume that fine organic matter particles enter the filter paper even after the soil digest solution has settled for 6 hours. This then causes the pores of the paper to clog to a certain amount which limits the spread of the liquid in comparison to a sample that has fewer organic matter particles in it. Moreover, I suspect that the more particles are deposited at the edge of the chromatogram, the more the advancing liquid has to push them forward, resulting in a thick, wavy and bulged outer zone edge. Finally, I noticed that the brown colour tone of a chromatogram is due to organic matter, whereas grey to grey-lilac shades indicate a high mineral content in the soil, as Pfeiffer (1984) also describes.



The chromatogram on the left reflects the forest soil with high organic matter content, the chromatogram on the right the rain garden soil with low organic matter and a high mineral content. *Photos & Chromatograms: Opitz, 2023.*

Second experiment – Student workshop

Context

During a workshop with students at NMBU, that I prepared and conducted, I was able to gain further experience with the PCC method. The workshop was part of the GLA304 course in "Landscape Architecture for Global Sustainability" and the aim was to visualise, represent and compare soils from different places with "Brachen" characteristics using the PCC method. A total of 12 students took part in the workshop, each bringing their own soil sample. The day before, I had already prepared the experiment setup so that we were able to start immediately with the preparation of the soil digest solution and the impregnation of the filter papers with silver nitrate. Based on the experience from my previous experiment and the limited time, we reduced the swirling of the soil dissolved in sodium hydroxide to two times to allow the soil to settle for at least one hour. In the further course, we followed the protocol as closely as possible.

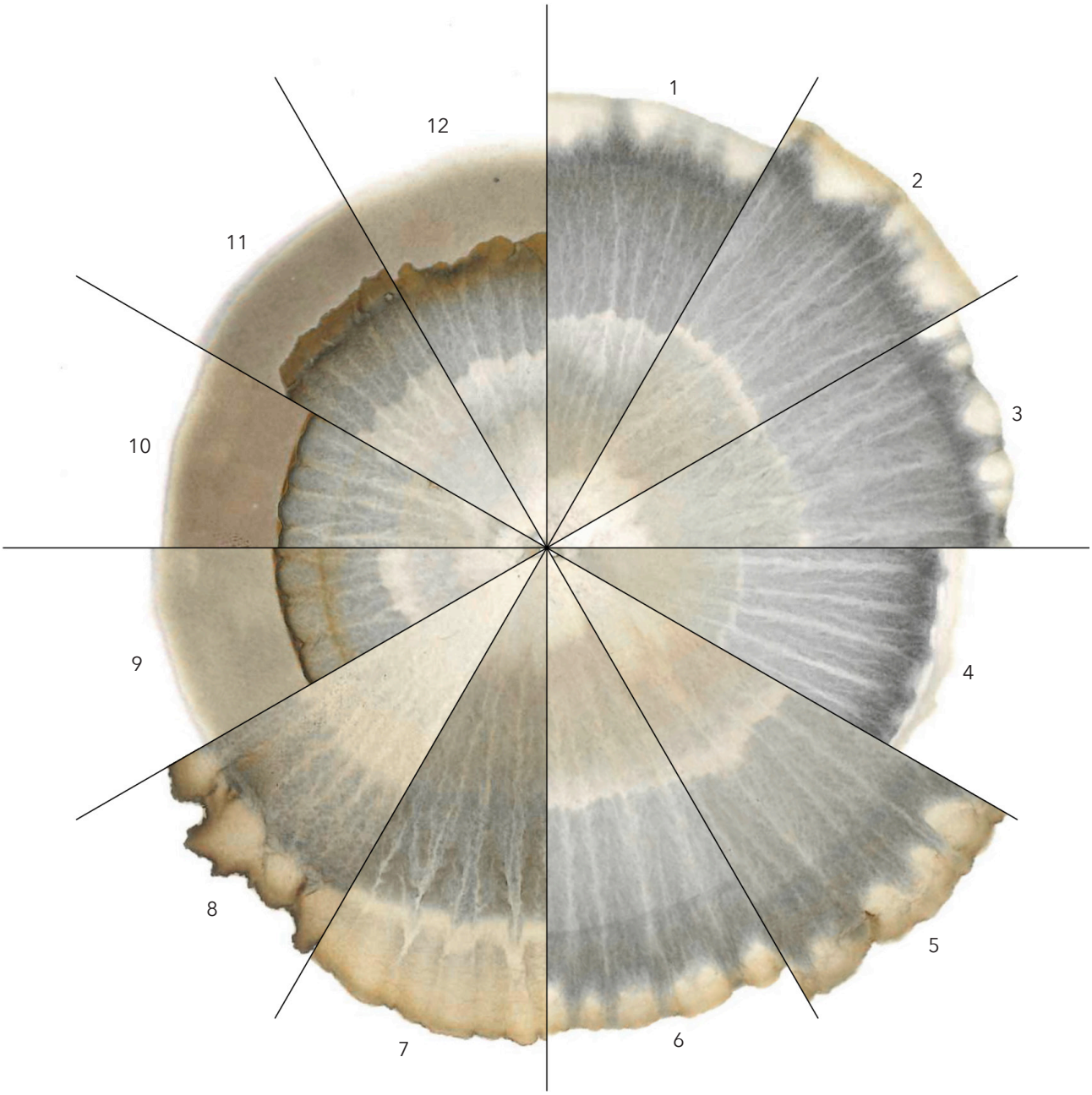
Observations

The filter papers were still slightly damp after we had left them to dry for only one hour. Nevertheless, they absorbed the soil digest solution relatively quickly at the beginning, but after a few minutes the spread of the liquid in the filter paper slowed down. Even after more than an hour, not all filter papers had absorbed the liquid as intended. Because of this, the variance in total radius is clearly recognisable between the chromatograms, as the compilation of all 12 chromatograms on the next page shows. The difference in colours is also clearly visible as well as the expression of different chromatogram features: the colours range from grey over brown to beige-cream in different intensities. In addition, there is a great variance in the expression of channels, spikes and the size of zones with abrupt or soft colour transitions. A phenomenon that I have not observed since this workshop and that I cannot explain is the formation of a brown, wide colour zone around the actual outer zone of chromatograms 9 to 12.

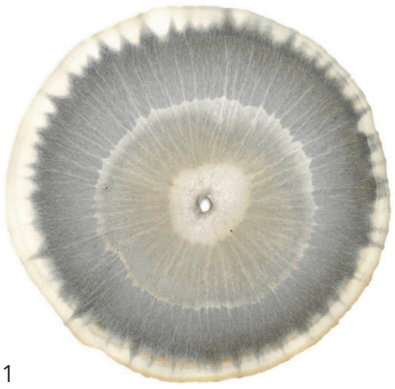
Conclusions

In general, this workshop confirmed my observations that the chromatograms reflecting a soil with a rather high organic matter content show on average a lower total radius and brown colour tones than those with a higher mineral content, which are characterised by a comparatively large total radius and rather grey colouring (chromatograms 7-12 vs. chromatograms 1-6). Another finding was that filter papers that are still wet may slow down the absorption of the soil digest solution and possibly distort the results. In addition, the small size of the petri dishes that I used for this workshop caused the filter papers to hang down in some places, allowing the liquid to spread out further in this direction following the force of gravity. This is clearly visible in the illustration of the 12 chromatograms on the second next page.

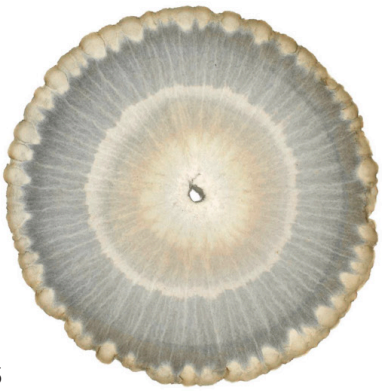
Apart from chromatogram-related perceptions, I found the workshop to be a great opportunity to engage with the topic of soil together and discuss differences in the chromatograms reflecting the soil. Working with a group also requires good preparation, enough space and preferably several people who know about chromatography. In addition, a clearly written experimental protocol is very helpful, which can be printed out, written on a board, or shown on a monitor. In addition, a follow-up discussion after the complete development of the chromatograms would be useful to review the initial observations and to compare the colour development over time.



Compilation of the chromatograms to compare the varying radii. *Photos & Graphic: Opitz, 2023. Chromatograms: GLA304, 2023.*



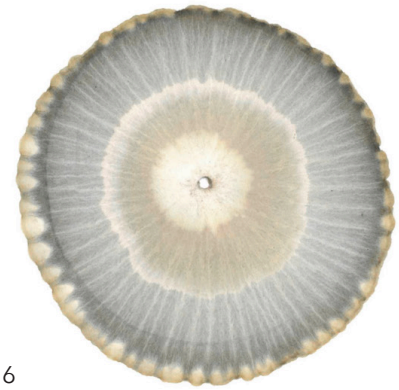
1



5

9

2



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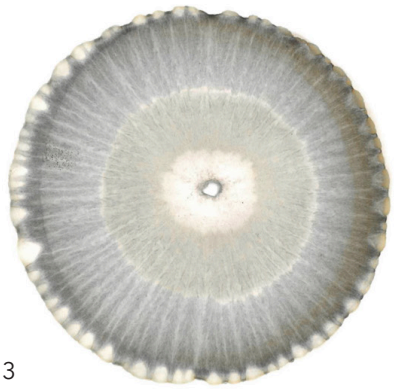
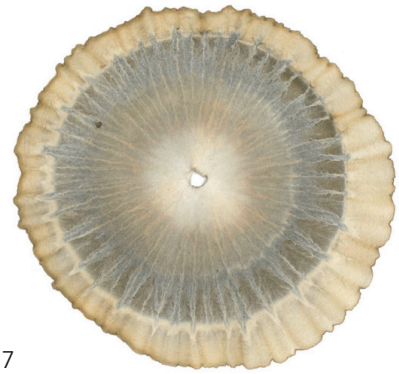
10



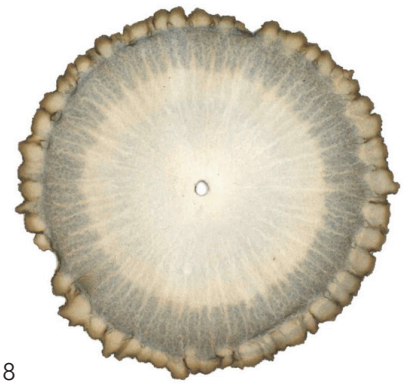
3



7

11

4



8

12

The results of the workshop as part of the GLA304 course at NMBU. *Photos: Opitz, 2023. Chromatograms: GLA304, 2023.*

Third experiment

Context

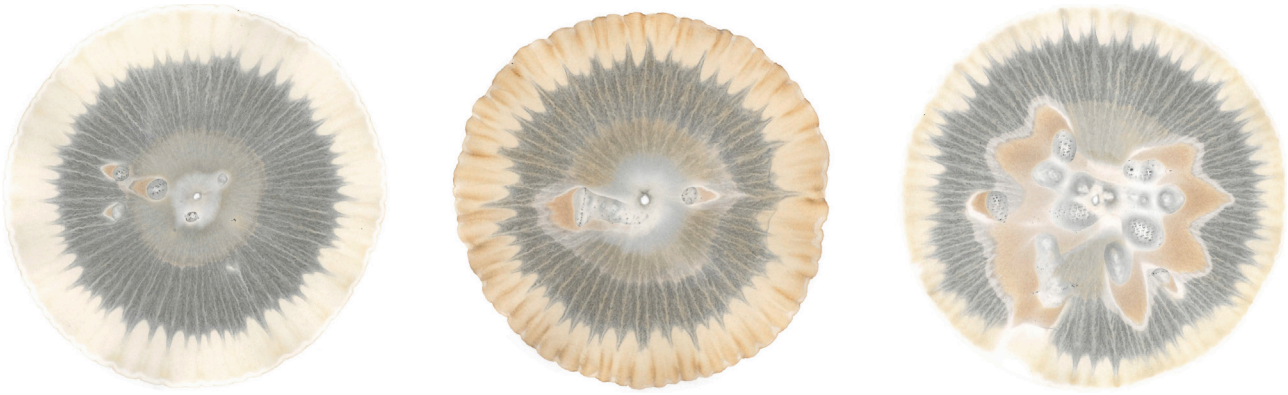
The goal of this experiment was to analyse the soil samples that I collected in Munich. Based on my experiences, I paid particular attention to following the experimental protocol as strictly as possible, especially to let the soil soak and settle for 6 hours, let the filter papers fully dry in the dark overnight and make sure that they lay flat on the petri dishes to absorb the soil digest solution evenly.

Observations

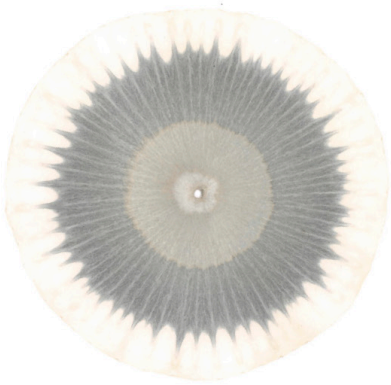
Although I let the filter papers dry overnight, they were still damp when I started the experiment the next morning. While using the damp filter papers in this experiment, I noticed that differently pronounced stains on most of the chromatograms appeared. These became larger and more intense over time and clearly distorted the chromatogram features. I was therefore not able to use the chromatograms from this experiment to analyse and interpret them later.

Conclusions

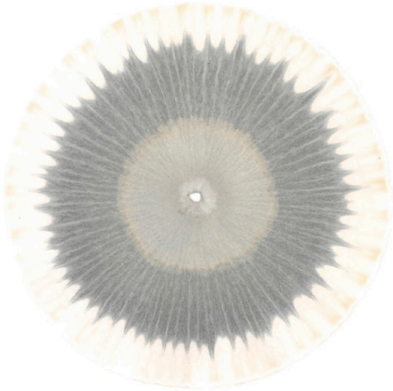
According to my observations, the reason why the filter papers did not dry as intended was the large quantity of moist filter papers (15 in total) that I stacked on top of each other. Especially the papers in the middle of the pile remained very moist. Since the liquid had presumably also accumulated in the depressions of the papers, which were rippled by the moisture, a particularly large amount of silver nitrate collected here. I assume that this triggered the formation of the stains later. Another factor could have been that the silver nitrate was no longer fresh enough and reacted differently than if the papers had been impregnated earlier. Although some of the literture about PCC that I evaluated recommended drying the filter papers over night in a pile, it did not work for me. Therefore I changed the experimental protocol to let the filter papers dry individually on top of a petri dish for only a couple of hours in the dark.



The resulting chromatograms with stains that differ in their shape and intensity. *Photos & Chromatograms: Opitz, 2023.*

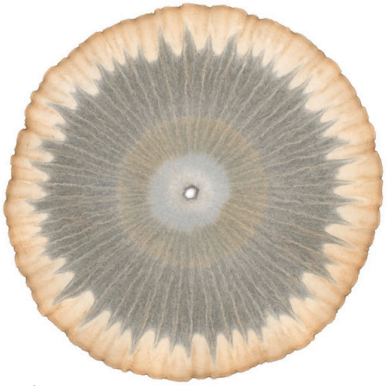


M1a (1)

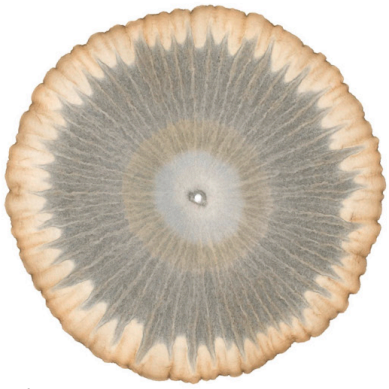


M1a (2)

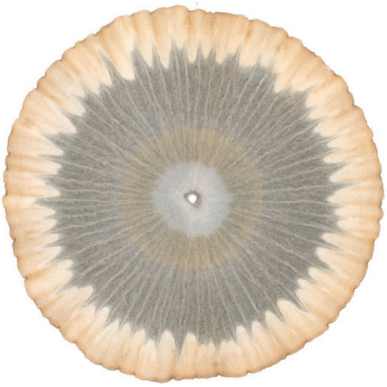
M1a (3)



M1b (1)

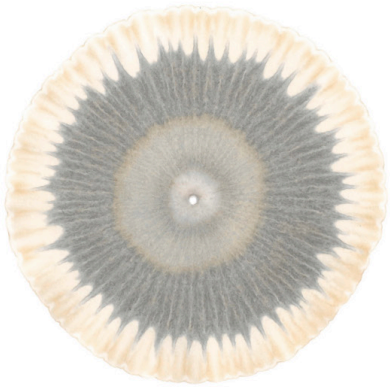


M1b (2)

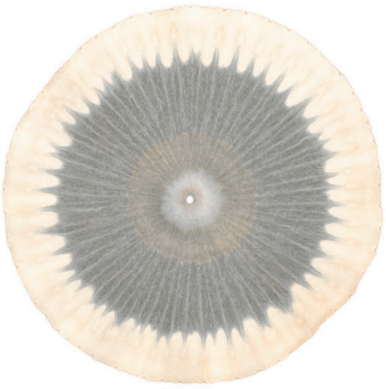


M1b (3)

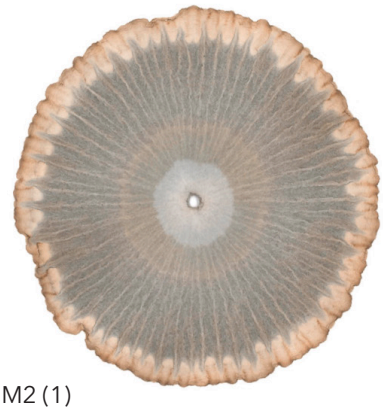
M1c (1)



M1c (2)



M1c (3)

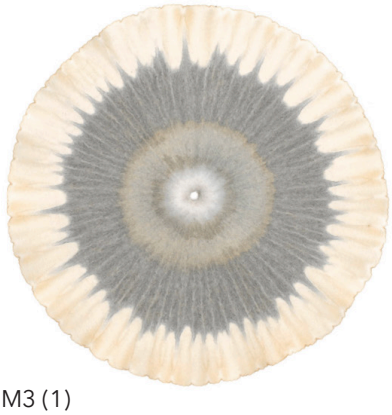


M2 (1)

M2 (2)

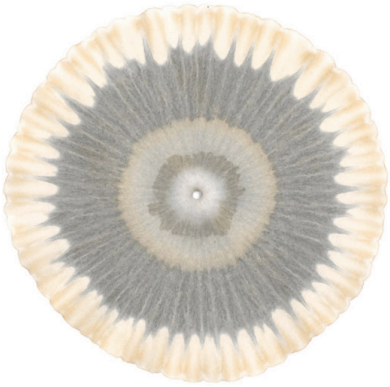
M2 (3)

The chromatograms from the Munich soil samples in triplicate. *Photos & Chromatograms: Opitz, 2023.*



M3 (1)

M3 (2)



M3 (3)

Fourth and fifth experiment

Context

Although experiment number three was not successful, I was able to learn a lot from it for the following procedures. In the fourth and fifth experiment with the soil samples from Munich and Berlin, I again strictly followed the protocol, this time also making sure that the filter papers impregnated with silver nitrate were completely dry before I used them. In addition, I no longer divided the experiment into two days but carried it out in one day: in the morning I impregnated the filter papers with silver nitrate and then prepared the soil digest solution. While the soils soaked and settled, the filter papers dried individually on petri dishes in the dark. In the afternoon they then were ready for absorbing the soil digest solution.

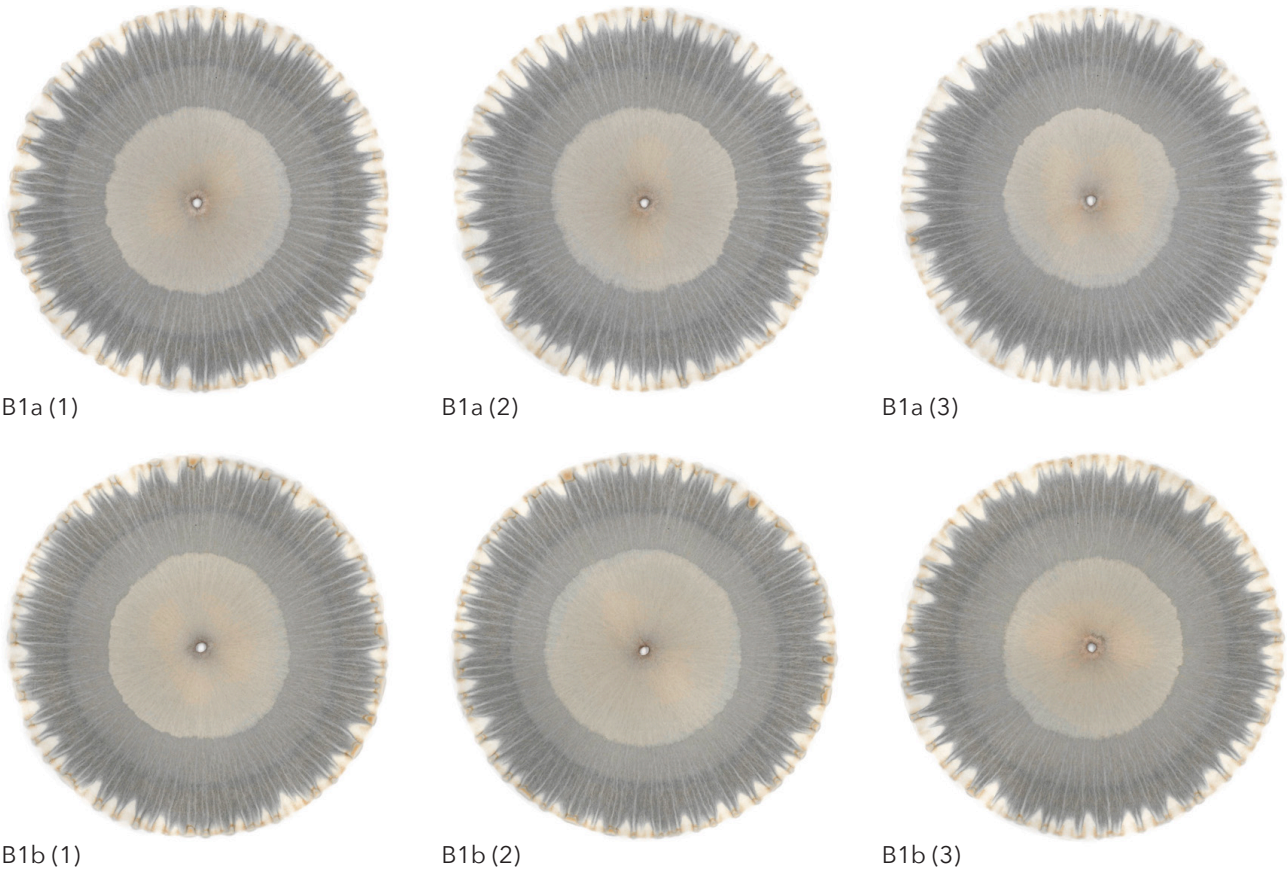
Observations

This time the filter papers dried completely. They paper was a bit more wavy than in the previous experiments, but this had no influence on the further course of the experiment. All filter papers absorbed the soil digest solution as intended up to the corresponding mark. A slight difference in the speed with which the filter papers absorbed the liquid could still be observed between soil samples with rather high or low organic matter content. All chromatograms developed very evenly this time and showed a large variance in their colouring after complete development in indirect daylight after 7 days. It was interesting to observe that the chromatograms representing soils from the same city were similar in certain characteristics. The chromatograms with soil samples from Berlin all have a slightly darker overall shade and an intensive, far-reaching middle zone. The soil samples from Munich, on the other hand, produced chromatograms with slightly lighter colours and a larger outer zone.

Conclusions

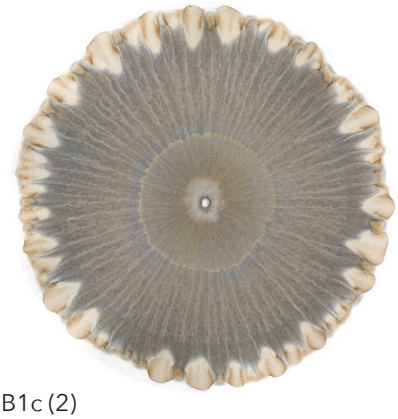
Both experiments turned out as planned with very good results. The differences in the general colouring and expression of the chromatograms of the two cities could be explained by the fact that

the soil type varies considerably between Berlin (relatively sandy) and Munich (relatively loamy with gravel). The chromatograms within a city may therefore share a certain similarity because they belong to a similar soil type. Another important aspect that I noticed in both experiments was that it is important to decant the soil digest solution carefully so that no undissolved organic particles enter the petri dish. Therefore, it would be sensible to use a pipette for the experiment, so that only the surface part of the liquid is collected without any sediments or organic matter particles. In addition, working with latex gloves turned out to be useful, as this prevents fingerprints on the chromatograms and makes it easier to grip them.



The chromatograms from the Berlin soil samples in triplicate. *Photos & Chromatograms: Opitz, 2023.*

B1c (1)



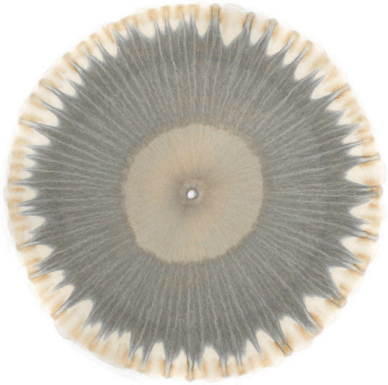
B1c (2)

B1c (3)

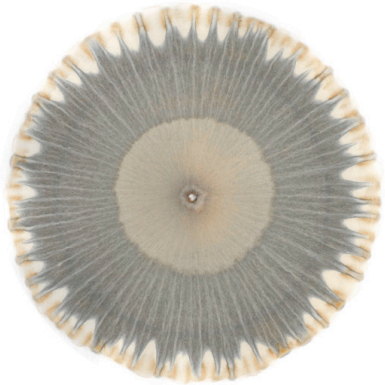
B2 (1)

B2 (2)

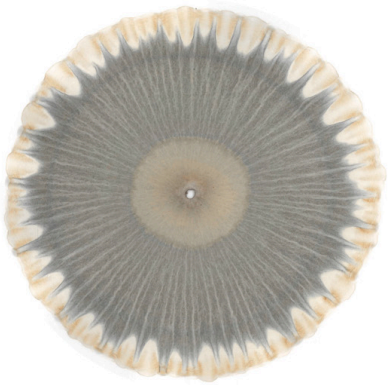
B2 (3)



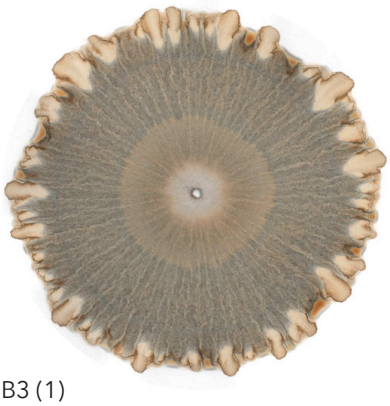
B3 (1)



B3 (2)



B3 (3)



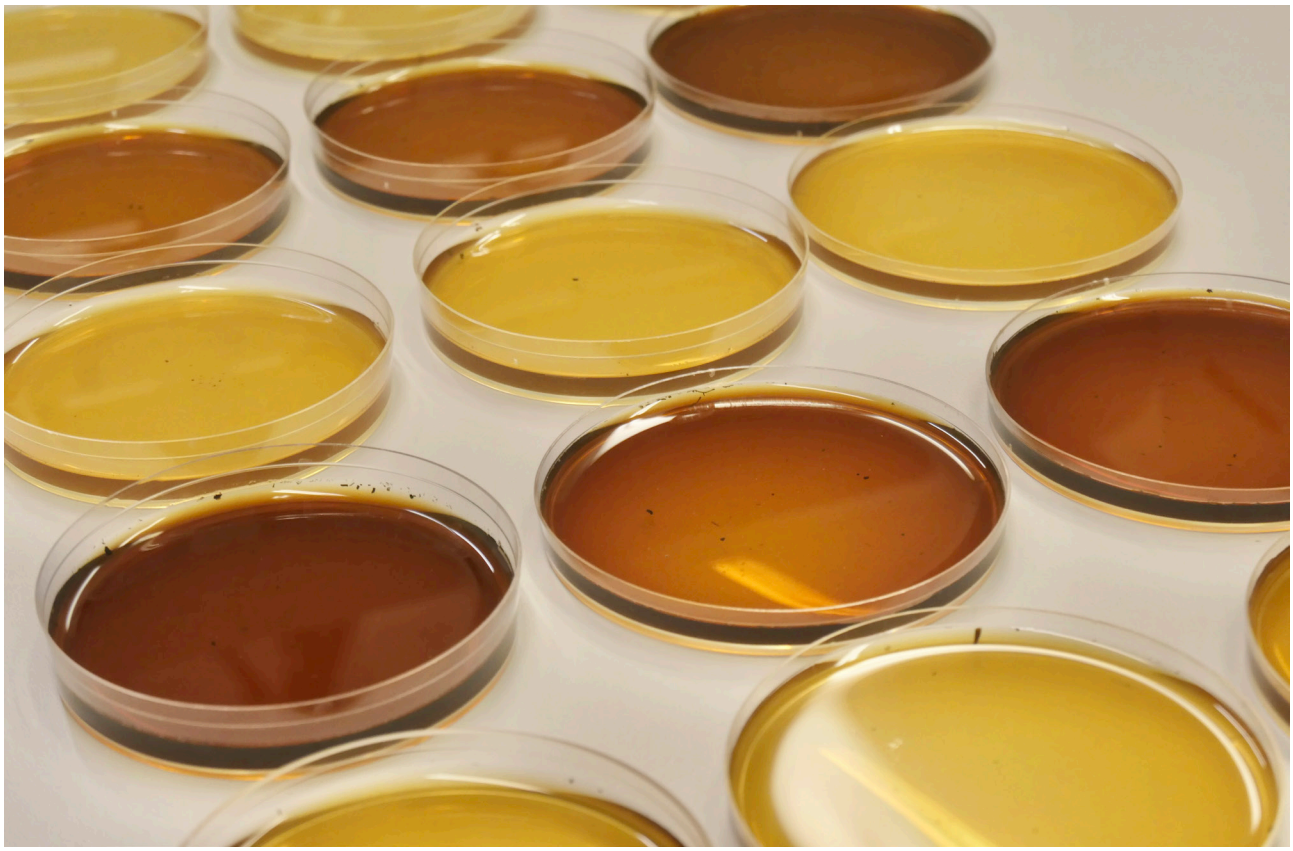
B3 (1)

B3 (2)

B3 (3)

Drying the soil samples from Munich in indirect light at room temperature. *Photo: Opitz, 2023.*

Weighing 5 grams of the soil before mixing it with the sodium hydroxide solution. *Photo: Opitz, 2023.*



The soil digest solutions resulting from the Munich soil samples. *Photo: Opitz, 2023.*

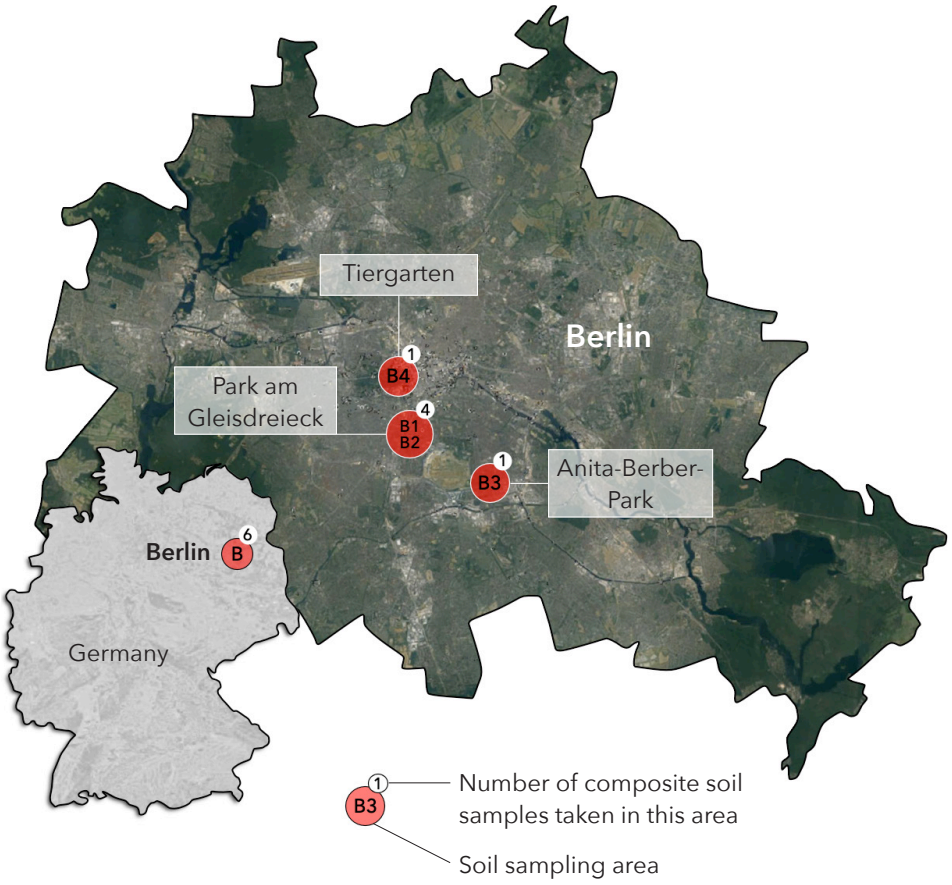
The chromatograms reflecting the Munich soil samples just after the experiment. *Photo: Opitz, 2023.*

Soil sampling in Berlin and Munich.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

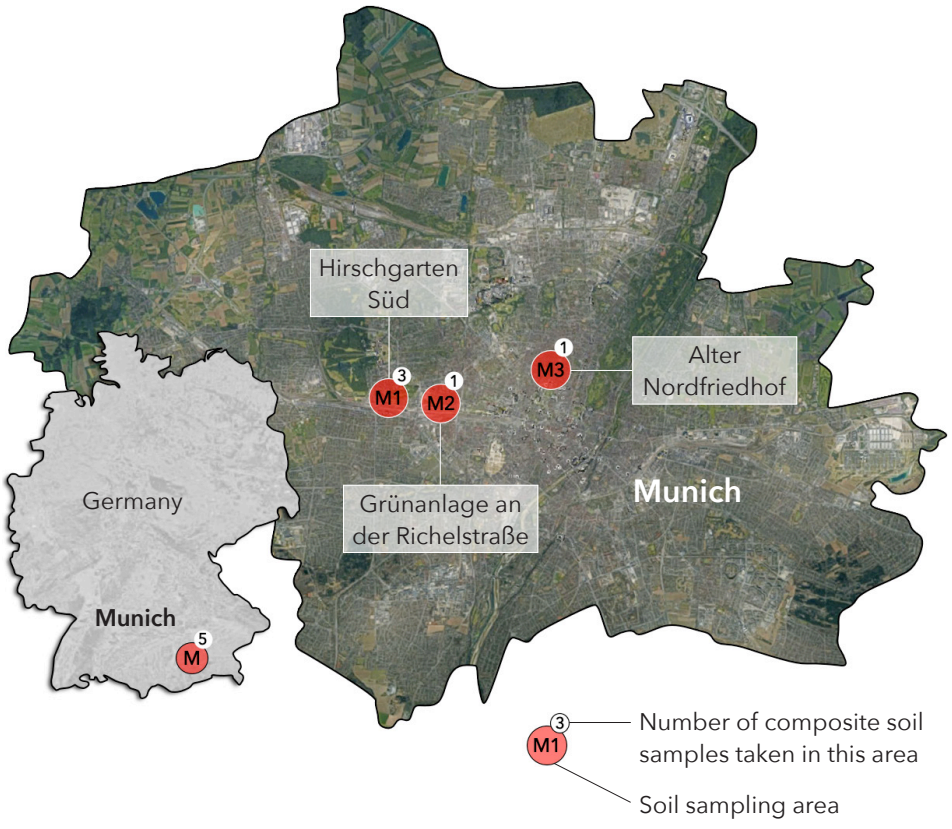
3.2 Catalogue of soil sampling locations and chromatogram interpretation

Following the first part of this chapter, in which I documented the PCC experiments and what I learned from them, I will now describe the different places in Berlin and Munich from which I took the soil samples. Subsequently, I interpret the chromatograms from the respective soil samples. I took a total of 11 composite soil samples from locations with different use and vegetation complexity. In Berlin, I took 6 composite samples, two of them from "fourth nature" areas, two from areas with meadow characteristics and one sample each from a lawn and an urban forest. In Munich, I took 5 composite samples, two of them from "fourth nature" areas, two from areas with urban forest characteristics and one sample from a lawn. For the soil sampling and interpretation I followed the instructions and schemes that I discribed in chapter 2.
In the following, I will always introduce the respective soil sampling site in general and then go into more detailed characteristics such as types of land use, vegetation and visually perceived soil properties. After that I show the results of the PCC work with the respective soil in a photo collage and interpret the chromatograms. The focus is on organic matter and with that on microbial activity in the soil.



Overview of the soil sampling areas in Berlin.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.



Overview of the soil sampling areas in Munich.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Top view of the three soil sampling locations at area B1.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Park am Gleisdreieck/ Ostpark - Area B1

General notes

The Park am Gleisdreieck was built on the former site of the Anhalter and Potsdamer freight station in Berlin and was completed in 2014. The park design incorporates the old relics of railway use as well as the ruderal vegetation that has reclaimed the site since the end of it's industrial use in the 1980s. All the "fourth nature" areas of the park may not be entered in order to allow for an undisturbed development of vegetation and wildlife. For my investigations, I chose an area in the east (B1) with 3 sampling locations as well as another sampling location (B2) in the south of the park, which I will describe in more detail later.

Area B1 has the characteristic of combining very different vegetation types in a relatively small area: B1a is a lawn, B1b a meadow and B1c a "fourth nature" forest strip with original successional vegetation. In each of these three locations with very different vegetation complexity I took one composite soil sample consisting out of 5 individual soil samples from random spots.

Perspective of the three soil sampling locations at area B1.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

View over the lawn area with the adjacent extensive meadow, demarcated by wooden poles (1). *Photo: Opitz, 2023.*

A foot and cycle path separates the railway wilderness (left) from the meadow area (right) (2). *Photo: Opitz, 2023.*

Top view of the soil sampling location B1a.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B1a - Lawn

Location and types of use

This lawn is located at a central point in the park with relatively high usage pressure. It is allowed to enter the lawn and people use it with their dogs and as a place to sunbath or play.

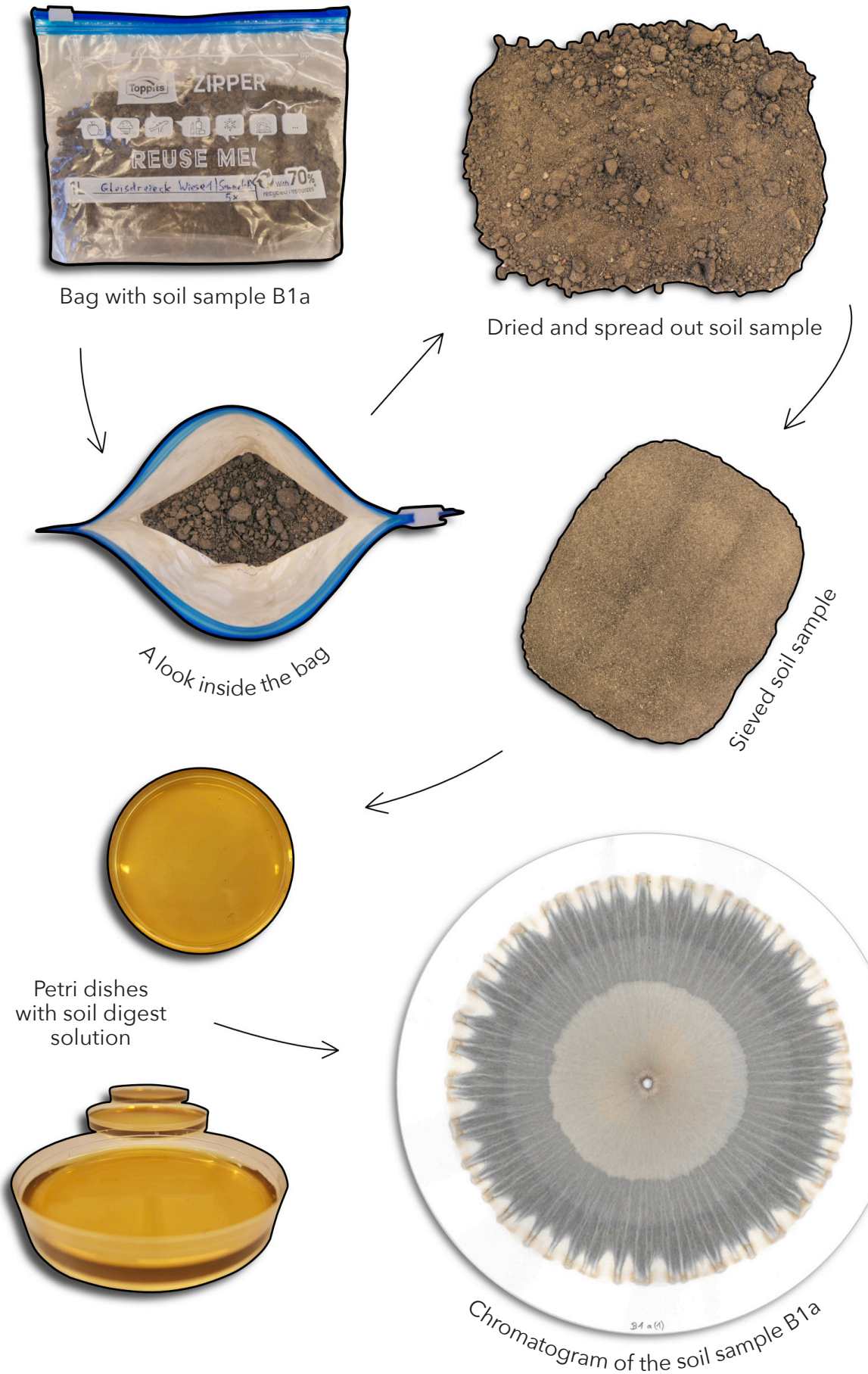
Vegetation

The area is characterized by medium dense lawn that is overgrown by several trees such as birch, robinia and pine. Aerial photos from Google Earth show that the lawn was newly established before the opening of this part of the park in 2011. The trees are much older and belong to the original successional vegetation that developed on the site after its use as a fright station stopped in the 1980s.

Soil

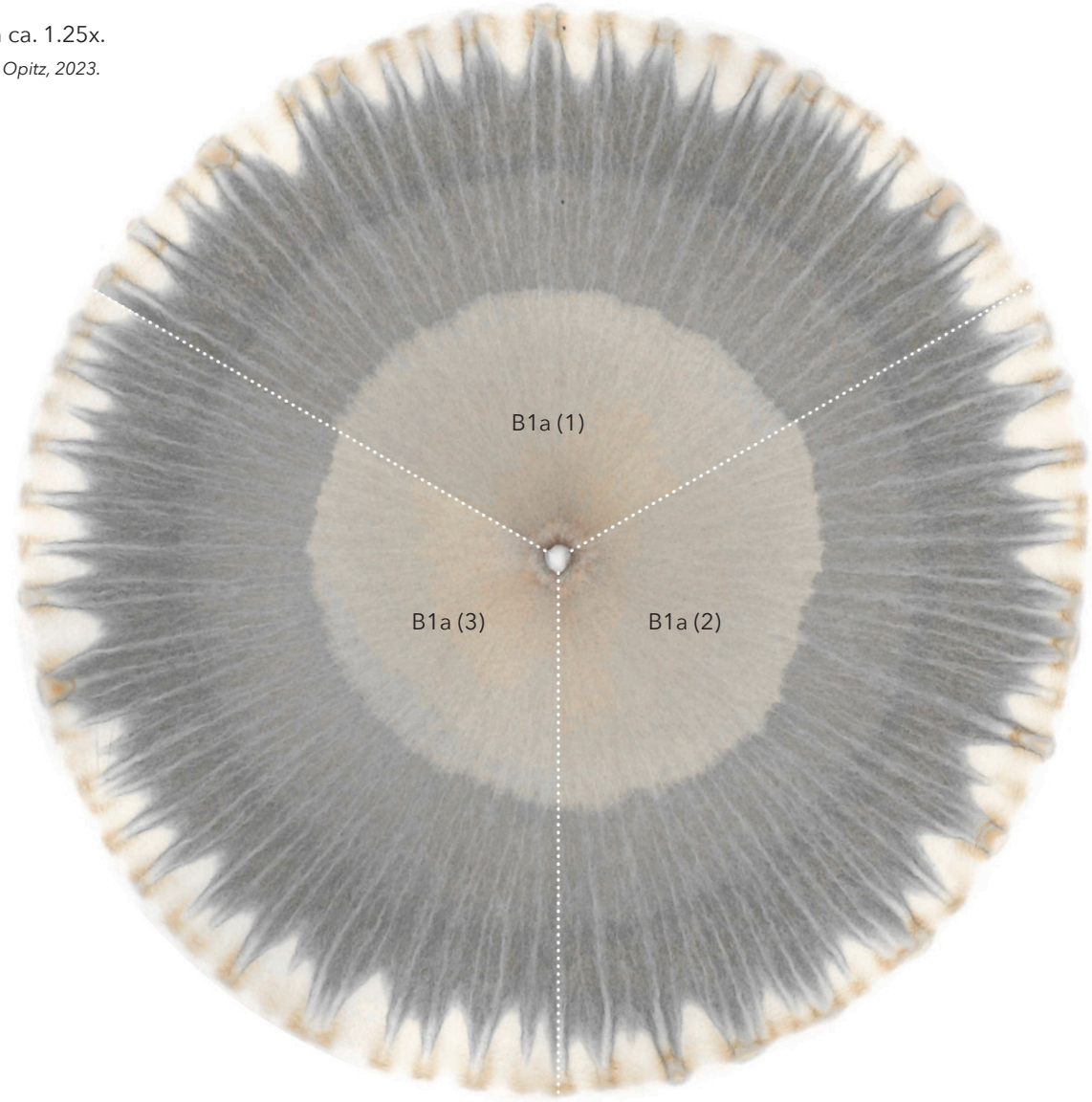
Sandy-loamy soil without any large stones. Relatively compact soil with rather weak growth of roots. The soil has a dark brown colouring with no recognizable zones. The soil was easy to penetrate with the hand spade.

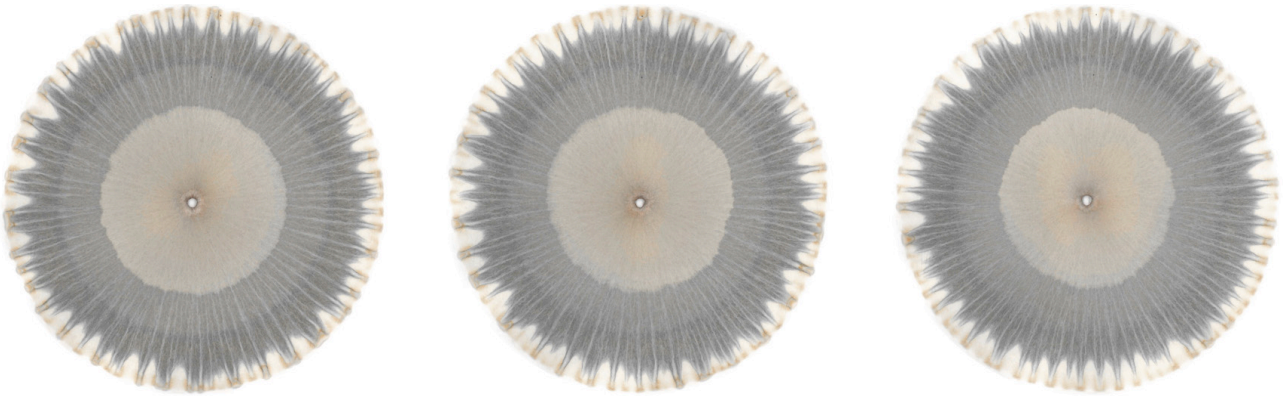
Taking a soil sample from the lawn.
Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B1a.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.





The three chromatograms B1a (1), B1a (2) and B1a (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B1a - Lawn

General visual interpretation

The B1a chromatograms are characterised by a low colour intensity with colours ranging from pale cream, cream-orange, light orange-brown to grey tones.

- Indicates a low soil organic matter content with low microbial activity.

Noticeable is the strong contrast in colours between central and middle zone as well as inside the middle zone. The visible channels in the middle zone end at the border of the central zone.

- Indicates possible exess of soluble minerals and a high mineral soil content and imbalance of soil components.

The middle zone is of grey colouration and the outer zone has a pale cream to cream-orange colour. The "clouds" around the ends of the spikes are visible but rather small.

- Indicates low soil organic matter content with low microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A wide total radius, a wide central zone radius, a narrow outer zone breadth, weak expression of channels and low to medium low colour intensity indicate a low soil organic matter content. The high spike number on the other hand points to a high organic matter content. However, as the other characteristics are very pronounced, they overrule.

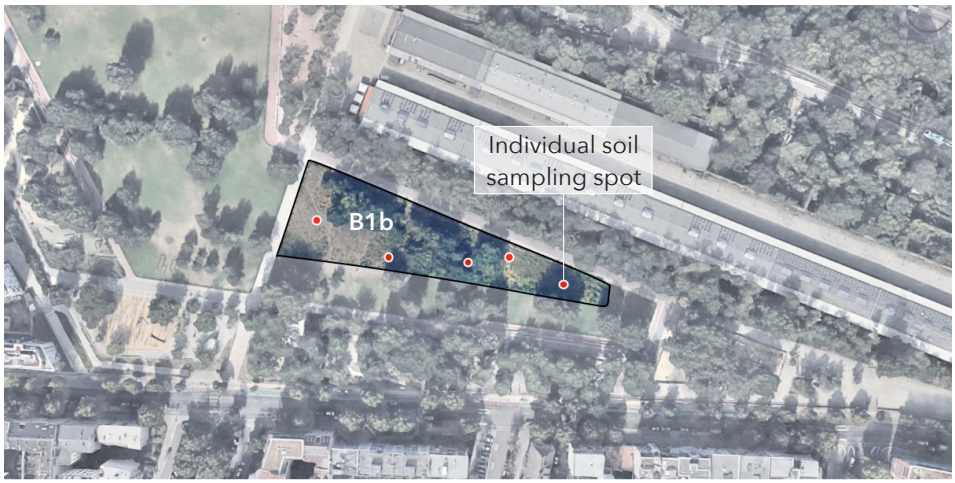
Conclusion

The chromatogram features point to a soil with a low content of organic matter. This indicates a **low microbial activity** in this lawn soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ● ●	● ● ●	●	●	●	● ● ●	●	Pale cream, cream-orange, light orange-brown grey tones	●	● ● ●
60,6 mm	27,8 mm	24,1 mm	8,7 mm	3 pts	64	1:1 - 1:2		2 pts	2
○ ○ ○	○ ○ ○	○ ○	○	○	○ ○ ○	○		○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B1a chromatograms.



Top view of the soil sampling location B1b.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B1b - Meadow

Location and types of use

This meadow is adjacent to the lawn B1a and is demarcated as a biotope by signs and wooden stakes. It is not allowed to walk on it to protect the plants and animals. Nevertheless, there are some footpaths on the meadow.

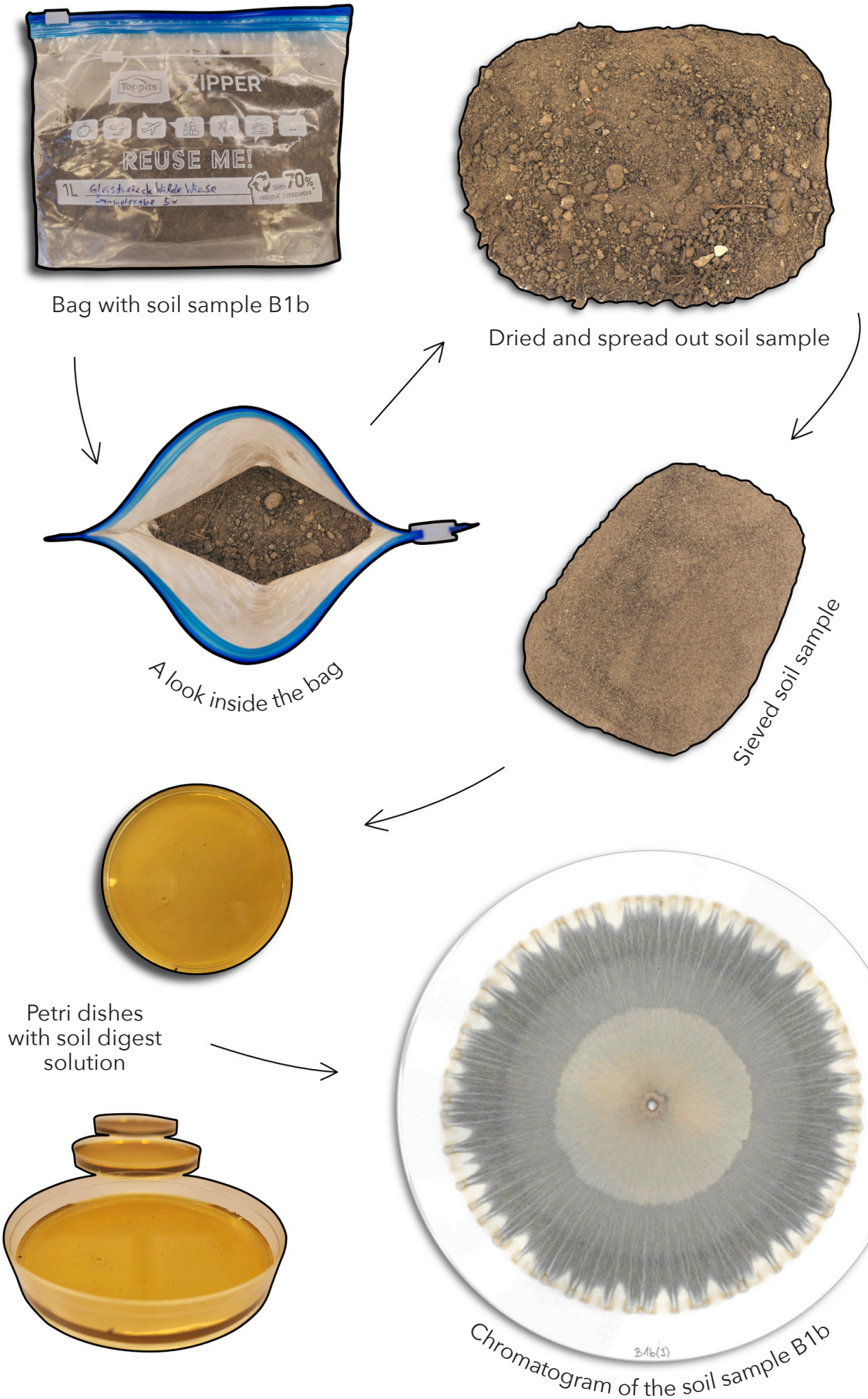
Vegetation

The area is characterised by a species-rich meadow that is maintained extensively. Several trees such as birch, robinia, pine, and partly scrubs overgrow the area. Aerial photos in Google Earth show that the meadow was newl established before the opening of this part of the park in 2011. The trees are much older and belong to the original successional vegetation that developed on the site after its use as a fright station stopped in the 1980s.

Soil

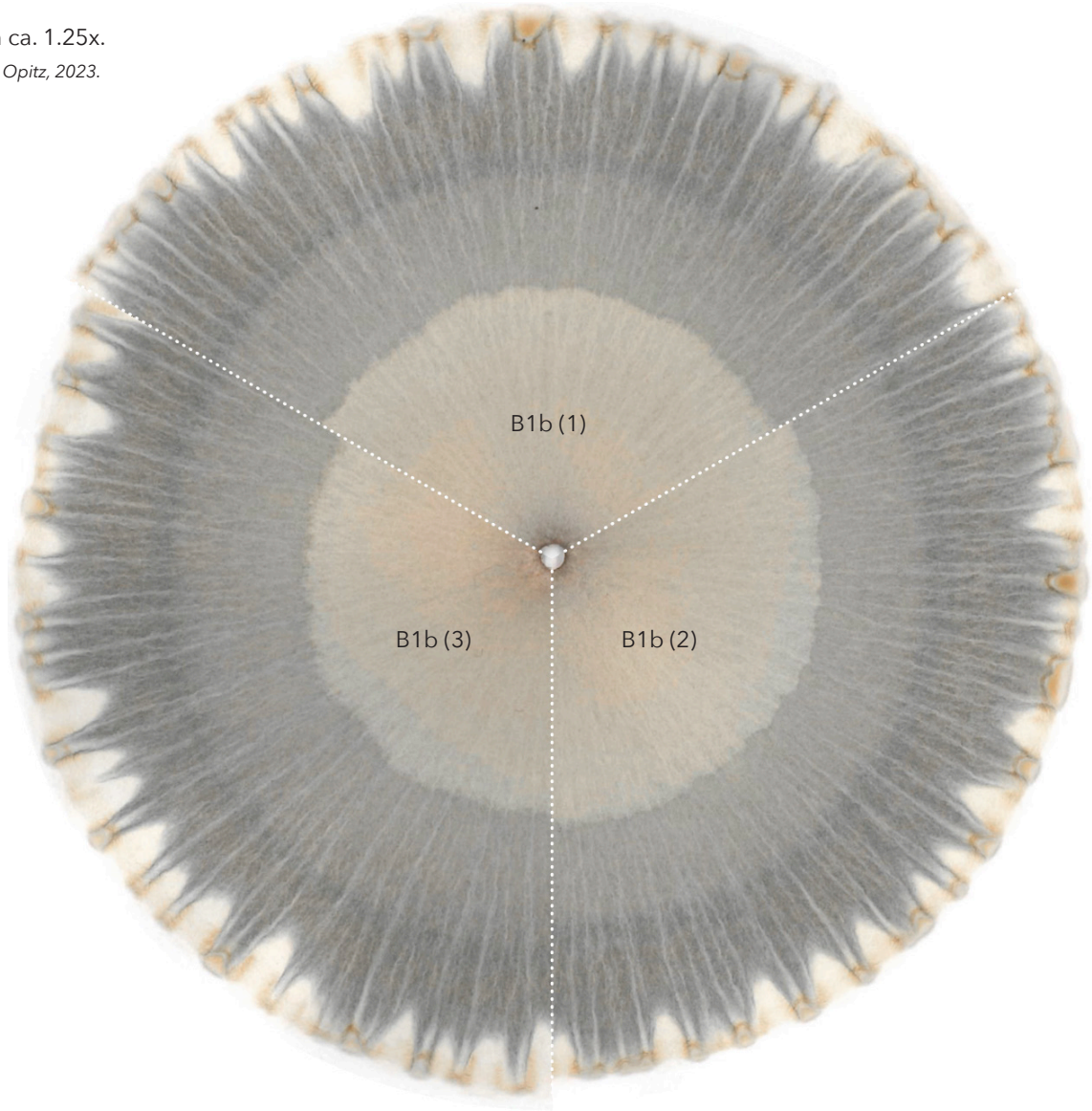
Sandy to sandy-loamy soil without any larger stones. Very loose soil with the growth of a lot of fine roots. The soil has a light to dark brown colouring with no recognizable zones. The soil was very easy to penetrate with the hand spade.

Taking a soil sample from the meadow.
Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B1b.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.



The three chromatograms B1b (1), B1b (2) and B1b (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B1b - Meadow

General visual interpretation

The B1b chromatograms are very similar to the chromatograms from sample B1a. The colour intensity is slightly higher (low to medium low) with colours ranging from pale cream, cream-orange, light orange-brown to grey tones.

- Indicates low to medium low soil organic matter content with low to medium low microbial activity.

Noticeable is the strong contrast in colours between central and middle zone as well as inside the middle zone. The visible channels in the middle zone end at the border of the central zone.

- Indicates possible exess of soluble minerals, a high mineral soil content and imbalance of soil components.

The middle zone is of grey colouration and the outer zone has a pale cream to cream-orange colour. The "clouds" around the ends of the spikes are visible but rather small. They are slightly stronger and darker then in sample B1a.

- Indicates low to medium low soil organic matter content with low to medium low microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A wide total radius, a wide central zone radius, a narrow outer zone breadth, weak expression of channels and low to medium low colour intensity indicate a low soil organic matter content. The high spike number on the other hand points to a high organic matter content. However, as the other characteristics are very pronounced, they overrule.

Conclusion

The chromatogram features point to a soil with a low to medium low content of organic matter. This indicates a **low to medium low microbial activity** in this meadow soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ● ●	● ● ●	●	●	●	● ● ●	● ●	Pale cream, cream-orange, light orange-brown grey tones	●	● ● ●
61,2 mm	28,9 mm	25,0 mm	7,2 mm	3 pts	64	1:1 - 1:3		2 pts	2
○ ○ ○	○ ○ ○	○ ○	○	○	○ ○ ○	○ ○ ○		○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B1b chromatograms.

Top view of the soil sampling location B1c.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B1c - "Fourth nature"

Location and types of use

The narrow strip of ruderal forest is located opposite the meadow B1b on the other side of a foot- and cyclepath. There are signs indicating that the area which is referred to as "railway wilderness" is not secured and therefore may not be entered.

Vegetation

The area is characterised by dense trees and undergrowth with bushes. The tree cover is dominated by robinia and birch. Aerial photos in Google Earth show that the whole vegetation belongs to the original successional vegetation that developed after its use as a fright station stopped in the 1980s.

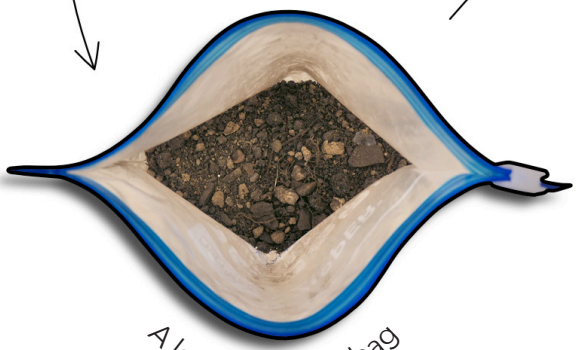
Soil

Sandy-loamy soil with a lot of stones in it (railway ballast). The soil is densely rooted. The soil has a dark to black-brown colouring with no recognizable zones. The soil was hard to penetrate with the hand spade because of the stones and roots.

Taking a soil sample from the "fourth nature" area.
Photo: Opitz, 2023.

Bag with soil sample B1c

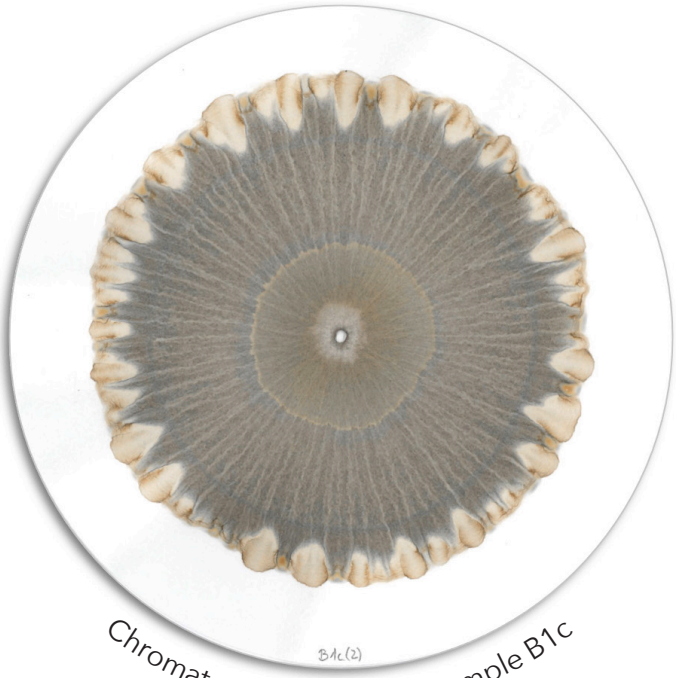
Dried and spread out soil sample



A look inside the bag

Sieved soil sample

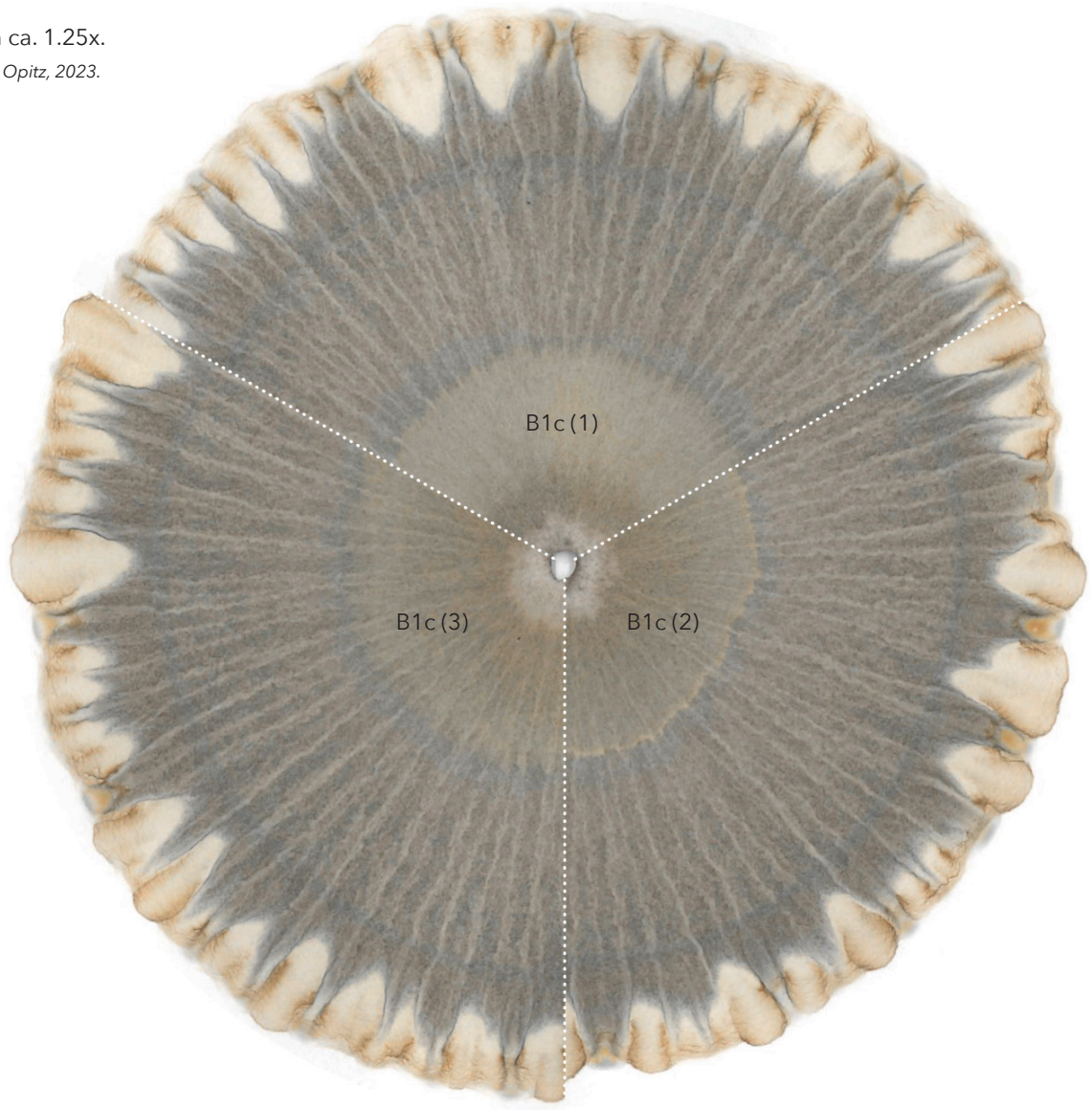
Petri dishes with soil digest solution



Chromatogram of the soil sample B1c

Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B1c.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.



The three chromatograms B1c (1), B1c (2) and B1c (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B1c - "Fourth nature"

General visual interpretation

The B1c chromatograms are characterised by a medium colour intensity with colours ranging from cream, brown-orange to grey-brown tones.

- Indicates a medium soil organic matter content with medium microbial activity.

Noticeable is the only slight contrast in colours between central and middle zone. The visible channels extend only weekly into the central zone and lose intensity. The central zone shows ring-shaped weak discolourations.

- Indicates a medium mineral soil content with only slight imbalances of soil components.

The middle zone is brown and the outer zone has a cream to brown-orange colouration. The "clouds" around the ends of the spikes have a brown-orange hue with medium to high colour intensity. They extend into a outer zone edge that is bulged.

- Indicates a medium to high soil organic matter content with medium to high microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A small total radius, a small central zone radius, a medium outer zone breadth, medium expression of channels and medium colour intensity indicate a medium soil organic matter content. The low spike number on the other hand points to a low organic matter content. However, as the other characteristics are very pronounced, they overrule.

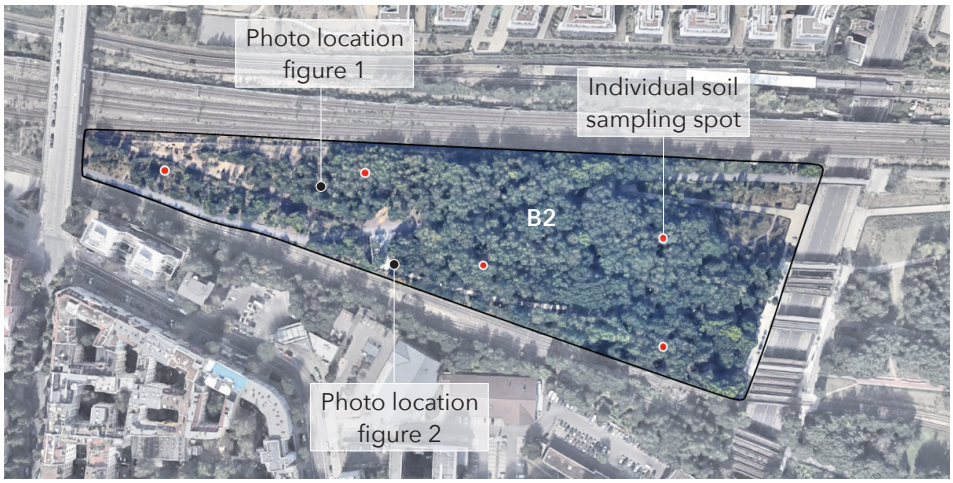
Conclusion

The chromatogram features point to a soil with a medium content of organic matter. This indicates a **medium microbial activity** in this "fourth nature" soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
●	●	● ●	● ●	● ●	●	● ●		● ●	● ● ●
57,7 mm	20,7 mm	26,2 mm	10,8 mm	4 pts	46	1:1 - 1:4	Cream, brown-orange, grey-brown tones	3 pts	2
○ ○ ○	○	○ ○ ○	○	○ ○	○	○ ○		○ ○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B1c chromatograms.



Top view of the soil sampling location B2

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Park am Gleisdreieck/ Flaschenhalspark - Soil sampling location B2

General notes

The Flaschenhalspark has its name because of its form that is shaped like a bottelneck. It is the most southern extension of the Park am Gleisdreieck and is connected to the other parts of the park via bridges. It was opened in 2014 as the last of three building phases. The bridges and paths make the area accessible and follow the original course of the railway tracks.

The Flaschenhalspark is characterised by its successional vegetation that covers the whole area. It developed after the former freight station was discontinued in the 1980s.

Compared to sampling location B1c, this sampling location is significantly larger so that the vegetation is presumably much more undisturbed. Five soil samples were taken at random spots and combined to form a composite sample.

Perspective of the soil sampling location B2.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

In the middle of the "railway wilderness" on the premises of the former Anhalter and Potsdamer freight station (1). *Photo: Opitz, 2023.*

The paths are carefully integrated into the successional vegetation (2). *Photo: Opitz, 2023.*

Top view of the soil sampling location B2.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B2 - "Fourth nature"

Location and types of use

This ruderal forest is surrounded on all sides by streets or train infrastructure. Foot- and cycle paths cross the area and are very frequently used by pedestrians and cyclists. There are signs indicating that the area which is referred to as "railway wilderness" is not secured and therefore may not be entered.

Vegetation

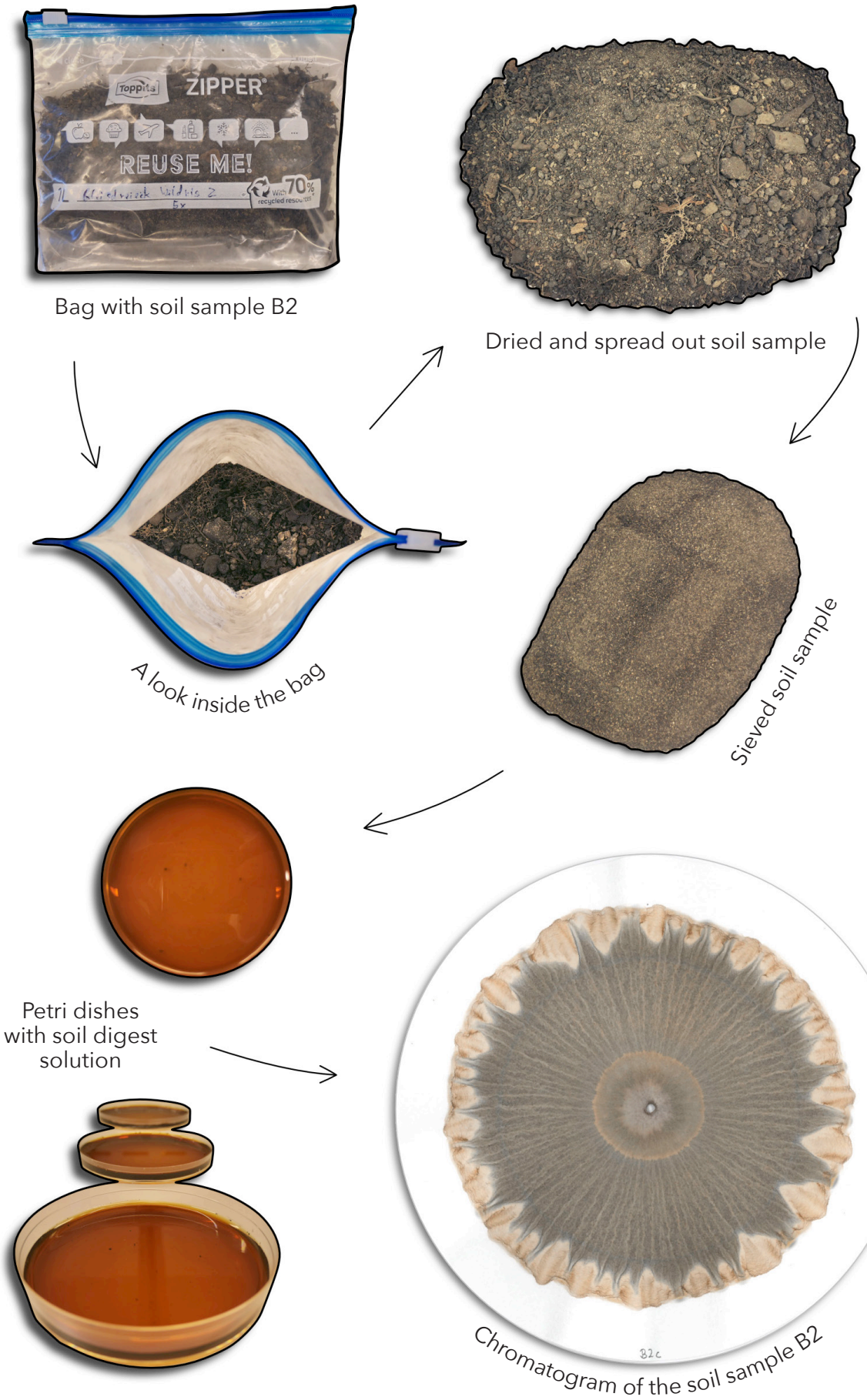
The area is characterised by dense trees and undergrowth with bushes. The tree cover is dominated by robinia, birch, pine, and oak. Aerial photos in Google Earth show that the vegetation belongs to the original successional vegetation that developed on the site after its use as a fright station stopped in the 1980s.

Soil

Sandy to sandy-loamy soil with and without stones in it (railway ballast). The soil is densely rooted. The soil has a dark to black-brown colour and shows a yellowish colouring in the lower part of the soil sample. The soil was medium hard to penetrate with the hand spade mostly because of the roots.

Taking a soil sample from the former fright station.

Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B2.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.

The three chromatograms B2 (1), B2 (2) and B2 (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B2 - "Fourth nature"

General visual interpretation

The B2 chromatograms are characterised by a medium to high colour intensity with colours ranging from cream-brown, brown-orange to brown tones.

- Indicates medium to high soil organic matter with high microbial activity.

Noticeable is the medium strong contrast in colours between central and middle zone. The very visible channels extend into the central zone but lose intensity. The central zone also shows medium to strong ring-shapeded discolourations.

- Indicates possible excess of soluble minerals, a medium mineral soil content with slight imbalances of soil components.

The middle zone is of dark brown colouration and the outer zone has a cream-brown colouring to dark orange-brown colour. The colour-intense "clouds" around the ends of the spikes extend into a outer zone edge that is slightly bulged.

- Indicates a high organic content in the soil with high microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A small total radius, a small central zone radius, a wide outer zone breadth, strong expression of channels and medium to high colour intensity indicate a high soil organic matter content. The low spike number on the other hand points to a low organic matter content. However, as the other characteristics are very pronounced, they overrule.

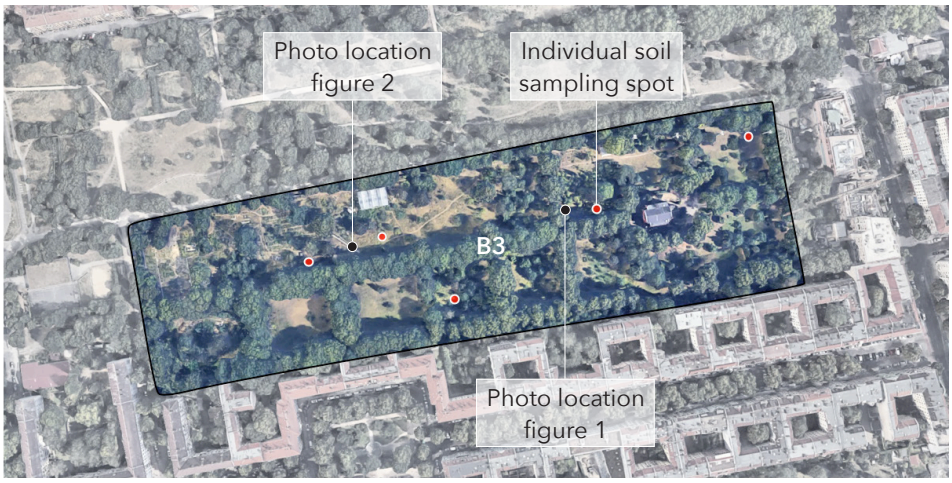
Conclusion

The chromatogram features point to a soil with a high content of organic matter. This indicates a **high microbial activity** in this "fourth nature" soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
●	●	● ● ●	● ● ●	● ● ●	●	● ● ●		● ●	● ● ●
58,1 mm	16,9 mm	29,9 mm	11,3 mm	4,5 pts	40	1:1 - 1:5	Cream-brown, brown-orange, brown tones	4 pts	2
○ ○ ○	○	○ ○ ○	○ ○	○ ○ ○	○	○ ○ ○		○ ○ ○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B2 chromatograms.



Top view of the soil sampling location B3.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

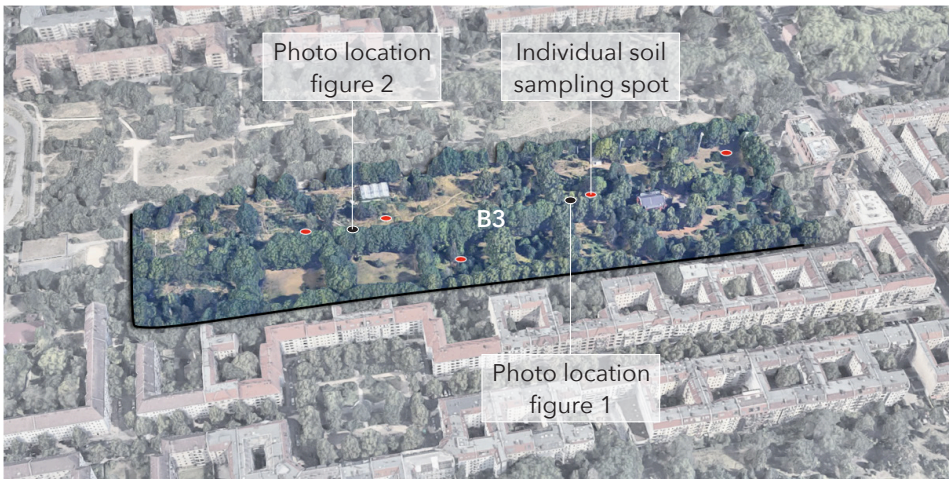
Anita-Berber-Park - Soil sampling location B3

General notes

The Anita-Berber-Park covers the former site of the eastern St.-Thomas-Kirchhof. It was part of several large cemeteries in this part of Berlin and directly borders the Tempelhofer Feld to the west. It was closed in 2012 and after a redesign opened as a park for the public. The part of the park where I took soil samples is situated aside of the main park and has hardly been changed since its former cemetery use, allowing the vegetation to develop undisturbed.

I chose this site as a sampling location because it has a very distinct own type of vegetation as a site that transitions from well-kept cemetery with typical cemetary plants to freely developing urban nature.

Five soil samples were taken at random spots and combined to form a composite sample.

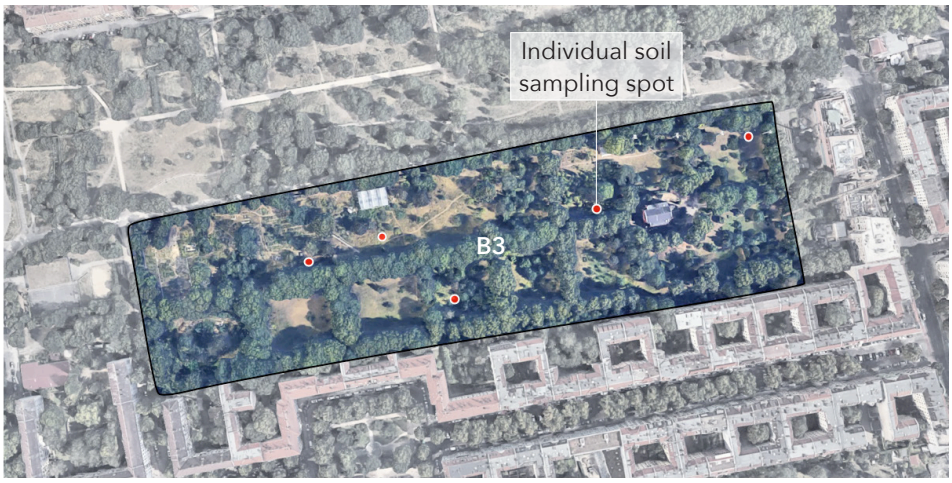


Perspective of the soil sampling location B3.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

The former cemetery with overgrown gravestones (1). *Photo: Opitz, 2023.*

This part of the Anita-Berber-Park is largely not maintained and is left to natural succession (2). *Photo: Opitz, 2023.*



Top view of soil sampling location B3.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B3 - Meadow

Location and types of use

This part of the former cemetery is open to the public, but it is separated from the rest of the park by a fence with only a few entrances. It is therefore rather quiet and mainly used by dog owners to let their dogs run free.

Vegetation

The area is covered with a mosaic of meadows, divided by rows of trees into the former cemetery fields. The relatively old tree population is characterised by deciduous and coniferous trees as well as the central linden avenue. Apart from an area that has been converted to urban gardening, the site is not maintained and the vegetation develops spontaneously, overgrowing the old graves.

Soil

Loamy soil without any larger stones. Relatively compact soil with rather weak growth of roots. The soil has a dark brown colouring with no recognizable zones. The soil was easy to penetrate with the hand spade.

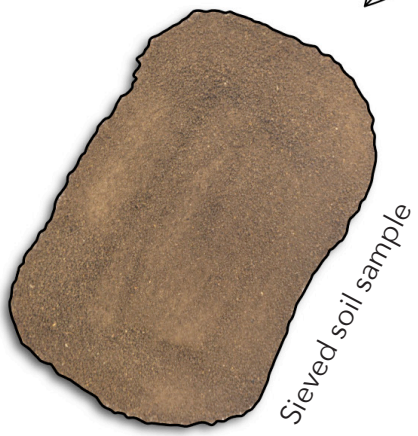
Taking a soil sample from the former cemetery.

Photo: Opitz, 2023.

Bag with soil sample B3

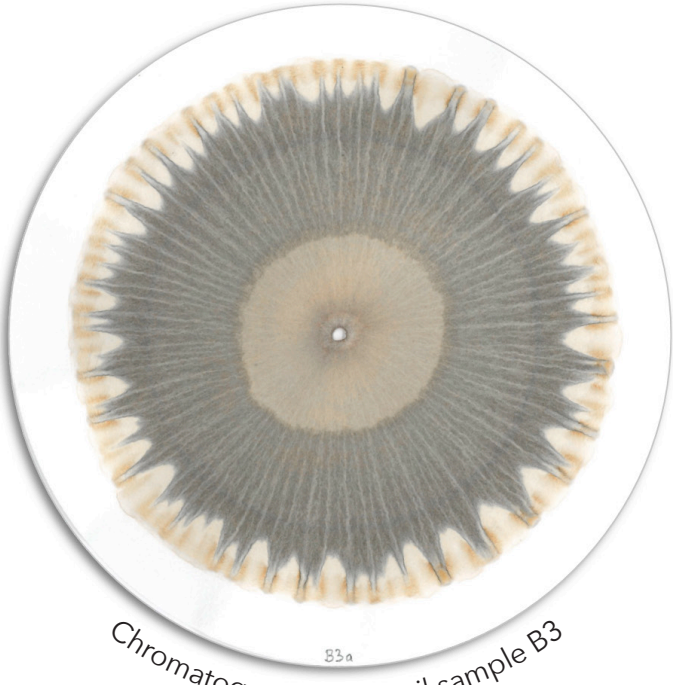
Dried and spread out soil sample

A look inside the bag



Sieved soil sample

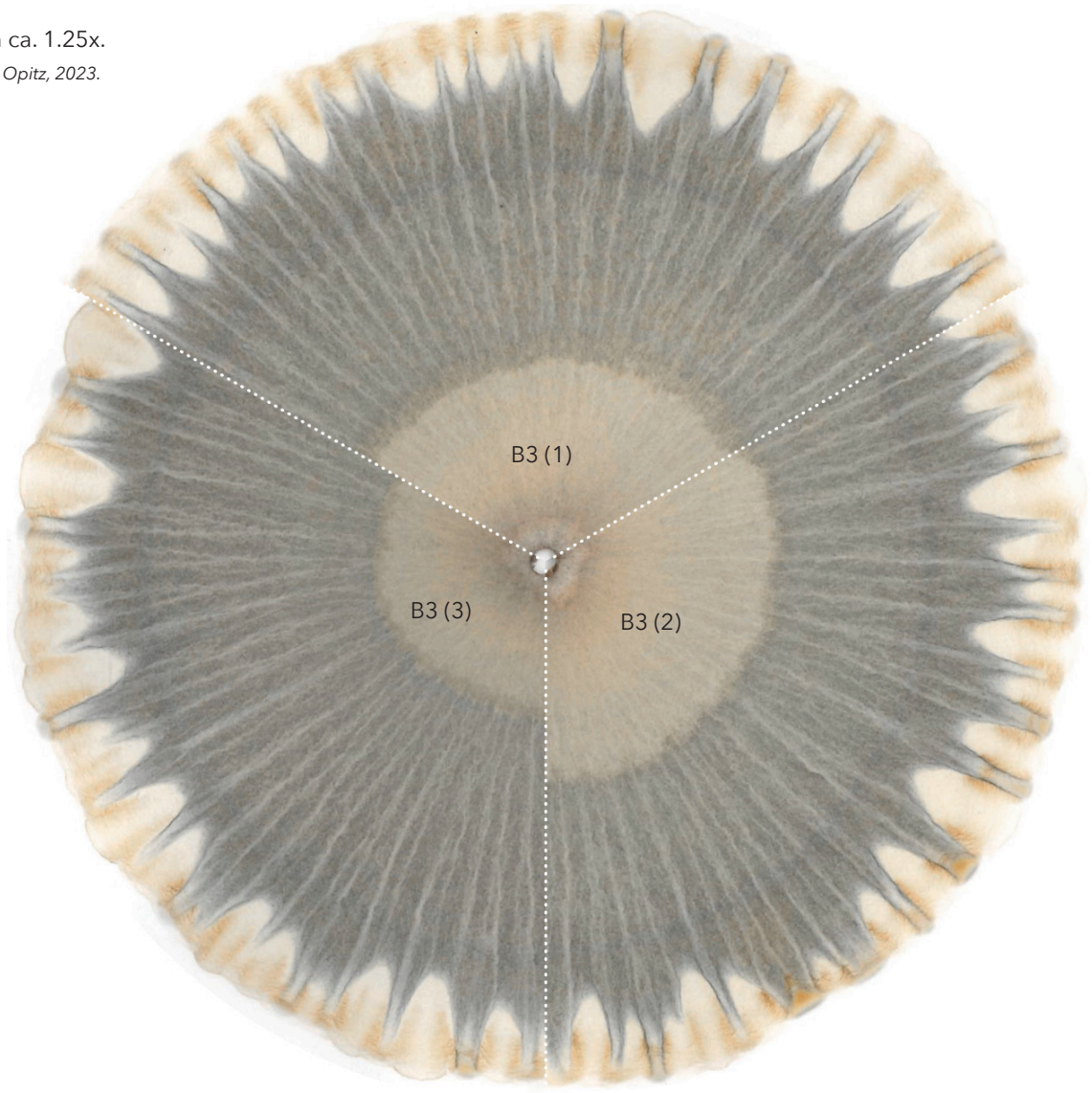
Petri dishes with soil digest solution

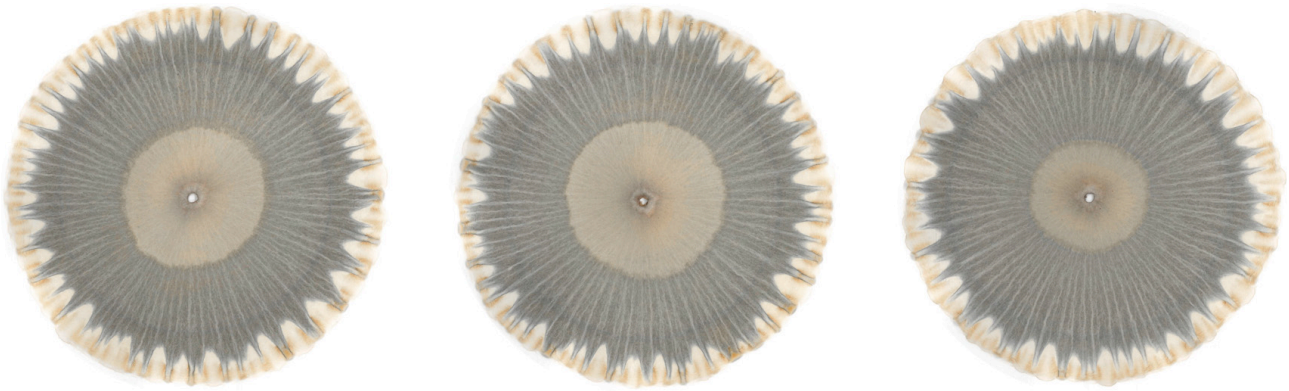


Chromatogram of the soil sample B3

Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B3.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.





The three chromatograms B3 (1), B3 (2) and B3 (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B3 - Meadow

General visual interpretation

The B3 chromatograms are characterised by a low to medium colour intensity with colours ranging from cream, cream-orange, grey-orange to grey tones.

- Indicates a low to medium soil organic matter content with low to medium microbial activity.

Noticeable is the strong contrast in colours between central and middle zone. The very visible channels extend into the central zone but lose most of their intensity.

- Indicates possible exess of soluble minerals, a medium to high mineral soil content and some inbalances of soil components.

The middle zone is of grey-brown colouration and the outer zone has a cream to cream-orange colour. The "clouds" around the ends of the spikes have a brown-orange hue with medium to high colour intensity. There is no visible outer zone edge.

- This indicates a medium soil organic matter content with medium microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation. A medium total radius, medium central zone radius, medium outer zone breadth, medium expression of channels, medium spike number and low to medium colour intensity indicate a medium soil organic matter content.

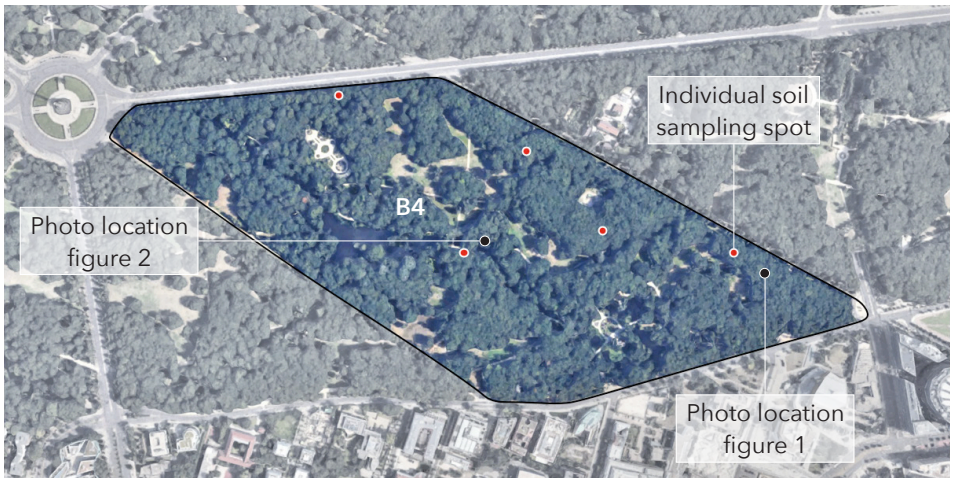
Conclusion

The chromatogram features point to a soil with a medium content of organic matter. This indicates a **medium microbial activity** in this meadow soil sample. I attribute **high confidence** to this interpretation, since 6 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ●	● ●	● ●	● ●	● ●	● ●	● ●		●	●
60,0 mm	20,8 mm	27,9 mm	11,2 mm	4 pts	54	1:1 - 1:3	Cream, cream-orange, grey-orange, grey tones	2,5 pts	1
○ ○ ○	○	○ ○ ○	○ ○	○ ○	○ ○	○ ○		○ ○	○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B3 chromatograms.

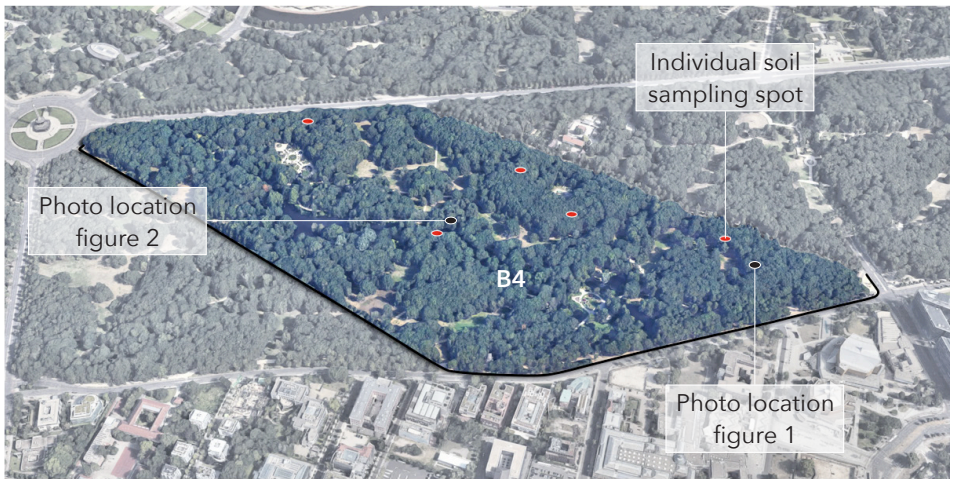


Top view of the soil sampling location B4.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Großer Tiergarten - Soil sampling location B4

The Große Tiergarten is located in the centre of Berlin and has an eventful history. Originally, the area that is now the park was a game area used for hunting. Later it was opened for public use as a baroque park and later as a landscape garden. In the course of the Second World War, the Große Tiergarten was severely damaged and used as a source of timber and as farmland in the post-war years. Later, the area was reforested and today the very extensive area is a refuge for many native forest species in the style of an English landscape garden.
I chose one area of the park for soil sampling because here, in contrast to "fourth nature" sites, a vegetation that resembles typical natural forests is promoted in the middle of the city.
Five soil samples were taken at random spots and combined to form a composite sample.



Perspective of the soil sampling location B4.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

View through the beech forest in the Großer Tiergarten (1). *Photo: Opitz, 2023.*

In front of a Rhododendron Grove in the park (2). *Photo: Opitz, 2023.*

Top view of the soil sampling location B4.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

B4 - Urban forest

Location and types of use

This soil sampling location is part of the Großer Tiergarten forest. The park is laid out in the style of a landscape garden and structured by paths that are used by people walking, jogging or walking their dogs, among others. The forest areas should not be entered in order to protect the flora and fauna.

Vegetation

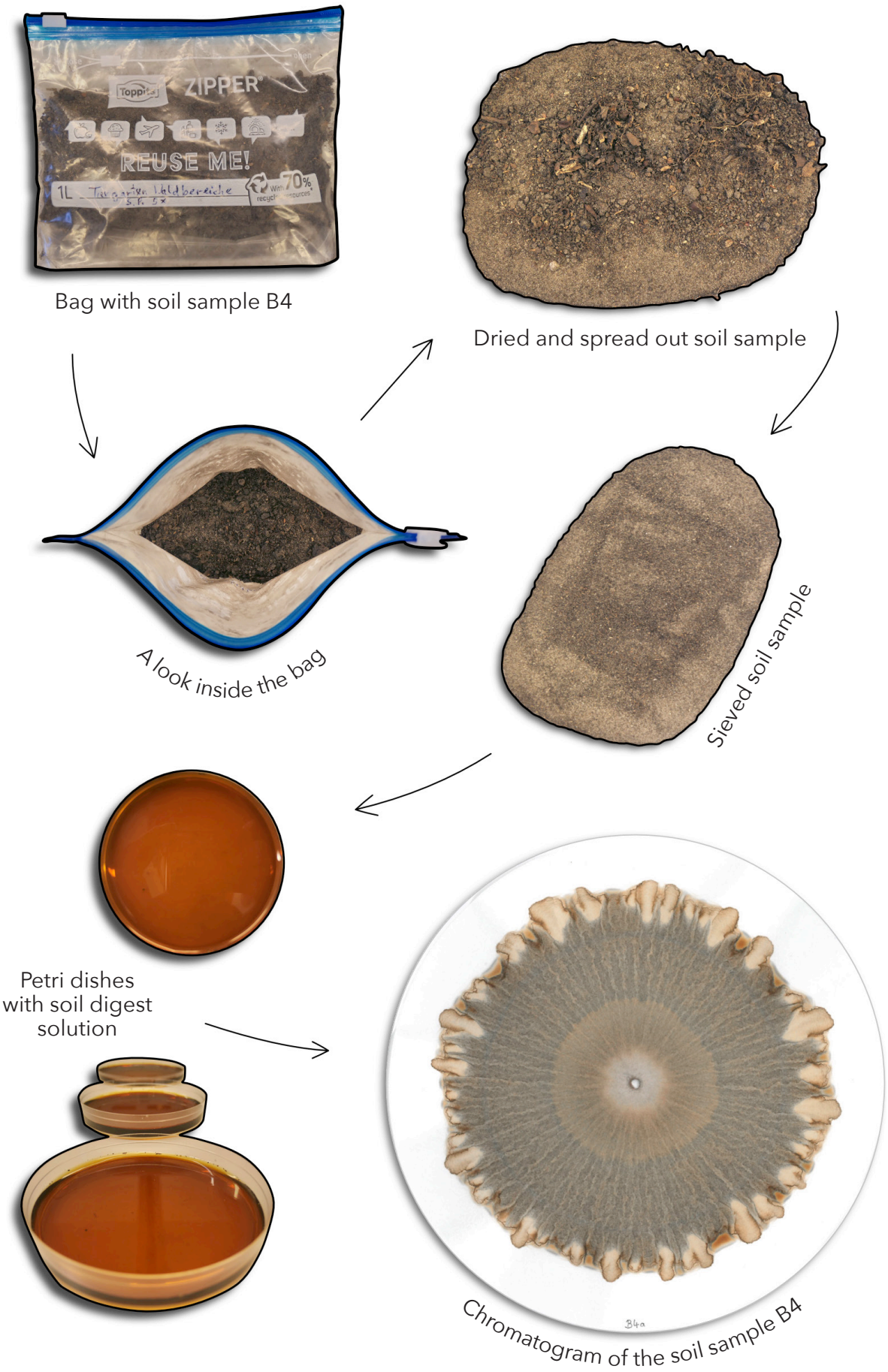
The area is characterised by tall trees with relatively little undergrowth. The tree population is dominated by typical forest trees like beech and oak. Even though the forst looks quite old, it is relatively young, as an aerial photograph from 1953 on Google Earth shows: most of the trees were not yet standing 70 years ago because of the deforestation during and after the Second World War.

Soil

Sandy to sandy-loamy soil without any stones in it. The soil is densely rooted. The soil has a dark brown colour and shows a light brown colouring in the lower part of the soil sample. The soil was medium hard to penetrate with the hand spade mostly because of the roots.

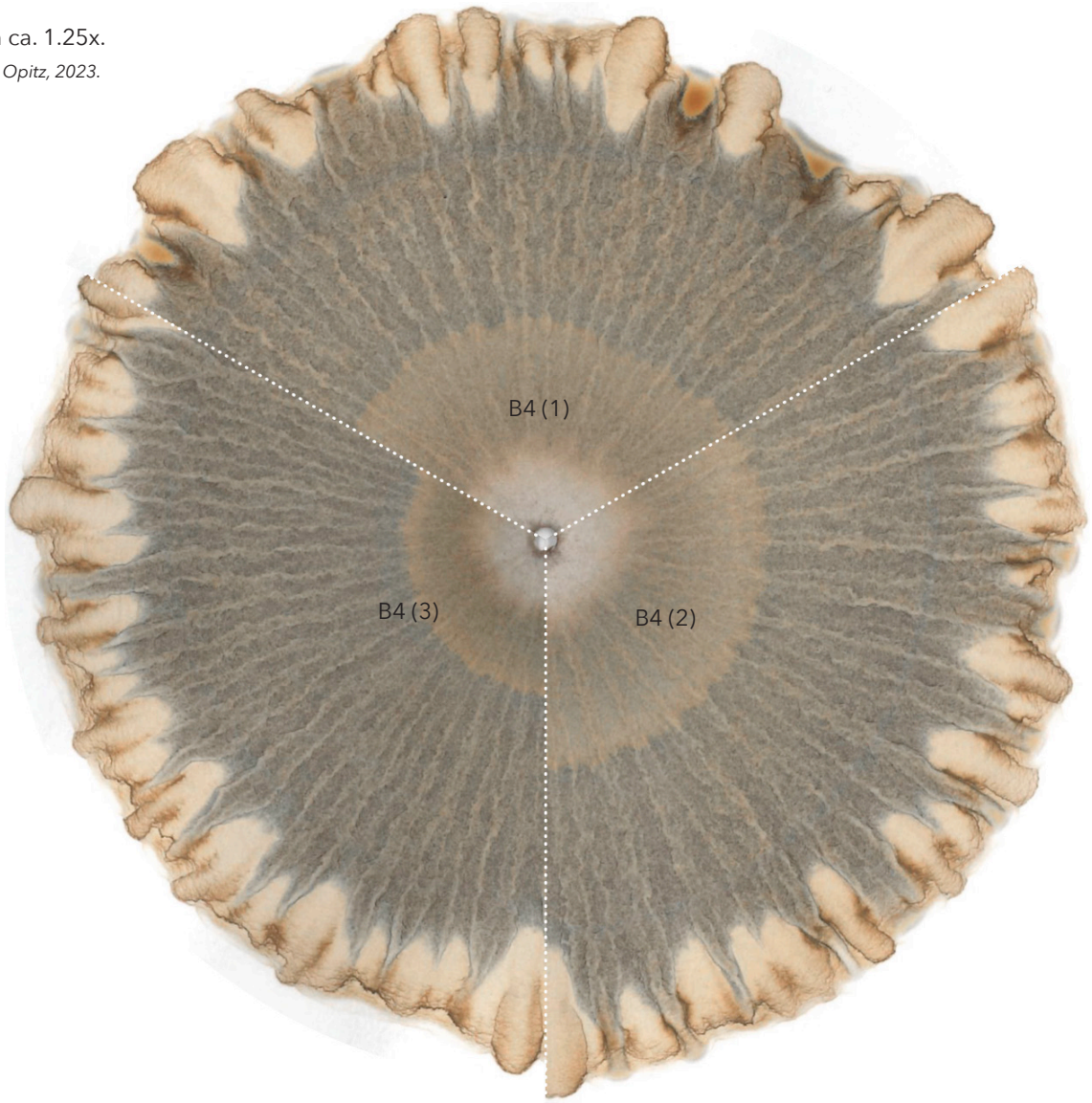
Taking a soil sample from the forest in the Tiergarten.

Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample B4.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.



The three chromatograms B4 (1), B4 (2) and B4 (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample B4 - Urban forest

General visual interpretation

The B4 chromatograms are characterised by a high colour intensity with colours ranging from cream-brown, brown-orange to brown tones.

- Indicates a high soil organic matter content with related high microbial activity.

Noticeable is the medium strong contrast in colours between central and middle zone. The very visible channels extend into the central zone but lose intensity. The central zone also shows ring-shaped discolourations.

- Indicates a normal mineral soil content with some imbalances of soil components.

The middle zone is of brown to brown-grey colouration and the outer zone has a cream-brown, brown-orange to dark-brown colour. The colour-intense "clouds" around the ends of the spikes extend into a dark outer zone edge that is strongly bulged.

- Indicates a high soil organic matter content with high microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A small total radius, a medium central zone radius, a wide outer zone breadth, medium expression of the channels and high colour intensity indicate a medium to high soil organic matter content. The low spike number on the other hand points to a low organic matter content. However, as the other characteristics are very pronounced, they overrule.

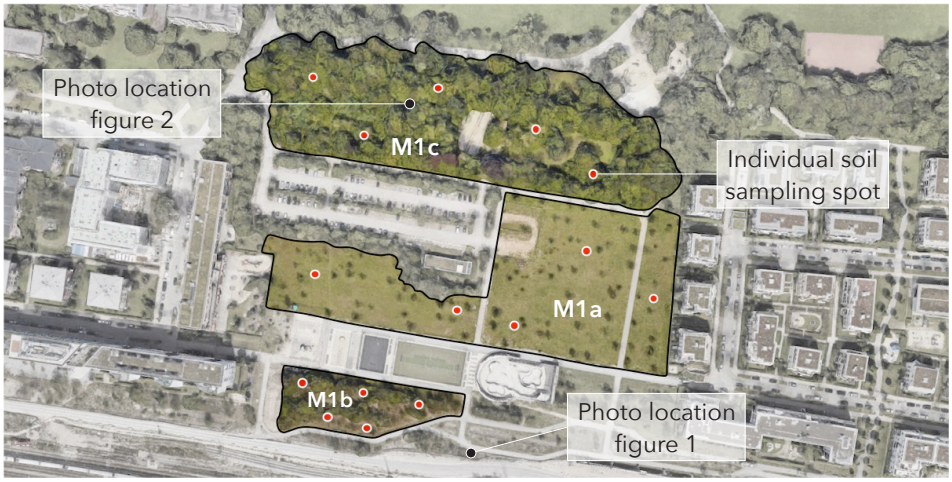
Conclusion

The chromatogram features point to a soil with a high content of organic matter. This indicates a **high microbial activity** in this forest soil sample. I attribute **medium confidence** to this interpretation, since since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
●	● ●	●	● ● ●	● ●	●	● ● ●		● ● ●	● ● ●
59,2 mm	23,2 mm	25,3 mm	13,3 mm	4 pts	43	1:1 - 1:5	Cream-brown, brown-orange, brown tones	5 pts	2
○ ○ ○	○ ○	○ ○	○ ○	○ ○	○	○ ○ ○		○ ○ ○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data average from B4 chromatograms.



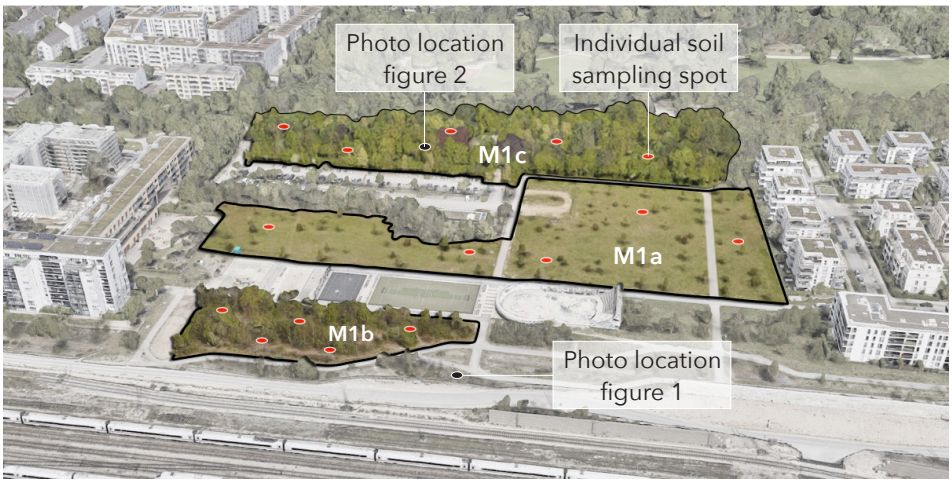
Top view of the three soil sampling locations at area M1.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Hirschgarten Süd - Soil sampling area M1

The Hirschgarten Süd in Munich is an extension of the historic Königlicher Hirschgarten towards the south. This area was formerly used as a marshalling yard by the Deutsche Bahn. Later it was converted into a park that connects to a long green corridor that links this part of the city to the east. Large residential quarters were also built alongside the green corridor. Relics of the former railway activities and parts of the succession vegetation that developed during and after the railway use were integrated into the design of the park. The extensive gravel areas with railroad ballast provide refuges for sand lizards.

This part of the park has the characteristic of combining very different vegetation types in a relatively small area. M1a is a lawn, M1b a patch of "fourth nature" vegetation and M1c an urban forest area that merges into the "Königlicher Hirschgarten". In each of these three locations with very different vegetation complexity I took one composite soil sample consisting out of 5 individual soil samples from random spots.

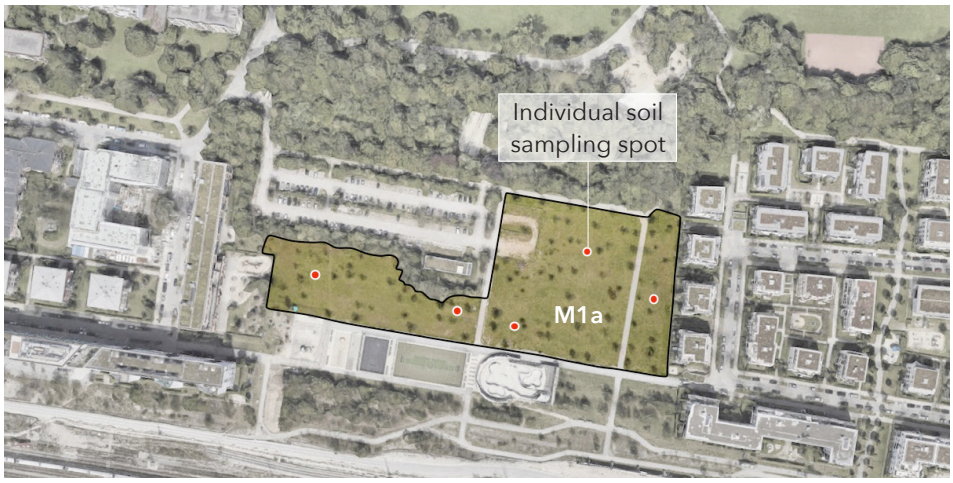


Perspective of the three soil sampling locations at area M1.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

View over the former railway site with pioneer vegetation, relicts from the former use and new established paths (1). *Photo: Opitz, 2023.*

Forest strip with old oaks between the former railway site and the Königliche Hirschgarten (2). *Photo: Opitz, 2023.*



Top view of the soil sampling locations M1a.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

M1a - Lawn

Location and types of use

The lawn is located between the historic Königlicher Hirschgarten park in the north and the railway tracks in the south. It is allowed to enter the lawn and people use it with their dogs and as a place to play. I estimate the pressure of use to be much lower compared to the lawn area in Berlin.

Vegetation

The area is characterised by medium dense to sparse lawn mixed with moss and wild herbs. It is overgrown by a variety of young orchard tree species. The lawn and the trees were newly planted in the course of the park construction. Aerial photographs from Google Earth date this back to around 2009. Before that, the area acted as a buffer zone between the historic park and the railway grounds which extended much further north than they do today.

Soil

Loamy soil mixed with gravel (Munich gravel plain). Very compact soil with rather weak growth of roots. The soil has a brown colour in the upper layer and transitions into a light brown to beige colour the more one penetrates into the gravel layer below. The soil was medium hard to penetrate with the hand spade because of the gravel.

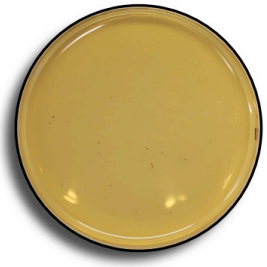
Taking a soil sample from the lawn.
Photo: Opitz, 2023.

Bag with soil sample M1a

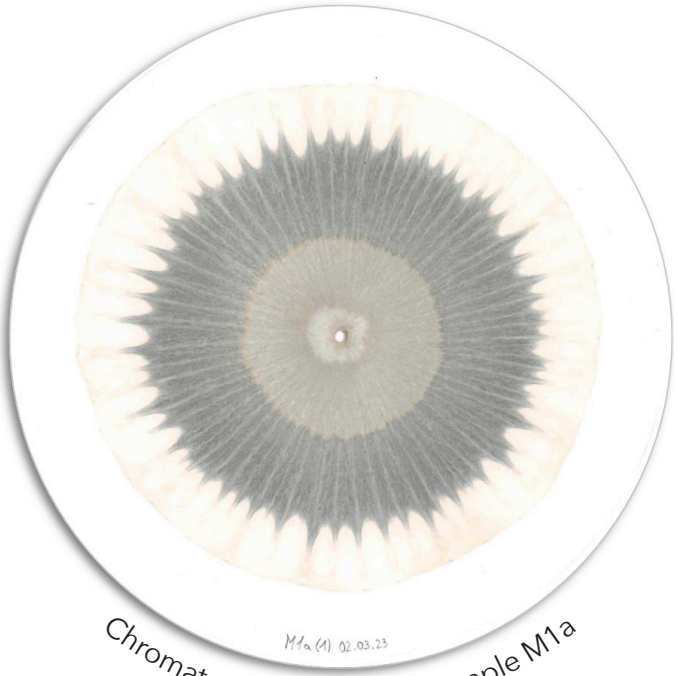
Dried and spread out soil sample

A look inside the bag

Sieved soil sample



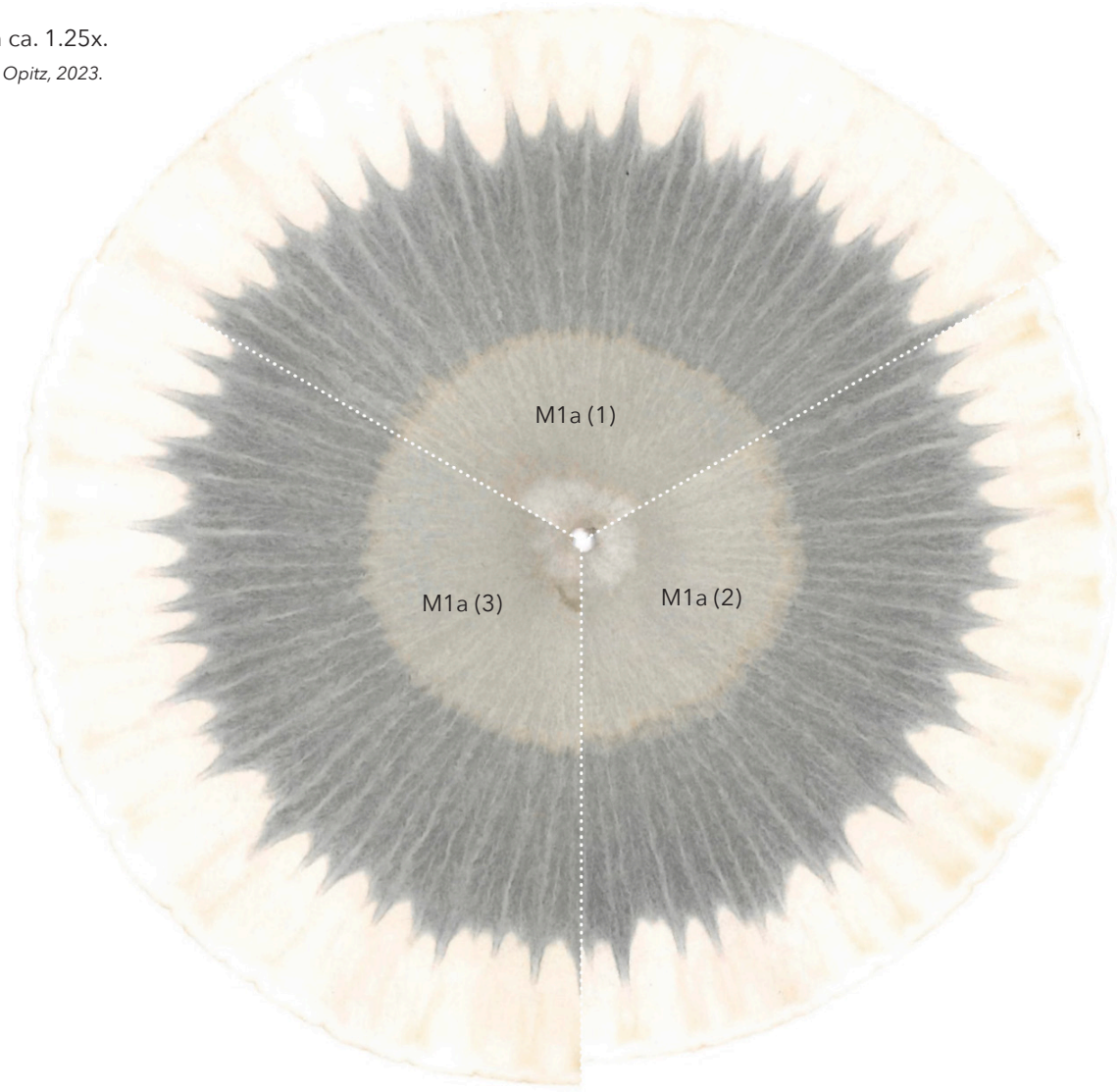
Petri dishes with soil digest solution

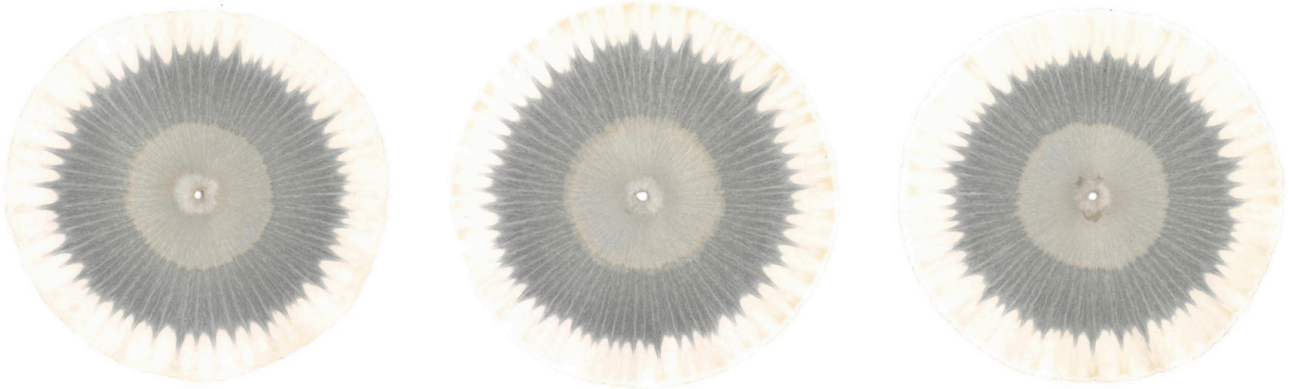


Chromatogram of the soil sample M1a

Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample M1a.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.





The three chromatograms M1a (1), M1a (2) and M1a (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample M1a - Lawn

General visual interpretation

The M1a chromatograms are characterised by a very low colour intensity with very pale cream, cream-grey and grey tones.

- Indicates a low soil organic matter content with low microbial activity.

Noticeable is the strong contrast in colours between central and middle zone. The visible channels only slightly extend into the central zone.

- Indicates possible exess of soluble minerals, a high mineral soil content and imbalance of soil components.

The middle zone is of grey colouration and the outer zone has a very pale cream colour. The "clouds" around the ends of the spikes are very faint and there is nearly no outer zone edge.

- Indicates a low soil organic matter content with related low microbial activity.

Verification through analytical interpretation

The analytical interpretation only partly confirms the general visual interpretation. A relatively large total radius, medium to weak expression of channels, and low colour intensity indicate a low to medium low soil organic matter content. The medium wide central zone radius, medium wide outer zone breadth and high spike number on the other hand point to a medium to high organic matter content. However, as the other characteristics are very pronounced, they overrule.

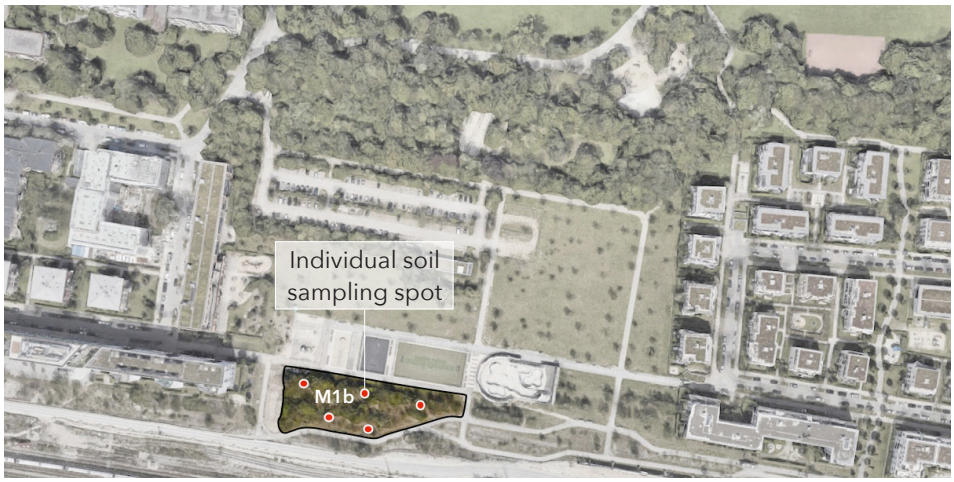
Conclusion

The chromatogram features point to a soil with a low content of organic matter. This indicates a **low microbial activity** in this lawn soil sample. I attribute **medium low confidence** to this interpretation, since 3 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ● ●	● ●	● ● ●	● ●	●	● ● ●	●		●	● ● ●
57,6 mm	21,6 mm	20,3 mm	15,7 mm	3,5 pts	65	1:1 - 1:2	Very pale cream, grey tones	1 pts	2
○ ○ ○	○ ○	○	○ ○ ○	○ ○	○ ○ ○	○		○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

This table shows the data average from M1a chromatograms.



Top view of soil sampling locations M1b.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

M1b - "Fourth nature"

Location and types of use

This small "fourth nature" patch is located between the lawn M1a and the railway tracks. It is surrounded from all sides by foot- and cycle paths.

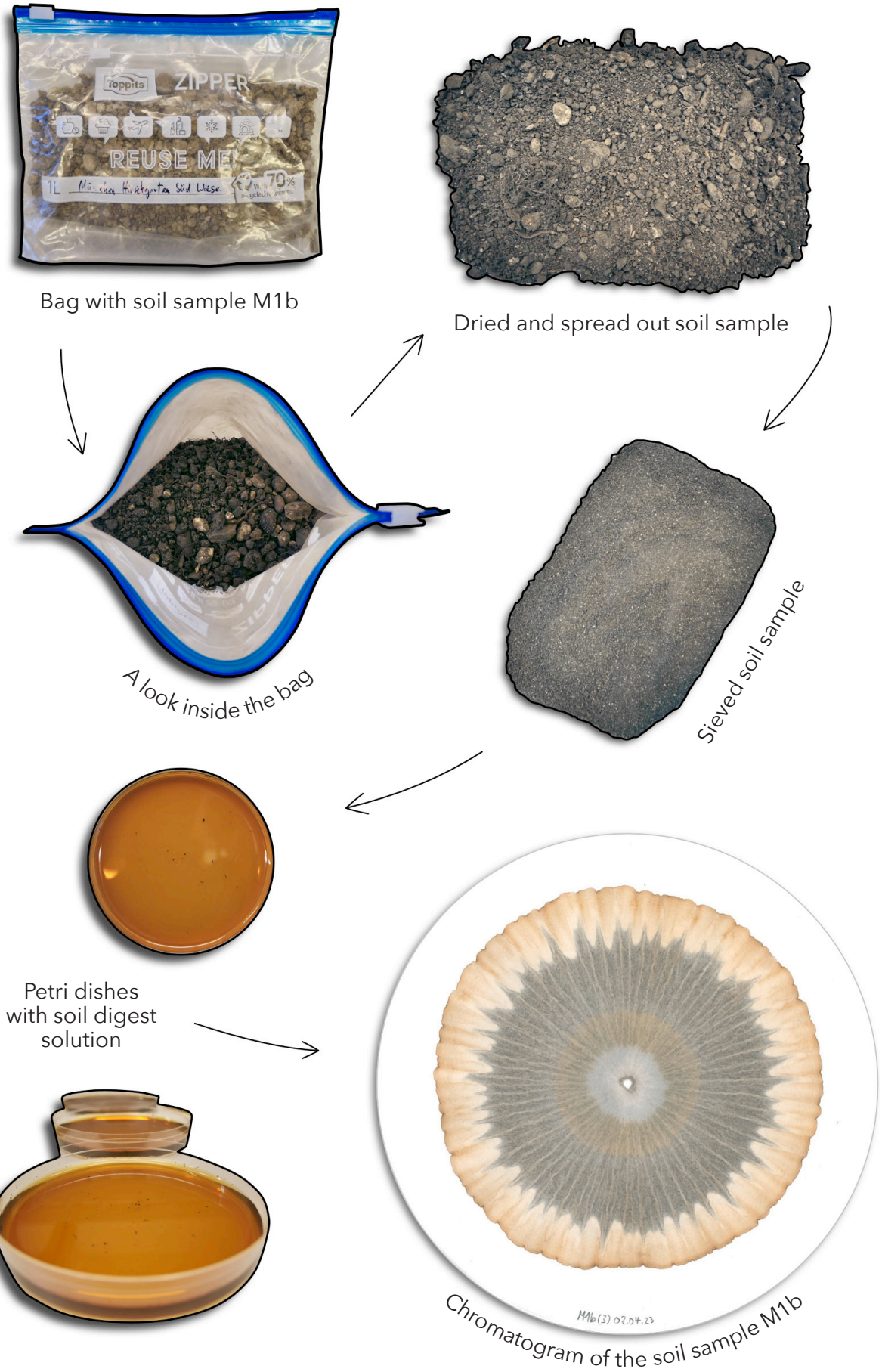
Vegetation

The vegetation is very dense and characterised by rather young trees and undergrowth with bushes. The tree cover is dominated by birch, oak, hazel and poplar. Aerial photos from Google Earth show that part of the vegetation belongs to the old succession nature that developed alongside the railway tracks already during its use. After the rails were removed during the construction of the park between 2007 and 2010, the vegetation developped freely also inbetween the already existing one.

Soil

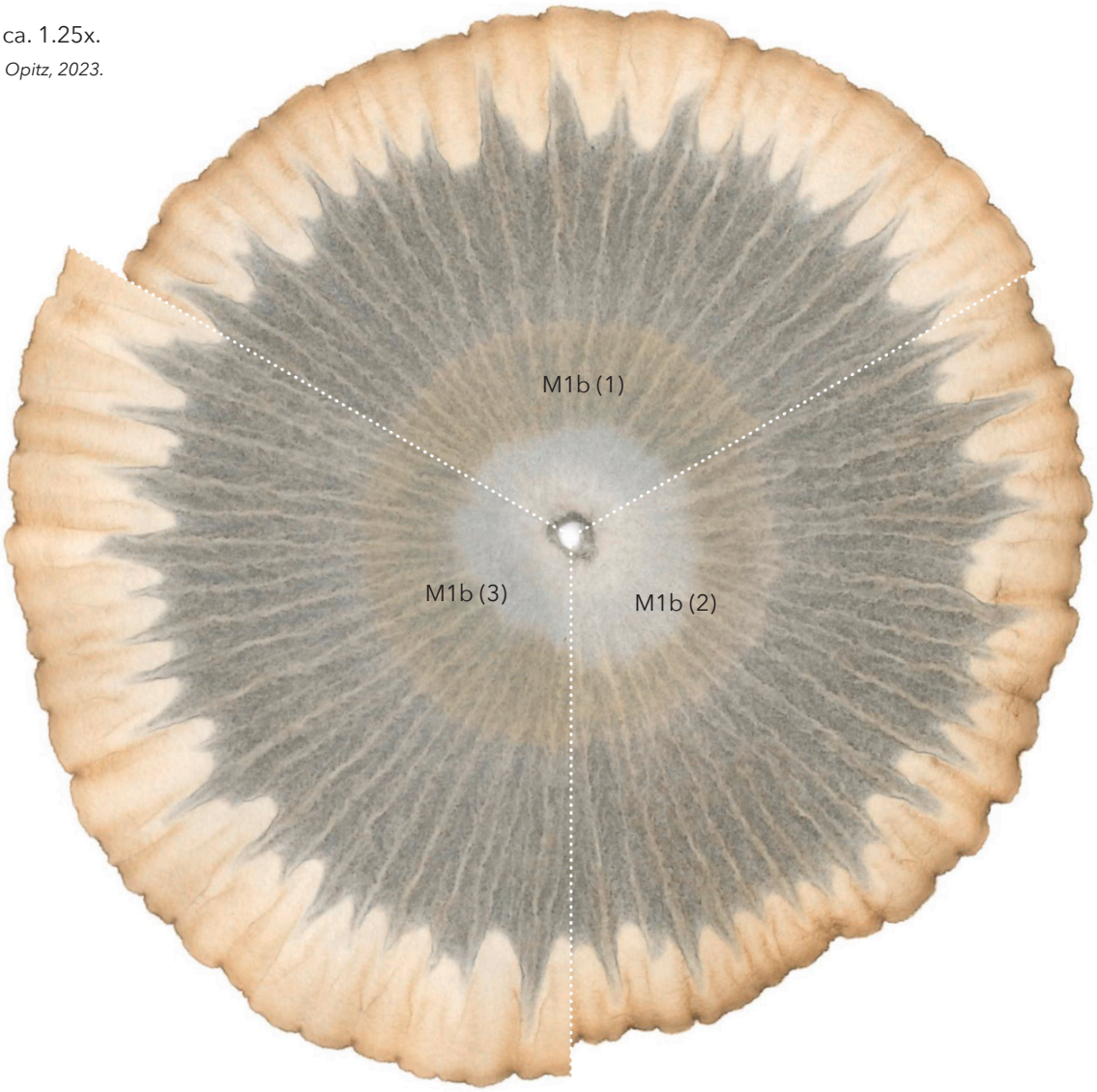
Sandy-loamy soil mixed with railway ballast. The soil is densely rooted and has a dark black-brown colour. The ground was very hard to penetrate with the hand spade because of the stones and roots.

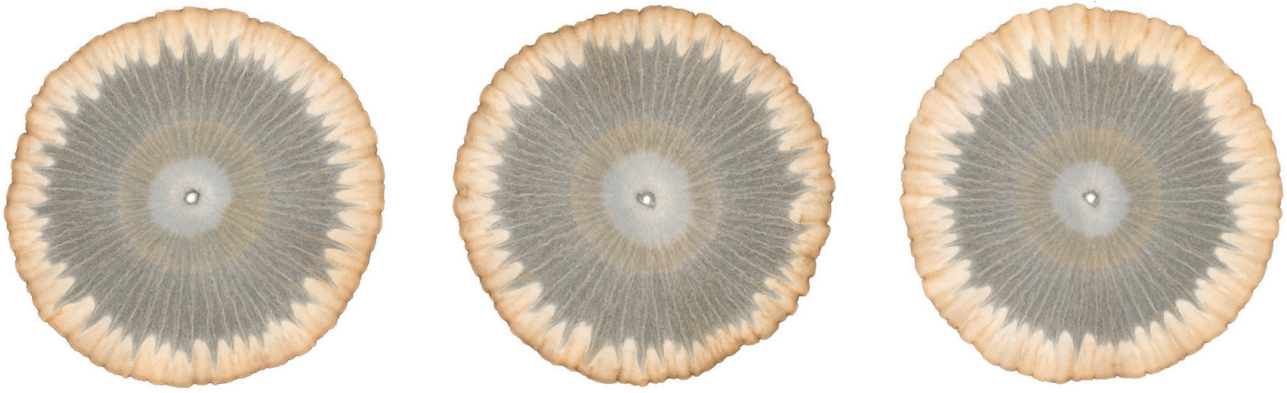
Taking a soil sample from the former railway area.
Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample M1b.
Magnification ca. 1.25x.
Photos & Graphic: Opitz, 2023.





The three chromatograms M1b (1), M1b (2) and M1b (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample M1b - "Fourth nature"

General visual interpretation

The M1b chromatograms are characterised by a high colour intensity with colours ranging from cream-brown, brown-orange to grey-brown and brown tones.

- Indicates a high soil organic matter content with related high microbial activity.

Noticeable is the relatively slight contrast in colours between central and middle zone. The intense and clearly visible channels extend into the central zone.

- Indicates a normal mineral soil content with good integration of soil components.

The middle zone is brown to grey-brown and the outer zone has a noticable intense cream-brown to cream-orange colouration. The "clouds" around the ends of the spikes have a brown-orange hue with high colour intensity. They merge into a dark outer zone edge that is slightly bulged.

- Indicates a high soil organic matter content with related high microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A medium total radius, small central zone radius, medium outer zone width, strong expression of channels and high colour intensity indicate a medium to high soil organic matter content. The low spike number on the other hand points to a low organic matter content. However, as the other characteristics are very pronounced, they overrule.

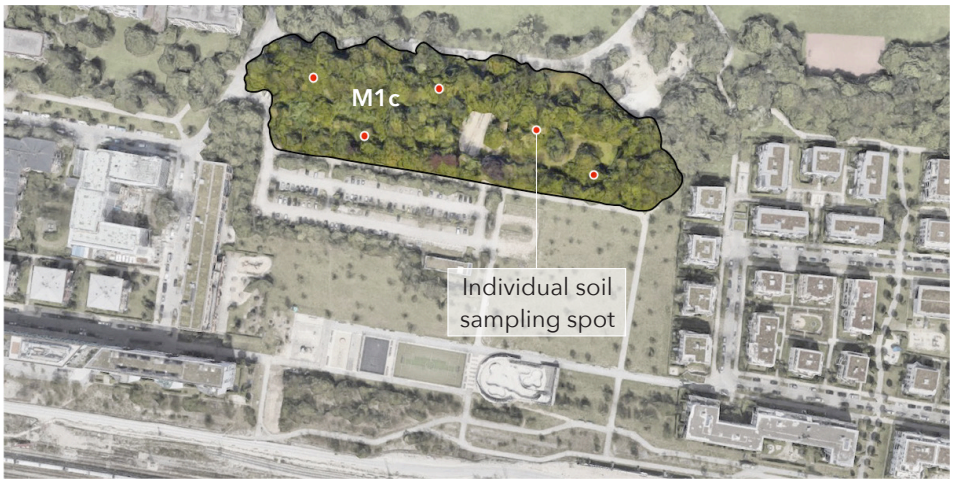
Conclusion

The chromatogram features point to a soil with a high content of organic matter. This indicates a **high microbial activity** in this "fourth nature" soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ○	●	● ○	● ○	● ● ●	●	● ● ●		● ● ●	●
54,4 mm	20,2 mm	19,2 mm	15,0 mm	5 pts	53	1:1 - 1:3	Cream-brown, brown-orange, grey-brown tones	5 pts	1
○ ○	○	○	○ ○ ○	○ ○ ○	○ ○	○ ○ ○		○ ○ ○	○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

This table shows the data average from M1b chromatograms.



Top view of the soil sampling locations M1c.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

M1c - Urban forest

Location and types of use

This sampling location is situated north of the lawn M1a and acted as a buffer between the Königliche Hirschgarten and the former railway area. The forest patch is partly used as a forest playground by the neighbouring kindergarten.

Vegetation

The area is characterised by a mix of very old oaks and beech trees but mostly consists out of younger individuals. They are dominated by robinia, hazel, and maple and few conifers. Between the trees there is a dense undergrowth with bushes.

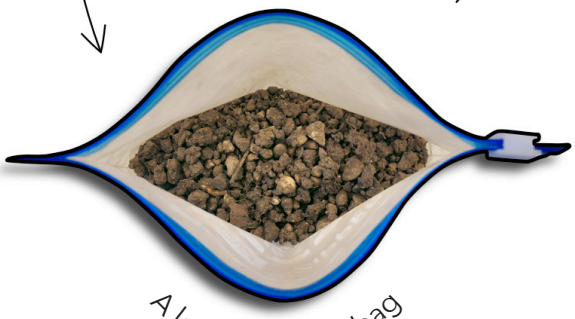
Soil

Loamy soil mixed with some gravel (Munich gravel plain). Very compact soil with rather strong growth of roots. The soil has a light brown colour with no recognizable zones. The soil was very hard to penetrate with the hand spade because of the roots and gravel.

Taking a soil sample from the park forest.
Photo: Opitz, 2023.

Bag with soil sample M1c

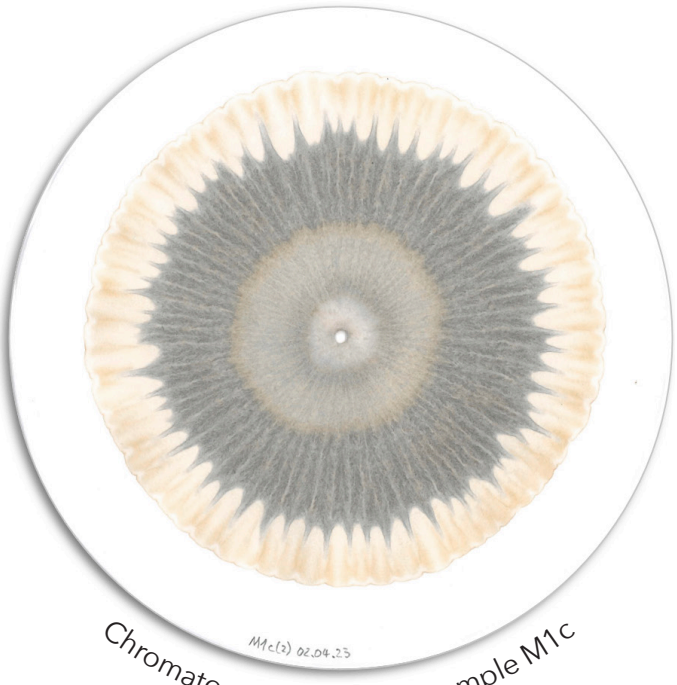
Dried and spread out soil sample



A look inside the bag

Sieved soil sample

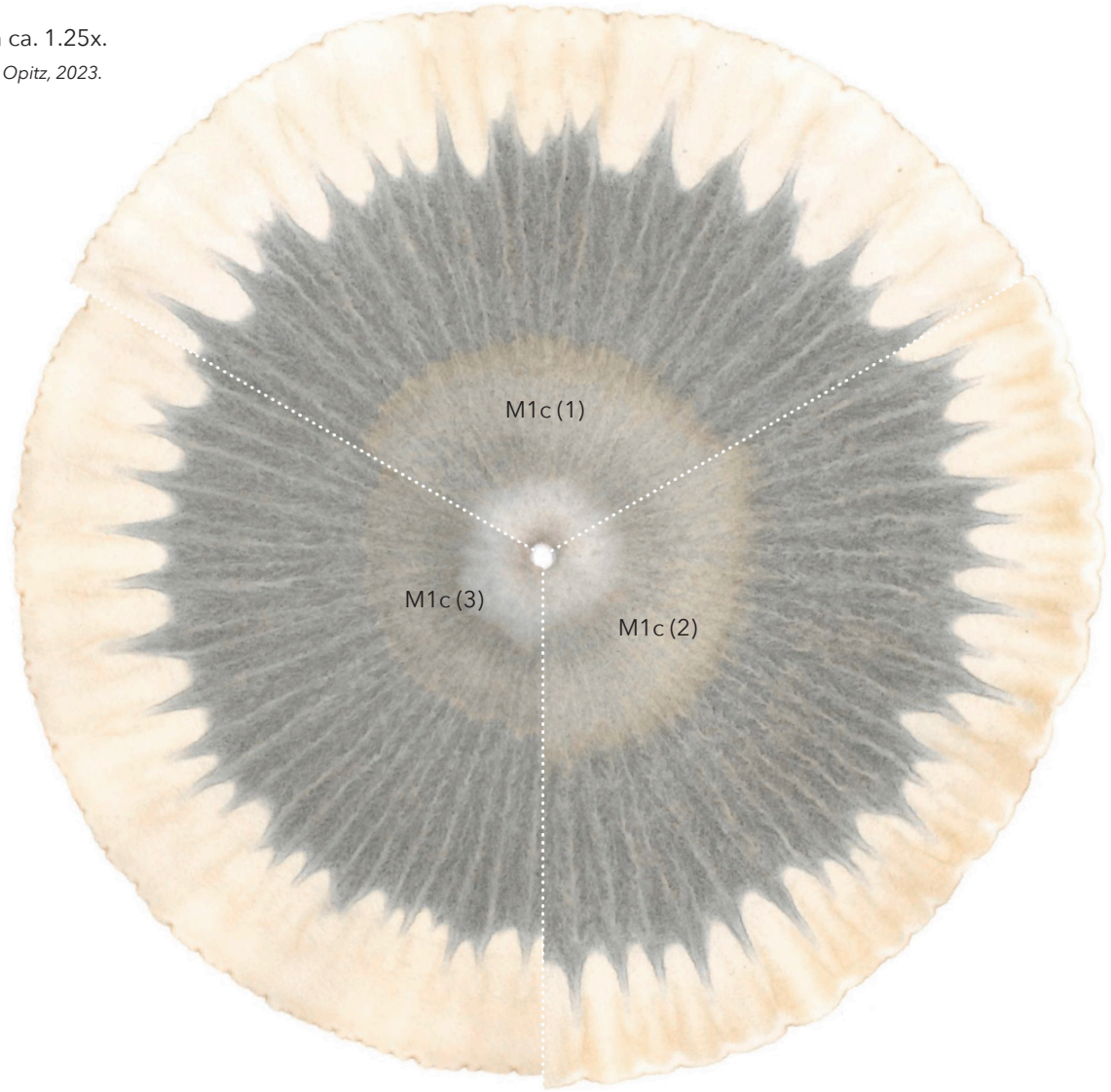
Petri dishes with soil digest solution

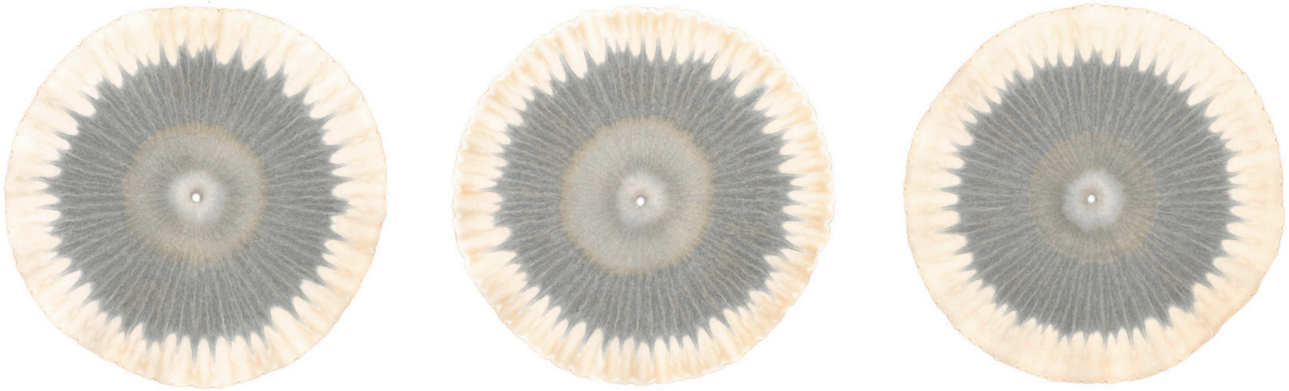


Chromatogram of the soil sample M1c

Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample M1c.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.





The three chromatograms M1c (1), M1c (2) and M1c (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample M1c - Urban forest

General visual interpretation

Chromatogram M1c is characterised by a rather low colour intensity with colours ranging from pale cream, pale grey-orange to grey tones.

- Indicates low soil organic matter with related low microbial activity.

Noticeable is the strong contrast in colours between central and middle zone. The visible channels do only weakly extend into the central zone. The central zone shows ring-shaped weak discolourations.

- Indicates possible excess of soluble minerals, a medium to medium high mineral soil content with some imbalances of soil components.

The middle zone is of grey colouration and the outer zone has a pale cream to pale cream-orange colour. The "clouds" at the ends of the spikes are rather faint and extend into avery thin, dark outer zone edge that is lightly waved.

- Indicates a low to medium organic content in the soil with related low to medium microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation to the most part. A medium total radius, small central zone radius, medium expression of channels, medium spike number and low colour intensity indicate a low to medium organic matter content in the soil. A relatively wide outer zone breadth on the other hand points to a high organic matter content. However, as the other characteristics are very pronounced, they overrule.

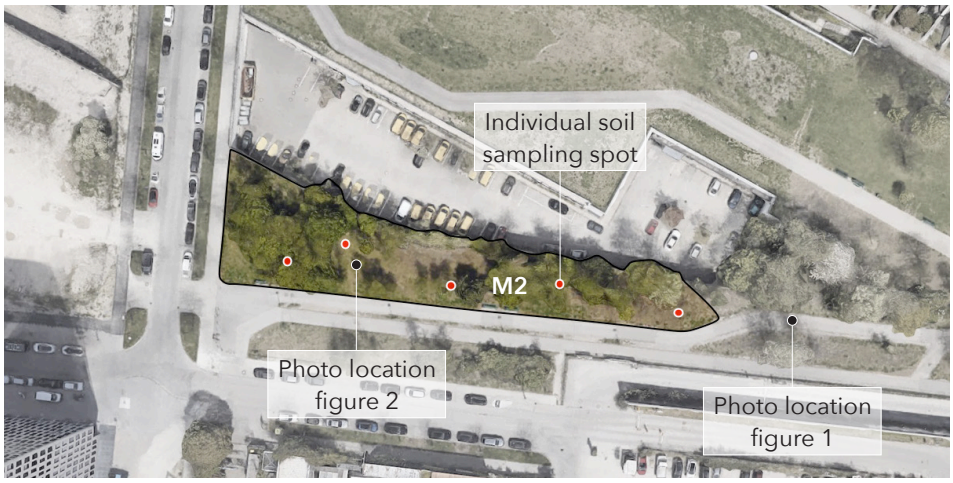
Conclusion

The chromatogram features point to a soil with a low to medium organic matter content. This indicates a **low to medium microbial activity** in this forest soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
● ●	●	● ● ●	● ● ●	● ●	● ●	● ● ●	Pale cream, pale grey-orange, grey tones	●	● ● ●
56,2 mm	19,9 mm	19,7 mm	16,5 mm	4 pts	60	1:1 - 1:3		1,5 pts	2
○ ○	○	○	○ ○ ○	○ ○	○ ○ ○	○ ○		○	○ ○ ○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

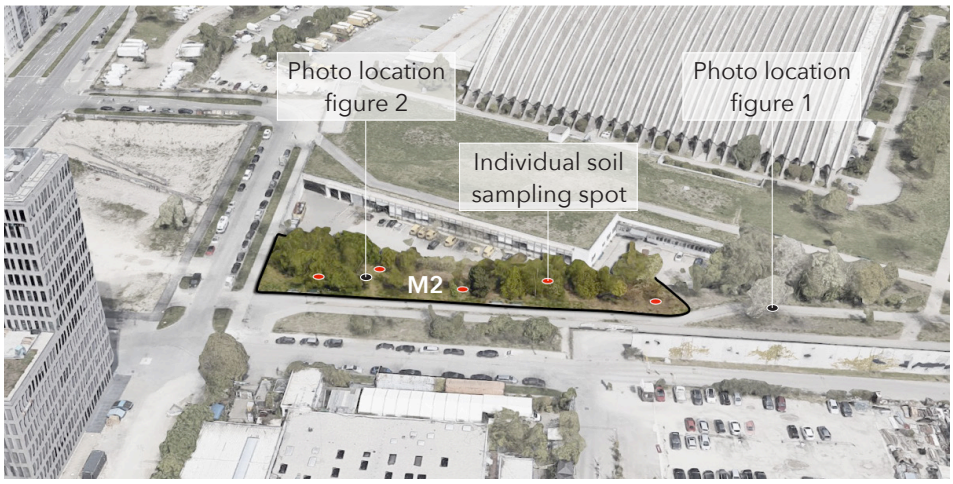
This table shows the data average from M1c chromatograms.



Top view of the soil sampling location M2.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Grünanlage an der Richelstraße - Soil sampling location M2

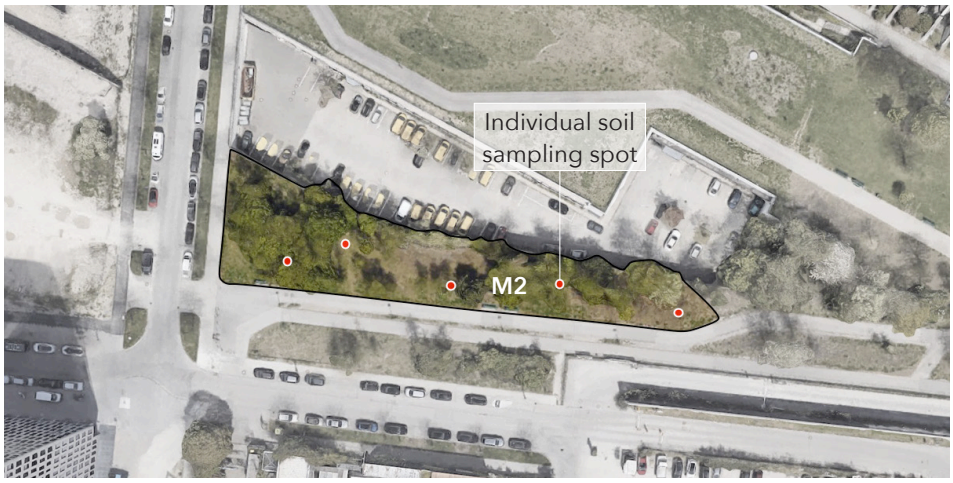
The Grünanlage an der Richelstraße is part of an extensive green connection along the central railway areas in Munich. It connects the western part of Munich to the east towards the city centre. Similar to Hirschgarten Süd, successional vegetation and some relics of the former railway use have been preserved and integrated into the green space. In addition, new trees have been planted in recent years. The footpath and cycle path that borders the green space to the south towards the railway tracks is always heavily frequented and used by cyclists, pedestrians and people walking their dogs. Since the end of the use of this area as railway grounds, the surrounding area has undergone a major structural change with the construction of many new buildings. The park is characterised by large lawns and a children's playground.
Five soil samples were taken at random spots and combined to form a composite sample.



Perspective of the soil sampling location M2.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

View along the former railway site with the new established foot and cycle path that is framed by ruderal vegetation (1). *Photo: Opitz, 2023.*

One of the sample spots characterised by grassland and typical pioneer species (2). *Photo: Opitz, 2023.*



Top view of soil sampling location M2.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

M2 - "Fourth nature"

Location and types of use

This small ruderal forest patch is located between a parking lot and cultural centre and is bordered by a foot- and cycle path along its entire length. The area is subject to high pressure of use due to concerts taking place in the cultural centre opposite and by the use of dog owners with their dogs.

Vegetation

The vegetation is quiet open and characterised by a meadow that is overgrown by trees and bushes. The tree cover is dominated by birch, hazel, maple, pine and white poplar. Aerial photos from Google Earth show that this area was part of an industry area adjacent to the railroad tracks in the south. During that time the vegetaion was nearly non-existant and developed only approximately since 2000.

Soil

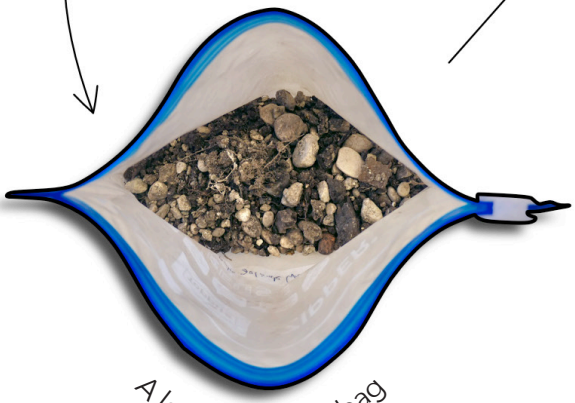
Loamy soil mixed with gravel (Munich gravel plain) and rubble. Very compact soil with medium growth of roots. The soil has a dark brown colour in the upper layer and transitions into a grey to beige colour the more one penetrates into the gravel layer below. The soil was hard to penetrate with the hand spade because of the gravel.

Taking a soil sample from the "fourth nature" area.

Photo: Opitz, 2023.

Bag with soil sample M2

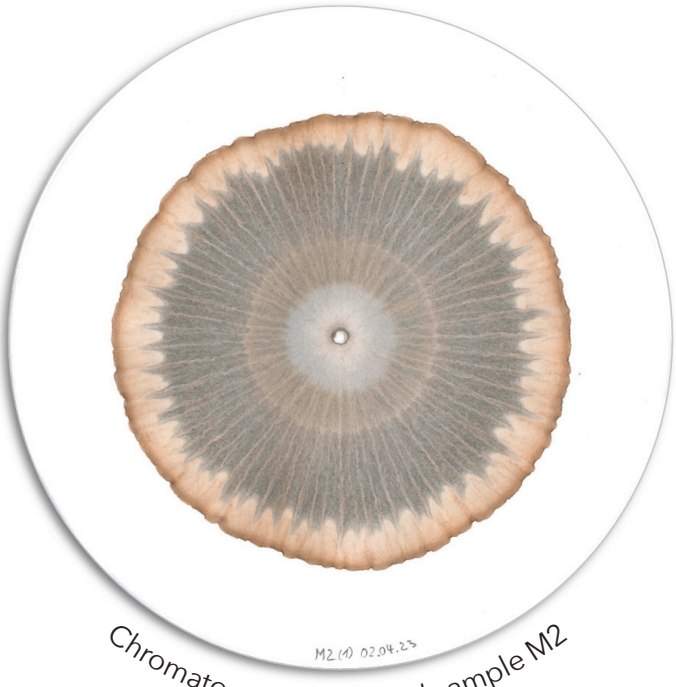
Dried and spread out soil sample



A look inside the bag

Sieved soil sample

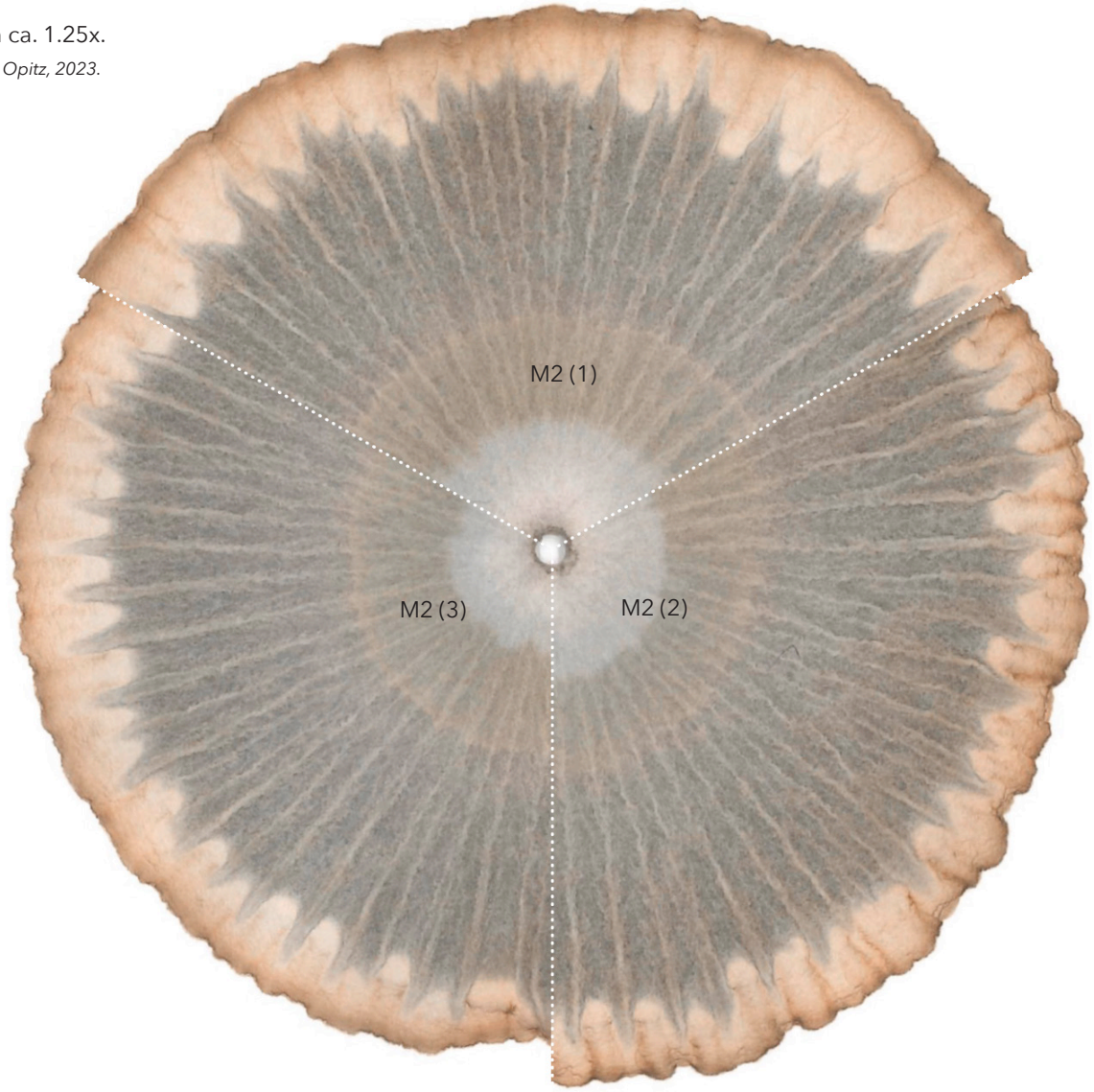
Petri dishes with soil digest solution



Chromatogram of the soil sample M2

Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample M2.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.



The three chromatograms M2 (1), M2 (2) and M2 (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample M2 - "Fourth nature"

General visual interpretation

The M2 chromatograms are characterised by a high colour intensity with colours ranging from brown-red, brown-orange to brown tones.

- Indicates a high soil organic matter content with related high microbial activity.

Noticeable is the very slight contrast in colours between central and middle zone. The intense and clearly visible channels extend into the central zone.

- Indicates a normal mineral soil content with good integration of soil components.

The middle zone is brown to orange-brown and the outer zone has a noticable intense cream-orange to cream-red colouration. The "clouds" around the ends of the spikes merge into a dark red-brown outer zone edge that is slightly bulged.

- Indicates a high soil organic matter content with related high microbial activity.

Verification through analytical interpretation

The analytical interpretation only partly confirms the general visual interpretation. A relatively small total radius, a small central zone radius, strong expression of the channels and high colour intensity indicates a high soil organic matter content. The relatively small outer zone breadth and average spike number on the other hand points to a medium to low organic matter content. However, as the other characteristics are very pronounced, they overrule.

Conclusion

The chromatogram features point to a soil with a high content of organic matter. This indicates a **high microbial activity** in this "fourth nature" soil sample. I attribute **medium confidence** to this interpretation, since 4 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
●	●	● ● ●	●	● ● ●	● ●	● ● ●		● ● ●	●
50,3 mm	19,2 mm	20,6 mm	10,5 mm	5 pts	58	1:1 - 1:3	Brown-red, brown-orange, brown tones	5 pts	1
○	○	○	○	○ ○ ○	○ ○ ○	○ ○		○ ○ ○	○

●/ ● ●/ ● ● ● = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

This table shows the data average from M2 chromatograms.

Top view of the soil sampling location M3.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

Alter Nordfriedhof - Soil sampling location M3

The Alte Nordriedhof is located in the centre of the Maxvorstadt, the university district of Munich with a lot of historical significance and and a animated cultural life. The surrounding area is dominated by dense residential development, making it the only green space in the entire district.

The cemetery was built in the 19th century and is characterised by its many historical gravestones and monuments. During the Second World War, the Alte Nordfriedhof was heavily damaged and was not used as a cemetery again after the war. It was restored and is now open to the public as a park and monument with a special atmosphere. The whole area is a protected landscape feature with very old trees and represents a lively habitat for birds. To the northwest, a playground borders the former northern cemetery.

Five soil samples were taken at random spots and combined to form a composite sample.

Perspective of the soil sampling location M3.

Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

View over the former cemetery, which is now used as a memorial and recreational area (1). *Photo: Opitz, 2023.*

The cemetery is a protected landscape feature in a residential area in the middle of Munich (2). *Photo: Opitz, 2023.*

Top view of the soil sampling location M3.
Graphic: Opitz, 2023. Underlaying map: Google maps, 2023.

M3 - Urban forest

Location and types of use

This former cemetery is open to the public and is used as a memorial and park. The space is is intensively used by people walking and jogging and offers benches to sit down. It is allowed to also walk besides the paths to observe the gravestones and memorials.

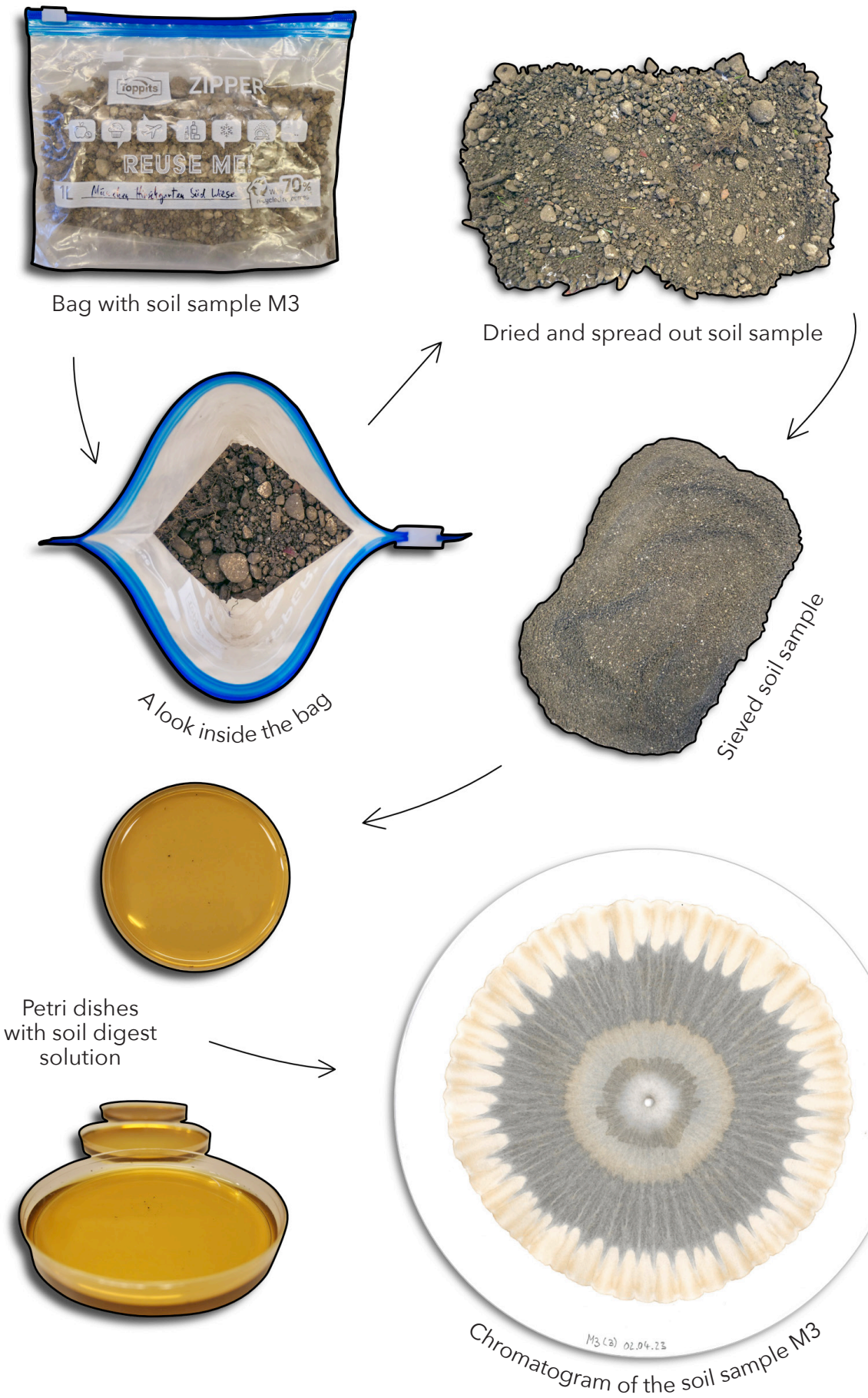
Vegetation

The area is divided by the cemetery fields that are overgrown by an old tree population. They are characterised by beech, oak, maple and other typical deciduous and coniferous cemetry trees. The park is maintained by the municipality.

Soil

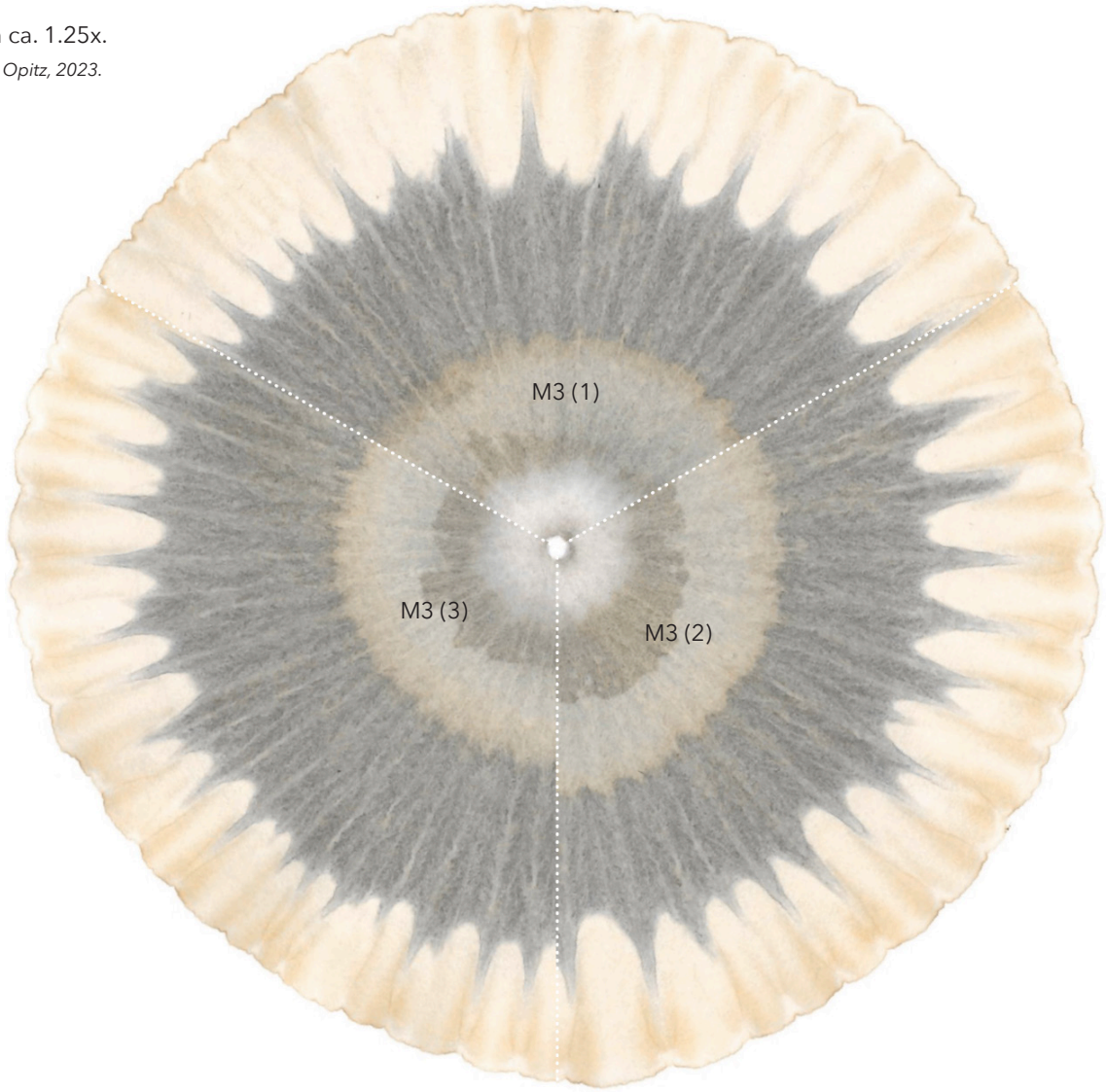
Loamy soil mixed with gravel (Munich gravel plain). Compact soil with medium growth of roots. The soil has a light brown colour in the upper layer and transitions into a beige colour the more one penetrates into the gravel layer below. The soil was medium hard to penetrate with the hand spade because of the gravel.

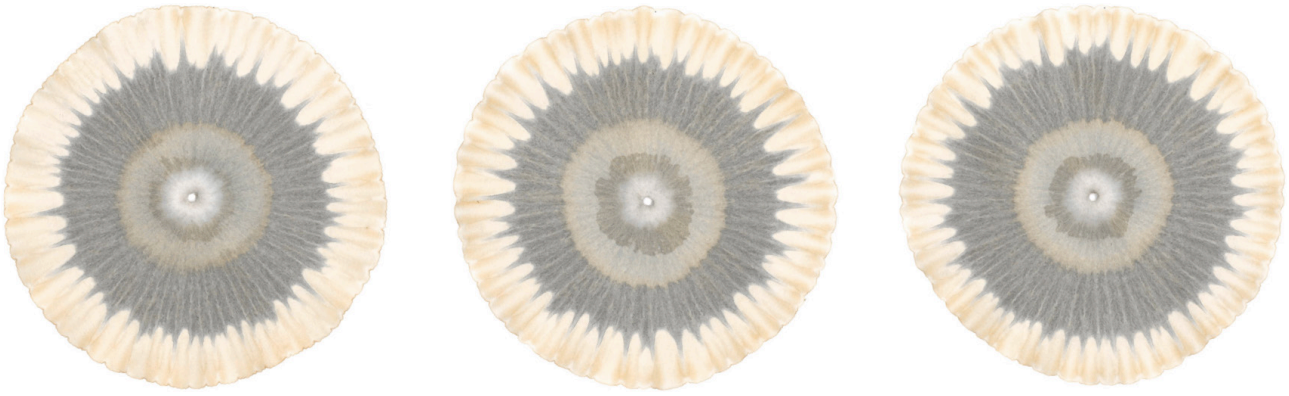
Taking a soil sample from the former cemetery.
Photo: Opitz, 2023.



Relationships between colours and shapes. *Photos & Graphic: Opitz, 2023.*

Compilation of the three chromatograms from soil sample M3.
Magnification ca. 1.25x.
Photo & Graphic: Opitz, 2023.





The three chromatograms M3 (1), M3 (2) and M3 (3). *Photos: Opitz, 2023.*

Chromatogram interpretation of soil sample M3 - Urban forest

General visual interpretation

The M3 chromatograms are characterised by a rather low colour intensity with colours ranging from pale cream, pale grey-orange to grey tones.

- Indicates a low soil organic matter content with related low microbial activity.

Noticeable is the strong contrast in colours between central and middle zone. The weak but visible channels in the middle zone do not extend into the central zone. The central zone shows ring-shaped discolourations.

- Indicates possible exess of soluble minerals, a high mineral soil content and imbalance of soil components.

The middle zone is of grey colouration and the outer zone has a pale cream to pale cream-organge colour. The "clouds" around the ends of the spikes are rather faint and extend into a very thin dark outer zone edge that is slightly waved.

- Indicates a low to medium soil organic matter content with related low to medium microbial activity.

Verification through analytical interpretation

The analytical interpretation confirms the general visual interpretation for the most part. A relatively wide total radius, wide central zone radius, weak expression of channels, low spike number and low colour intensity indicate a low soil organic matter content. A realtively wide outer zone breadth on the other hand points to a high organic matter content. However, as the other characteristics are very pronounced, they overrule.

Conclusion

The chromatogram features point to a soil with a low content of organic matter. This indicates a **low microbial activity** in this urban forest soil sample. I attribute **medium high confidence** to this interpretation, since 5 out of 6 of the considered chromatogram features support this statement.

Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
○ ○ ○	○ ○ ○	○	○ ○ ○	○	○	○ ○ ○	Pale cream, pale grey-orange, grey tones	○	○ ○ ○
59,1 mm	23,3 mm	17,3 mm	18,5 mm	3 pts	55	1:1 - 1:3		2 pts	2
○ ○ ○	○ ○	○	○ ○ ○	○	○ ○	○ ○		○	○ ○ ○

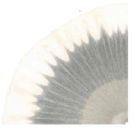
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○ ○/ ○ ○ ○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

This table shows the data average from M3 chromatograms.

	Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
 **B1a** Lawn Low microbial activity Medium high conf.	○○○ 60,6 mm ○○○	○○○ 27,8 mm ○○○	○ 24,1 mm ○○	○ 8,7 mm ○	○ 3 pts ○	○○○ 64 ○○○	○ 1:1 - 1:2 ○	Pale cream, cream-orange, light orange-brown grey tones	○ 2 pts ○	○○○ 2 ○○○
 **B1b** Meadow Low to medium low microbial activity Medium high conf.	○○○ 61,2 mm ○○○	○○○ 28,9 mm ○○○	○ 25,0 mm ○○	○ 7,2 mm ○	○ 3 pts ○	○○○ 64 ○○○	○○ 1:1 - 1:3 ○○○	Pale cream, cream-orange, light orange-brown grey tones	○ 2 pts ○	○○○ 2 ○○○
 **B1c** "Fourth nature" Medium microbial activity Medium high conf.	○ 57,7 mm ○○○	○ 20,7 mm ○	○○ 26,2 mm ○○○	○○ 10,8 mm ○	○○ 4 pts ○○	○ 46 ○	○○ 1:1 - 1:4 ○○	Cream, brown-orange, grey-brown tones	○○ 3 pts ○○	○○○ 2 ○○○
 **B2** "Fourth nature" High microbial activity Medium high conf.	○ 58,1 mm ○○○	○ 16,9 mm ○	○○○ 29,9 mm ○○○	○○○ 11,3 mm ○○	○○○ 4,5 pts ○○○	○ 40 ○	○○○ 1:1 - 1:5 ○○○	Cream-brown, brown-orange, brown tones	○○ 4 pts ○○○	○○○ 2 ○○○
 **B3** Meadow Medium microbial activity High conf.	○○ 60,0 mm ○○○	○○ 20,8 mm ○	○○ 27,9 mm ○○○	○○ 11,2 mm ○○	○○ 4 pts ○○	○○ 54 ○○	○○ 1:1 - 1:3 ○○	Cream, cream-orange, grey-orange, grey tones	○ 2,5 pts ○○	○ 1 ○
 **B4** Urban forest High microbial activity Medium conf.	○ 59,2 mm ○○○	○○ 23,2 mm ○○	○ 25,3 mm ○○	○○○ 13,3 mm ○○	○○ 4 pts ○○	○ 43 ○	○○○ 1:1 - 1:5 ○○○	Cream-brown, brown-orange, brown tones	○○○ 5 pts ○○○	○○○ 2 ○○○

●/ ●●/ ●●● = Low/ middle/ high value compared to other chromatograms from Berlin.
○/ ○○/ ○○○ = Low/ middle/ high value compared to other chromatograms from Berlin and Munich.

This table shows the data averages from Berlin (B) chromatograms.

	Total radius	CZ radius	MZ breadth	OZ breadth	Chan-nels	Spike numb.	S.-c. ratio	Colour range	Colour intens.	Con. rings
 **M1a** Lawn Low microbial activity Medium low conf.	○○○ 57,6 mm ○○○	○○ 21,6 mm ○○	○○○ 20,3 mm ○	○○ 15,7 mm ○○○	○ 3,5 pts ○○	○○○ 65 ○○○	○ 1:1 - 1:2 ○	Very pale cream, grey tones	○ 1 pts ○	○○○ 2 ○○○
 **M1b** "Fourth nature" High microbial activity Medium high conf.	○○ 54,4 mm ○○	○ 20,2 mm ○	○○ 19,2 mm ○	○○ 15,0 mm ○○○	○○○ 5 pts ○○○	○ 53 ○○	○○○ 1:1 - 1:3 ○○○	Cream-brown, brown-orange, grey-brown tones	○○○ 5 pts ○○○	○ 1 ○
 **M1c** Urban forest Low to medium microbial activity Medium high conf.	○○ 56,2 mm ○○	○ 19,9 mm ○	○○○ 19,7 mm ○	○○○ 16,5 mm ○○○	○○ 4 pts ○○	○○ 60 ○○○	○○○ 1:1 - 1:3 ○○	Pale cream, pale grey-orange, grey tones	○ 1,5 pts ○	○○○ 2 ○○○
 **M2** "Fourth nature" High microbial activity Medium conf.	○ 50,3 mm ○	○ 19,2 mm ○	○○○ 20,6 mm ○	○ 10,5 mm ○	○○○ 5 pts ○○○	○○ 58 ○○○	○○○ 1:1 - 1:3 ○○	Brown-red, brown-orange, brown tones	○○○ 5 pts ○○○	○ 1 ○
 **M3** Urban forest Low microbial activity Medium high conf.	○○○ 59,1 mm ○○○	○○○ 23,3 mm ○○	○ 17,3 mm ○	○○○ 18,5 mm ○○○	○ 3 pts ○	○ 55 ○○	○○○ 1:1 - 1:3 ○○	Pale cream, pale grey-orange, grey tones	○ 2 pts ○	○○○ 2 ○○○

●/ ●●/ ●●● = Low/ middle/ high value compared to other chromatograms from Munich.
○/ ○○/ ○○○ = Low/ middle/ high value compared to other chromatograms from Munich and Berlin.

This table shows the data averages from Munich (M) chromatograms.

4 Discussion and conclusion

Recap and context

After working extensively with Pfeiffer's circular chromatography soil test in the third chapter of this thesis and using this method to analyse and interpret soil samples from sites with "fourth nature" characteristics as well as contrasting vegetation, I will now conclude by discussing the results.

The key findings of my literature review indicate that diverse microbiomes in the city promote human health and ecosystem functions. To enable this, measures and strategies are needed to realise the existence of such diverse microbiomes. From the perspective of microbial ecology research, a possible approach is to support microbiome rewilding through active habitat restoration. In addition, the reviewed literature suggests the potential of "fourth nature" to promote the occurrence of diverse microbiomes in the city. However, the literature also emphasises that these are approaches and hypotheses that need to be confirmed and refined through further research. Parallel to these findings of microbial ecology, the urban ecological movement in landscape architecture is also concerned with "fourth nature". The focus here is on the general ecological value of spontaneously emerging vegetation as well as its cultural meaning. The possible significance of "fourth nature" for diverse microbiomes, which in turn positively influence human health and ecosystem functions, has not yet been explicitly discussed from a landscape architecture perspective.

Exactly at this point the theoretical part of this thesis begins, which aims to link these two development lines to find out whether "fourth nature" vegetation promotes microbial activity. Since microorganisms are present everywhere, even in the air, I decided to focus on microbial activity in the soil. As a method to "expand my senses" to visualise the invisible spheres of urban nature, I used Pfeiffer's circular chromatography soil test (PCC). In chapter 2, I explained what influenced my decision to use this method. As far as I know, it is not possible to specifically test microbial activity in soil with the help of PCC. But the method does provide general insights into soil components such as organic matter. Organic matter is an important factor for microbial

activity and serves in this thesis as a indicator and approximation for determining the microbial activity of the respective soil. By comparing all chromatograms representing the different soil samples, it is possible to conclude whether the respective soil has a rather high, medium, or low organic matter content compared to the others, with associated rather high, medium, or low microbial activity.
Before I discuss the results of the chromatogram interpretation and formulate related research questions and conclusions for planning, I first look back at the findings of the work with Pfeiffer's circular chromatography soil test.

4.1 Reflections on the application of Pfeiffer's circular chromatography soil test in practice

Pfeiffer's circular chromatography soil test (PCC) proved to be a very multifaceted method in the course of this thesis. The description of all the experiments that I conducted traces the learning curve that I experienced regarding the execution of them (Chapter 3). Even though the experimental procedure is complex with a lot of room for error, after conducting a total of five experiments, I reached a point where I had control over most of the variables and was able to achieve and repeat a constant result. The resulting chromatograms were then analysed and interpreted.
The analysis and interpretation of the chromatograms is complex, as there are only a few measurable variables, and it is more important to interpret the overall picture. Without the adequate experience and possibilities for comparison, this turned out to be challenging. To verify my general visual interpretation of the chromatograms, I developed an analytical interpretation procedure based on measurements, counting and a "scoring" system. Using this, it was possible to compare the chromatograms with each other based on the respective "score" and to determine whether the respective value was rather high or low compared to the other chromatograms. With the help of the correlation table based on Kokornaczyk et al. (2017), I was then able to draw conclusions about the soil components present, focusing primarily on the soil organic matter content.
The analytical interpretation as an addition to the general visual interpretation also allowed the determination of the "confidence" in the interpretation. The more the analytical interpretation corresponded to the general visual interpretation, the higher I estimated the "confidence" in the interpretation. Furthermore, the analytical interpretation allowed for the following findings and conclusions:

- The chromatogram feature "spikes" contradicted the statements of all other features in 8 of 11 chromatograms. Therefore, contrary to the findings of Kokornaczyk et al. (2017), in this thesis, the spikes do not seem to have a positive correlation with the soil organic matter content. According to my observations, fine spikes/ a high spike number indicate rather low soil organic matter. On the other hand, large and wide spikes/ low spike number indicated high soil organic matter.

- The introduced category "spike-channel ratio" supports this observation. The more channels that lead into the same spike, the wider the spike appears, pointing to a higher soil organic matter content.

- In five of the six Berlin chromatograms, only the "spike" feature contradicted the general visual interpretation; for the sixth chromatogram, the analytical interpretation was in complete agreement with the general interpretation.

- The analytical interpretation of the Munich chromatograms was not as clear as in Berlin. Nevertheless, the interpretation was still possible from my point of view, as colour and colour intensity turned out to be reliable indicators for soil organic matter.

Outlook

In conclusion, Pfeiffer's circular chromatography soil test (PCC) enabled me to gain fascination for soils and their properties without a background in soil sciences. Especially the visual quality of the chromatograms created a distinctive character of the soil depicted in each case. According to my observations, PCC provided a general overview of soil properties and thus allowed only for a general approximation of microbial activity in the soil. However, in my view, this is also a reason why PCC is particularly suitable as an introduction to soil sciences, as a bridge between science and other disciplines, for comparing soils from different locations or for observing a soil over a long period of time. PCC can also complement more precise methods with purely number-based results as a holistic and very visual approach.
To further develop the method for landscape architecture, more experiments with urban soil are needed, where the results are verified by precise methods. In this way, it would be possible to create a reference catalogue to compare chromatograms with. This would contribute to refine the interpretation scheme that I have developed for this thesis.

4.2 Reflections on field work and interpretation of chromatograms

Context

To obtain relevant soil samples for the investigation of microbial activity in the soil of "fourth nature" sites, I conducted field work in Berlin and Munich. The selected "fourth nature" sites in both cities had in common that they were subject to former railway use and are thus comparable. As a reference, I also took soil samples from locations with "urban forest", "meadow" and "lawn" characteristics. The reason for selecting these types of locations was to cover a wide range of different vegetation complexities. I attributed the highest complexity to "fourth nature" and urban forests, followed by meadows and lawns with medium to very low complexity (the more species-richness and height difference there is, the more complex the vegetation). I then compared the reference areas with the "fourth nature" sites to find out how the different vegetation affects microbial activity in the soil.
In Berlin and Munich, I decided to select soil sampling locations throughout the city as well as one site each where several very different land use types are present in a confined space. The idea behind this was to find out how much historical and current land use and vegetation types influence the soil within a limited area, and how this affects the soil organic matter content (SOM content) and the associated microbial activity (hereafter referred to as SOM content/microbial activity). In Berlin, I chose a site in the Park am Gleisdreieck/ Ostpark (Area B1), where lawn (B1a), meadow (B1b) and "fourth nature" (B1c) were directly adjacent. In Munich, I chose a place south of the Königlicher Hirschgarten (Area M1), which combined lawn (M1a), "fourth nature" (M1b) and urban forest (M1c).

Findings Berlin

In Berlin, there was a very evident contrast between the open lawn and meadow areas (B1a and B1b) with low or low to medium SOM content/ microbial activity and the "fourth nature" vegetation (B1c) with medium SOM content/ microbial activity. Just by looking at the soil, the difference in SOM could be recognised by a clearly darker colouring of the soil in B1c compared to B1a and B1b. Nevertheless, a slight difference in SOM content/ microbial activity was also visible when comparing the open areas: B1b had a slightly higher SOM content/ microbial activity compared to B1a. This can be explained by the fact that B1b is a protected meadow biotope which, unlike the lawn, is only extensively maintained, so that more organic matter remains on the site. Location B1c, on the other hand, has been able to develop freely for about 40 years since the railway use in the area ended, so that all organic matter has remained in place. With regard to these three sites, it can be observed that the

more the complexity of the vegetation increases and the maintenance intensity decreases, the more the organic matter content in the soil increases and thus presumably also the microbial activity.
The other soil sampling locations in Berlin confirm this observation. Soil sampling location B2, compared to B1c with even more undisturbed and complex "fourth nature" vegetation, had a high SOM content/ microbial activity. Soil sampling location B3, which I categorised as "meadow" had a medium to low vegetation complexity with extensive meadow areas. This sampling location had a medium SOM content/ microbial activity. Finally, urban forest site B4 in the Tiergarten in Berlin should be mentioned. Here, a beech forest typical of Germany's natural landscape has been deve-loping in the middle of the city since reforestation after the Second World War. The forest areas in the park are developed very naturally to promote typical forest plant species and had a high SOM content/ microbial activity.

Findings Munich

Like in Berlin, I also selected a site in Munich (Area M1) with a strong contrast in the vegetation complexity of three adjacent soil sampling locations. The lawn area M1a had the lowest SOM content/ microbial activity compared to all other areas from Munich and Berlin. The adjacent "fourth nature" site M1b, on the other hand, had a high SOM content/ microbial activity and the "urban forest" M1c had a low to medium SOM content/ microbial activity. This again confirms that the area with the lowest vegetation complexity also had the lowest SOM content/ microbial activity.
The second "fourth nature" location in Munich M2 also had a high SOM content/ microbial activity, whereas location M3, an urban forest on the site of the Alte Nordfriedhof, had a low SOM content/ microbial activity. The large differences in the results of the urban forests in Berlin and Munich with regard to SOM content/ microbial activity could be explained by the fact that the locations within the "urban forest" category were very different in comparison to each other. For example, a forest-like character was much more given in location B4 than in M1c, which had rather younger trees. M3 had an old tree population, but the areas between the trees were cleared and characterised by lawn and open ground due to the former use of the cemetery. This gradient of vegetation complexity is also reflected in the results of the chromatogram interpretation: B4 with high SOM content/ microbial activity, M1c with low to medium SOM content/ microbial activity and M3 with low SOM content/ microbial activity.
As in Berlin, the "fourth nature" locations in Munich also had the highest values in SOM content/ microbial activity. The results of the analysis of the soil samples from Munich thus confirm the observations of the samples from Berlin, that the more the complexity of the vegetation

increases, and the maintenance intensity decreases, the more the organic matter content in the soil increases and thus presumably also the microbial activity.

Summary of findings

Overall, I come to the following conclusion regarding the field work in Berlin and Munich, the work with the soil in the context of the PCC experiments and the subsequent interpretation:

- The "fourth nature" locations in Berlin and Munich consistently showed a high SOM content/ microbial activity compared to all the other samples, despite their previous intensive use as railway land.
- The two lawn areas in Berlin and Munich had the lowest SOM content/ microbial activity compared to all other samples.
- The meadow areas in Berlin had a low to medium SOM content/ microbial activity.
- The urban forest areas ranged from high to low SOM content/ microbial activity and were therefore rather inconsistent. This is probably due to the large differences in vegetation complexity between the locations in this category.
- The results indicate that the more the complexity of the vegetation increases and at the same time the maintenance intensity decreases, the higher the SOM content and thus presumably also the microbial activity in the soil.
- The areas with high SOM content/ microbial activity also tend to be the areas that are not allowed to be entered to protect nature. This means that human contact with areas with lower SOM content/ microbial activity is more likely than with areas with high SOM content/ microbial activity.

Conclusion

In summary, **the results indicate that the soils of locations with urban spontaneous vegetation ("fourth nature") investigated in this thesis had a high content of organic matter compared to the reference sites and therefore promote microbial activity in the soil**. Although these areas were still subject to very intensive and extreme use as railway land a few decades ago, the spontaneously growing vegetation had changed the soil of these places without active human intervention in such a way that a high microbial activity can be assumed there today. This could have positive impacts on ecosystem functions and human health, which could transform the way we look at "fourth nature".
I understand this result as a confirmation to strengthen the collaboration with the microbial ecology discipline from a landscape architecture

perspective. In my view, this benefits both disciplines: the integration of microbiome-related knowledge into landscape architectural planning enables the promotion of diverse microbiomes for human health and ecosystem functions, which is one of the goals of microbial ecology research. The urban ecology movement in landscape architecture, in turn, will benefit from future findings of microbial ecology research if there is more and more knowledge about the role of "fourth nature" for microbiome rewilding. This would lead to a whole new level of argumentation to appreciate "fourth nature" as spontaneous, site-adapted vegetation with diverse microbiota that has a positive impact on ecosystem functions and human health in the city.

Further research questions and conclusions for landscape architectural planning

Further research questions

Despite this result, it should be underlined that the PCC method only allowed a very general approximation to the research questions of this thesis. Therefore, it would be of great value to conduct further studies using it in parallel to more precise and complex methods in the context of the question "Does 'fourth nature' promote microbial activity?". Besides the soil, it would also be sensible to examine the surface of plants or the air to draw conclusions about the extent to which "fourth nature" has an effect on the microbiomes in these habitats. The simultaneous application of PCC and methods with higher precision also has the value of verifying the accuracy of the PCC results. Furthermore, the following questions should be clarified through further studies:

- Which kinds of microorganisms are supported by "fourth nature"?
- How exactly do these affect the human immune, metabolic, and nervous systems?
- Do they also reduce the incidence of non-communicable diseases?
- To what extent do "fourth nature" microbiomes counteract microbial resistance?
- Which ecosystem functions do "fourth nature" microbiomes support?
- Is it possible to categorise "fourth nature" more precisely into groups and thus also their microbiomes?
- Can "fourth nature" also have a negative impact on human health due to, for example, pollution on the site?
- How can human contact with health-promoting "fourth nature" microbiota be intensified?

Although research on urban microbiome rewilding is still at the beginning and knowledge gaps exist, in my view this is no reason to wait. In today's world, which is characterised by climate change and health threats such as non-communicable diseases or antimicrobial resistance, I believe that the experimental approach of landscape architecture is more important than ever. Landscape architects can be pioneers by planning in the context of the microbial ecology knowledge that is currently available. Especially through the lessons learned from projects that specifically address the rewilding of microbiomes in the city, sustainable and experience-based knowledge can be generated. Through studies accompanying such projects, subsequent monitoring and follow-up projects that have been adjusted and improved on the basis of these findings, this knowledge is tested and further developed in iterations. In this way, a catalogue of possible actions is built up over time on how landscape architecture can contribute most effectively to microbiome rewilding and thus to the promotion of ecosystem functions and human health.

Further ideas for landscape architectural measures in the context of "fourth nature" and microbiome rewilding:

- Incorporation of existing "fourth nature" into planning instead of planting new vegetation
- Creation of "succession areas" within green spaces, where vegetation can develop spontaneously and is monitored at the same time.
- Developing "fourth nature" plant seed banks of species that are adapted to certain conditions through evolutionary processes (e.g. drought stress). These could be used for seeding appropriate areas to enable faster growth of the ideal vegetation (inoculation principle).
- Replanting of "fourth nature" trees from areas that are converted and using them as urban trees/street trees instead of nursery-grown trees.
- Spreading knowledge about diverse microbiomes for ecosystem functions and human health through information or art installations in open spaces.
- Establishing urban nature experience areas for children and adults in the city and developing further strategies that promote contact between people and urban nature.

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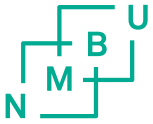

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway