Master's Thesis in Biomedical Engineering

Microbial ecosystem analysis of biochar-enriched soil in Kenya using microfluidic soil models

Erik Karlsson

MSc Engineering Nanoscience

Supervisors:

Pelle Ohlsson, PhD, Department of Biomedical Engineering, LTH

Edith Hammer, PhD, Department of Microbial Ecology, LU

Hanbang Zou, PhD, Department of Microbial Ecology, LU

Dries Roobroeck, PhD, International Institute of Tropical Agriculture

Geoffrey Maritim Kimutai, International Institute of Tropical Agriculture

Examiner:

Johan Nilsson, PhD, Associate Professor & Head of Department,
Department of Biomedical Engineering, LTH

Spring 2022

Department of Biomedical Engineering

Faculty of Engineering, LTH

Lund University

SoilChip in front of biochar-enriched maize fields, Nyabeda, Western Kenya.

Abstract

Healthy soil is rich in diverse microbial life, and a healthy microbial ecosystem is crucial for any well-functioning agriculture. Soil microbes are essential for organic decomposition and nutrient recycling in the soil, which make nutrients available for agricultural crops. Biochar-enrichment of soil has previously been found to increase crop yields in Kenya, and could potentially be a self-reliant and sustainable method for improving agricultural yields systematically in the region.

In this thesis, microfluidic soil model chips, called SoilChip, were utilized to investigate the effects of biochar-enrichment on the microbial ecosystem of agricultural soil in Western Kenya. Both in-situ and lab experiments were conducted where microbial abundance and microbial foraging capabilities were investigated. The SoilChip technology provides direct, in-situ, visualisation of the microbial community and its dynamics. SoilChips were fabricated in Lund, Sweden, and transported and successfully installed in Kenya.

We show that we can, in a robust and sterile manner, employ SoilChip technology across continents, and successfully analyze chip results in-situ by the fields using a portable field microscope. The results show significant impact of biochar-enrichment on chip colonization for both fungal hyphae and protist populations. Biochar-enriched soil showed fewer fungal hyphae, and more protists, populating the chips, and both populations showed increased foraging capabilities in biochar-enriched soil. No significant impact of biochar-enrichment could be found for nematode, cyst, or bacterial populations. The lab experiments also indicate that biochar-rich environments support rapid and dense fungal hyphae growth, although more research is required to draw any conclusions. We also present insights that the SoilChip technology can provide educational advantages when communicating soil ecology- and soil microbiology research.

Key words: Soil microbial ecosystem analysis, microbe abundance, microbe foraging, biochar, agriculture, microfluidics, in-situ visualization, Kenya, SoilChip, soil model, Minor Field Study.

This thesis has been a collaboration between Lund University, Sweden, and the International Institute of Tropical Agriculture (IITA), Kenya. Let it give rise to many more successful research collaborations in the future.

The thesis has been carried out in within the framework of the Minor Field Study (MFS) scholarship program funded by the Swedish International Development Cooperation Agency (Sida). Within the MFS scholarship, students from Swedish universities are given the opportunity to conduct fieldwork and data collection in a low- or middle income country for their Bachelor's or Master's thesis. The thesis topic should contribute to the Sustainable Development Goals set by the United Nations. The findings in this thesis is based on data gathered from 12 weeks of fieldwork in Kenya.

The thesis was made possible through the funding from:
The Swedish International Development Cooperation Agency (Sida),
The Swedish Association of Graduate Engineers,
The Swedish Foundation for Strategic Research (SSF),
Carl Erik Levins Stiftelse, and
Stiftelsen Gefle Köpmannaförening - Ernst, Sven och Jacob Engwalls Stipendiefond.

Acknowledgements

This thesis work is the sum of all of its contributors. The thesis could not have been completed without the help of many, my deepest gratitude goes to the following:

My supervisors, Pelle and Edith, who have shown nothing but support and encouragement from day one. Thank you for all your guidance. I could not have wished for better supervisors.

To Joe Hanbang Zou for all your time and valuable input in making this project both practically doable and successful.

The SoilChip team: Julia Duljas, Micaela Mafla Endara, and Milda Pucetaite, for showing me the ins and outs of fabricating chips and for always helping me in the lab.

To Axel Tojo for the help you gave me to mill out the PMMA support structures for the chips.

To Håkan Wallander for all your input early on during the initial planning stages of this project, and for connecting me with Dries and Geoffrey at IITA. And for your book of course!

To the Swedish International Development Cooperation Agency, The Swedish Association of Graduate Engineers, Carl Erik Levins Stiftelse, and Gefle Köpmannaförening Stipendiefond – Ernst, Sven och Jacob Engwalls Stipendiefond, who financially supported this project.

To my main contact persons and supervisors on-site in Kenya, Dries Roobroeck and Geoffrey Kimutai Maritim, for welcoming me to IITA and for making this study possible. Thanks for all the good discussions and for always checking up on me.

To my field assistant Richard Ouma who always made sure that I had everything I needed out on the field.

To Afrika Okello for all the valuable discussions and input on the questionnaire and social study.

To Sylas Obara and Linda Barasa at IITA for all the help you gave me with administrational and practical matters in Kenya, and for always greeting me with a big smile.

To the whole team of NemAfrica lab who welcomed me to their lab and always was there to assist me. Thanks Kanan, Sharline, Evans, Ivy, Calvin, Mellen, Simon, Newton, Judy, Celestine, Danny, and Solveig.

To all the people who gave me the warmest welcome in Siaya. A special thanks to Grace and Chrispin Bodi, and to the beautiful family of Alice, Paul, Skola, Konsalata, Clinton, Carol, and Beatrice who invited me to be a part of their family. Asante sana, tutakutana tena.

To my good friend Kasper who I started this journey with.

And finally, my deepest gratitude to Josefine and my family, for their never-ending support, both in good and bad.

Contents

1 Introduction **1**

1.1 Background 1

1.1.1 Agriculture, the food system, and the climate 1

1.1.1.1 Kenya - agriculture and malnourishment 1

1.1.1.2 Biochar-enrichment in agriculture 2

1.1.2 Soil at the microscopic level 2

1.1.3 Microfluidics and soil ecology 3

1.2 Purpose and research questions 4

1.3 Delimitations 4

2 Methodology **5**

2.1 Chip fabrication and preparation 5

2.1.1 Master production 5

2.1.2 In-situ chips 5

2.1.2.1 Chip design 5

2.1.2.2 Chip production 6

2.1.2.3 Preparation and transportation of chips 7

2.1.3 In-lab chips 9

2.1.3.1 Chip design 9

2.1.3.2 Chip production 10

2.1.3.3 Preparation of solutions and chips 10

2.2 Chip installation 11

2.2.1 In-situ experiments 11

2.2.1.1 Experimental plots design and description 11

2.2.1.2 Chip placement selection 11

2.2.1.3 Chip installation and plot details 12

2.2.2 In-lab experiments 12
2.2.2.1 Inoculation of soil 12
2.3 Data collection and analysis 13
2.3.1 In-situ experiments 13
2.3.1.1 Data description and collection 13
2.3.1.2 Measurement description 15
2.3.1.3 Statistics and data visualisation 16
2.3.2 In-lab experiments 16
2.3.2.1 Data description and collection 16
2.3.2.2 Measurements description 16
2.3.2.3 Statistics and data visualisation 17
2.4 Questionnaire 17
2.5 Supplementary methodological information 17

3 Results **18**

3.1 In-situ experiments 18
3.1.1 Qualitative results 18
3.1.2 Quantitative results 18
3.1.2.1 Microbial abundance 19
3.1.2.2 Chip colonization depth ratio (CCDR) 21
3.1.2.3 Fungal to bacteria ratio 23
3.2 In-lab experiments 24
3.2.1 Qualitative results 24
3.2.2 Quantitative results 25
3.3 Questionnaire 27
3.3.1 Quantitative results 27
3.3.2 Qualitative observations 28

4 Discussion **29**

4.1 Measurements - microbial abundance and CCDR 29

4.2 Biochar and its effect on microbial communities 30

4.3 Methodological approach and potential improvements 31

4.4 SoilChip as an analysis tool 33

5 Conclusion **34**

5.1 Conclusions 34

5.2 Future outlook 34

References **35**

A Experimental plot design **38**

B Questionnaire **39**

C In-situ raw data **41**

D p-values and F-ratios, in-situ experiments **43**

E In-lab data **44**

1 Introduction

1.1 Background

1.1.1 Agriculture, the food system, and the climate

Agriculture is the very foundation onto which our modern society is built upon. Some 10 000 years ago, agriculture allowed us to leave the hunter-gatherer lifestyle and it has since supported us in building the society we live in today. But it is not without its challenges. Although our agricultural system is more productive than ever, over 800 million people are still living in hunger [1], and to build on this, current agriculture practices are at the same time exerting an immense ecological pressure and is responsible for up to 29% of global greenhouse gases [2], and an unsustainable use of fertilizers causes huge disruptions in both soil and aquatic ecosystems, as it eutrophizes lakes and streams, favouring solely a few fast-growing species in the ecosystems, while the gross of biodiversity is quickly outcompeted [3, 4]. With an estimated global population of almost 10 billion people by 2050, there is mounting pressure to make agriculture and the food system both more efficient and sustainable.

One region that is particularly feeling the consequences of insufficient nutrition is the countries around the Horn of Africa in Eastern Africa. Recent droughts and shortages in grain and fertilization supply have brought a situation near crisis in the region, with an estimated 43 million people in both acute[5] and chronic[6] hunger of and many alarming recent reports of hunger crisis [7][8]. Kenya is one of the countries experiencing these challenges today.

1.1.1.1 Kenya - agriculture and malnourishment

Several studies have been performed to analyse the degree of malnourishment in the Kenyan population. These studies analyse if the population is suffering from e.g. stunting, a condition that is defined as the “impaired growth and development that children experience from poor nutrition, and inadequate psychosocial stimulation” [9]. Stunting can cause cognitive impairment, which increases the risk of having slowed development of motor functions, lowered brain function, and poor school performance [10]. Another condition that is investigated is wasting, which is a condition referring to children that are too thin for their height as a result of surrounding factors, e.g. malnutrition or illness. It is often seen as a rapid decline in nutritional status during a short time frame, especially for children below five years of age [10]. Furthermore, another defining factor often described in nutritional studies is the term underweight, meaning that an individual is weighing below two standard deviations from the expected median weight of the individual’s age, compared to the reference population [11]. Out of Kenya’s children under five years old, 26% are stunted, 4% are suffering from wasting, and 11% are underweight [12].

However, malnourishment is not the only issue present in Kenya. A multidimensional analysis performed by the Swedish International Development Cooperation Agency (Sida) showed that as many as 36.1% of the population in the years 2015/16 were impoverished [13]. Additionally, drought, more unpredictable weather patterns, and the significant population increase in Kenya, have pushed agriculture into arid lands, which has escalated conflict of natural resources [14].

Agriculture is the dominating economic activity in Kenya, contributing with approximately 33% of GDP, and employs over 70% of the rural population[15]. Small-scale agriculture is widespread and produce an estimated of 75% of Kenya's food. To increase efficiency and robustness in small-scale agriculture in Kenya is key, not only to combat hunger and malnourishment, but also to empower big portions of the Kenyan population. One approach that is being studied for making Kenyan agriculture both more productive and self-reliant is biochar-enrichment of the soil.

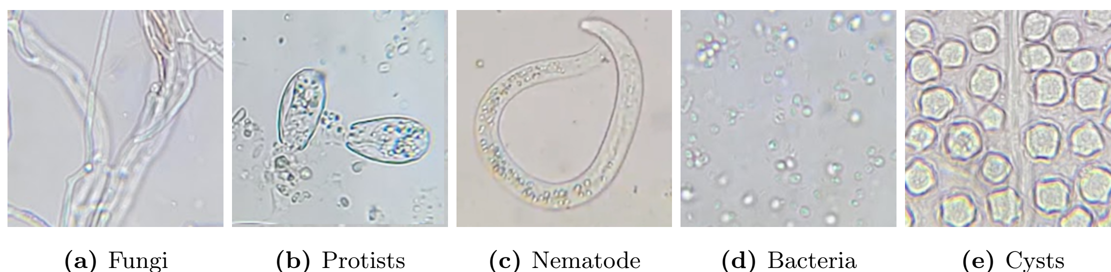
1.1.1.2 Biochar-enrichment in agriculture

Biochar is pyrolyzed biomass similar to charcoal, with the purpose to be used as a soil amendment that can increase, amongst others, the soil's water holding capacity[16], microbial biomass[17], ion exchange capacity[18] and general fertility[16]. Through previous research initiatives, biochar can be produced locally by the farmers by using specially-designed gasifier cooking stoves[19]. These stoves can also function as cooking and housewarming stations and, with a lower release of air pollutants, thereby improving the living standard of the families participating in this study.

Biochar addition to agriculture has been studied in Siaya County in Western Kenya since 2007, and it has been shown in previous studies that the addition of biochar to Kenyan soil has increased the long-term yield of various crops [20]. They showed that single-time application of biochar had both long-term and additive effects to crop yields together with fertilizer. If biochar-enrichment could be implemented systematically, it could improve local farmers' production capabilities which, in turn, could raise the economical and nutritional standard of the surrounding communities. [20]. Researchers from the International Institute of Tropical Agriculture (IITA) are continuously monitoring the effects of biochar-enrichment, through both ecological and sociological studies of biochar production and application for agriculture. This research conducted in this thesis has been made in collaboration with IITA, and aims to bring further insights to the effects of biochar-enrichment.

1.1.2 Soil at the microscopic level

Healthy soil is a complex and thriving landscape rich of life and diversity. It is said that in a single spoon of soil you can find more microbes than people on the planet. Soil microbes play a crucial role in decomposing organic matter and in nutrient recycling in the soil, which both are essential for making nutrients available for plants and crops. Soil microbial communities can conventionally be categorized into four different subgroups, namely: fungi, protists, nematodes, and bacteria. Fungi are eukaryotic mircroorganisms such as molds, yeasts, and mushrooms. Protists are free-living, aquatic, single-cell, eukaryotic organism, usually 5-50 μm in diameter. Nematodes are microscopic worm-like organisms, typically 50-1000 μm in size. All of these subgroups will be studied in this thesis, in addition to cysts, which is the dormant state of protists. See figure 1 below for images of the different groups of soil microbes.



(a) Fungi **(b)** Protists **(c)** Nematode **(d)** Bacteria **(e)** Cysts

Figure 1: Different subgroups of microorganisms living in the soil.

The microbial community can be studied in a numerous of methods, from over-arching analysis of ecosystem functions such as general microbial activity through soil respiration measurements[21], to biochemical methods for studying the diversity and changes within it at larger scale of groups of organisms of the microbial community[22], to more detailed studies of species composition at high phylogenetic resolution with help of molecular methods such as qPCR [23]. All different methods bring important insights to the microbial life in our soil, but all are based on indirect measurements, and none can offer direct, in-situ, visualisation of the community, and neither of those can study functions, interactions and processes embedded into their natural environment.

Soil is an incredibly complex environment, with clumps and fluid-filled channels ranging from centimeters to nanometers in scale[24], and soil microbes must traverse these obstacles to find nutrients. To study how microbes interact and behave in different complex soil environments has traditionally been difficult due to the soils high complexity and non-transparent composition. In recent years however, microfluidics has emerged as a promising tool to overcome these challenges.

1.1.3 Microfluidics and soil ecology

Microfluidics is the research field and technology where small volumes of liquids/fluids (10^{-9} to 10^{-18} litres) are studied and manipulated through the use of micrometer-scale channels and structures. The field has been growing rapidly since the late 1970's, with many applications in analytical chemistry, diagnostics, and biomedical research [25][26]. In addition to the self-evident advantages of requiring less space and sample due to the minuscule dimensions, it also draws benefits from laminar fluid flow[27], which allows for precise control and manipulation of fluids without the chaotic effects of turbulence. Microfluidic chips, often called lab-on-a-chip, are fabricated with technology originating from MEMS-technology for fabricating transistors and IC-chips. Through the use of soft lithography and the polymer polydimethylsiloxane (PDMS), fabrication of transparent and robust microfluidic chips with precise micrometer-scale structures is possible [28, 29].

Microfluidics has traditionally seen great utilization for analyzing human samples in medical research, but in recent years it has also branched out to study soil ecology[30]. At Lund University, an interdisciplinary research team has recently developed lab-on-a-chip techniques, called SoilChip technology, for studying soil microbes [31, 32, 33]. Analysis of both soil microbiology and ecology is possible using this method and the technology can be used to shed new light on different questions in the soil research field. Previously, the researchers have tested several different theses, such as: what microorganisms can travel into the chips depending on the channels' shape and size[34, 35]; the effects on nano- and microplastics on soil ecosystems; how small scale structures affect the nutrient uptake of large scale nutrient systems[33].

1.2 Purpose and research questions

The aim of this thesis is to utilize microfluidic SoilChip technology to investigate how biochar-enrichment of soil affects the microbial ecosystem. This will be done for both fertilized and un-fertilized soil for small-scale agriculture in Kenya. More specifically, the microbial abundance and microbial foraging characteristics will be investigated. Furthermore, the thesis aims to investigate whether visualisation of the soil microbial ecosystem can affect peoples' perception and evaluation of soil and soil management. Finally, this thesis will act as a feasibility study of using SoilChip technology in distant field studies in rural and low-resource areas, far from typical lab environments.

Research questions

1. How can microfluidic SoilChip technology be employed for studies in distant, rural, and low-resource areas?
2. What are the effects of biochar-enrichment on soil microbial abundance?
3. What are the effects of biochar-enrichment on soil microbial growth characteristics?
4. How do different soil microbes interact with biochar particles?
5. How can visualisation of the soil microbial ecosystem affect peoples' perception and evaluation of soil and soil management?

1.3 Delimitations

- Although rough classification of soil microbes will be done in this thesis work, there will be no further specific species identification beyond the following categories: fungi, nematodes, protists, cysts, and bacteria. Further specification is doable, but not feasible within the time-frame of this thesis work.
- The data gathered in this thesis will only come from a single geographical location: Nyabeda, Siaya county, Kenya.
- No other soil analysis will be conducted in this thesis.
- No new chip design will designed for the specific purpose of this study. Chip designs from previous studies will be used.

2 Methodology

This thesis consists of three different main experiments; in-situ experiments, in-lab experiments, and a questionnaire. The in-situ experiments had chips installed directly in the field, allowing microbes to colonize the chips. The in-lab experiment was used to gather time-resolved data, using harvested soil from the field. The questionnaire was designed to gather insights from the local farmers of visualisation of the microbial ecosystem. The methodology of this study consists of three different main steps: chip fabrication and preparation, chip installment, and lastly, data collection and analysis.

2.1 Chip fabrication and preparation

2.1.1 Master production

The master fabrication followed conventional SU8 microfabrication, and was prepared prior to this thesis. A negative photoresist (SU-8 5, MicroChem Corp, USA) was spin coated to a thickness of approximately 7 μm (60 seconds at 1250 rpm). The photoresist was then soft baked at 90°C for 5 minutes, patterned by UV exposure (Karl-Suss MA4 mask aligner), and baked again post UV exposure. After baking, the master was developed in mr-Dev 600 for three minutes, and then rinsed clean with isopropanol (IPA).

2.1.2 In-situ chips

The in-situ chips were prepared for the field experiments conducted in Nyabeda, Siaya county, Kenya during March-May 2022. These chips were installed in the field by burrowing them in the agricultural plots.

2.1.2.1 Chip design

The microfluidic chip design used in-situ experiments is a previously designed chip called the 'Obstacle chip' [36], see schematic in Figure 2. The chip design is 60x22 mm in dimensions, with microstructures of a height of 10 μm. The chip consists of an entrance system with pillars, which connects to five different set of geometrical structures. The chip entrance runs across the full length of the chip side, which is immediately followed by a pillar system with pillars 100 μm in diameter with a distance of 100 μm between each other. The pillar system is then followed by the set of different structures. The different geometrical structures are highlighted in Figure 2.

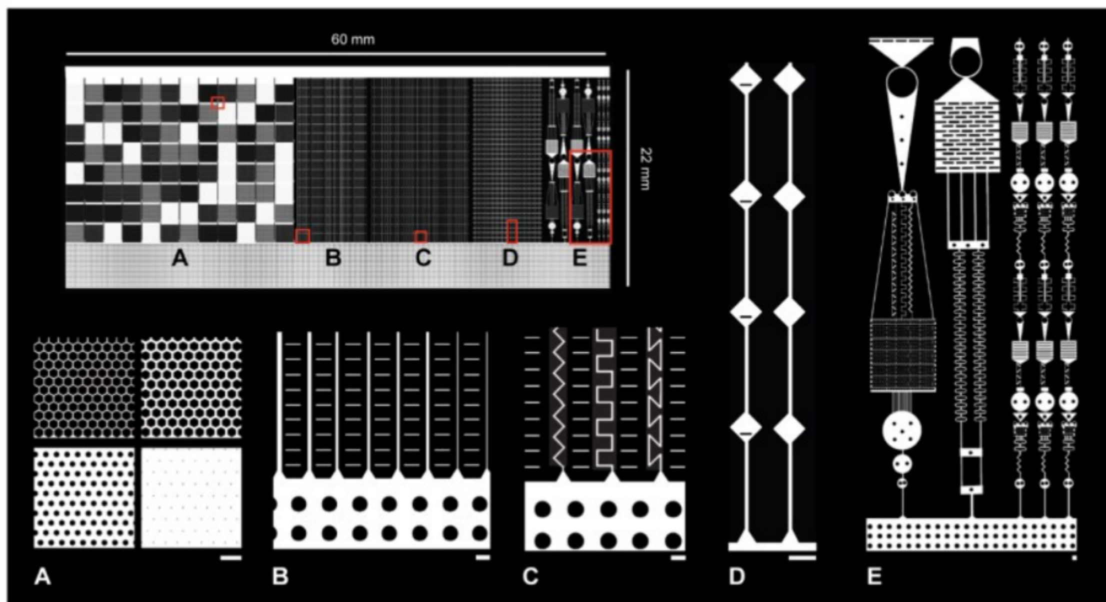


Figure 2: Schematic overview of the in-situ microfluidic chip design. pillars entrance system to different set of structures and obstacles. Structure *A* is hive-like structure with hexagonal channels with alternating widths, structure *B* are channels with alternating channel widths, structure *C* shows channels with alternating turning angles, structure *D* shows channels with diagonal boxes, and structure *E* shows a combination of different structure forming a complex obstacle course. Image taken from [32].

2.1.2.2 Chip production

PDMS slab preparation. PDMS monomer (Sylgard 184, Dow Corning, USA) was mixed with curing agent (Sylgard 184, Dow Corning, USA) at a 10:1 ratio. The mix was stirred thoroughly until the mixture was visually full of air bubbles. The PDMS mixture was poured over the master taped to the bottom of a Petri dish until a minimum PDMS height of 5 *mm* was reached. The Petri dish was placed in a vacuum chamber for a minimum of three hours, or until all residual air bubbles had left the PDMS mix. Any remaining air bubbles were removed physically from the PDMS using a polymer pipette tip. Once the PDMS was completely free of air bubbles, the Petri dish was placed in a 60°C oven for a minimum of 12 hours to cure. Once cured, a slab with the approximate dimensions of 30x50 *mm* was cut out using a scalpel. The cut-out PDMS slab was transferred to a storage container with structures facing up. Tape (Scotch Magic™ Tape) was placed on top of the structures to avoid dust contaminating the chips. The chips were left as this until the following preparation steps took place, a maximum wait of 30 days. A total of four different masters with the same design was used for the chip production.

Bonding. The PDMS slabs were bonded onto glass coverslips. Coverslips (54x70 mm, 0.13-0.16 *mm* thick, Epredia) were cut to 45x54 *mm* using a handheld glass cutter. After cutting, the coverslip was cleaned using isopropyl alcohol (IPA) and blowdried using nitrogen gas. The tape that was previously placed on the PDMS slab for dust protection was removed, and a new piece of tape (Scotch Magic™ Tape) was placed across the outermost 5-10 *mm* of the pillar system, close to the chip entrance, of the PDMS slab to hinder activation and bonding of this part. The clean

coverslip was placed in a plasma chamber (Diener Electronic Zepto) and activated for 60 seconds. The PDMS slab, put on a glass slide with structures facing up, was then also placed in the plasma chamber together with the coverslip and plasma activated for 18 seconds. After activation, the tape was removed from the PDMS slab and the activated PDMS slab and coverslip were gently pushed together to allow bonding. A distance of at least 1 *cm* of coverslip from the chip entrance was ensured to allow of soil placement. The chip was then placed on a glass slide on top of a 80°C heating plate for a minimum of 3 minutes. Bond strength was tested, and if the bonding strength was deemed to low, additional uncured PDMS solution was added to all outer walls of the PDMS slab, and then the chip was replaced on the 80°C heating plate for a minimum of 30 minutes. The ready chip was stored in room temperature until further preparation steps took place.

2.1.2.3 Preparation and transportation of chips

The chip preparation process consists of four different steps: filling the chips with Milli-Q water, preparing the chips for transportation, diffusing nutrient solvent into the chips, and finalizing the chip with support structures. The preparation process was initialized in Lund, Sweden, due to equipment requirements, and later finalized in Nyabeda, Kenya, to minimize the risk for contamination in the chips. This meant that the chips had to be transported semi-finalized from Sweden to Kenya. The following requirements were set up for the transportation, which had to be considered during the chip production and preparation process.

- The chips must withstand any physical forces during transportation to avoid breaking.
- The chips must be filled with liquid in Lund, and they must not dry out during transport.
- The chips must be kept sterile to avoid contamination of bacteria and any other microbes before the chips are to be installed in Kenya.

Fill chips with Milli-Q water (Lund)
After chip production, as described above in section 2.1.2.2, the bonded chips were filled with Milli-Q water using the plasma oven. A small cut was made with disinfected scalpel by the corner of the chip entrance and pillar system. The full length of the entrance was not cut opened as it would increase the risk of the chip drying out during transportation. For approximately one quarter of the chips, both corners of the chip entrance were cut opened due to a scratch by the pillar system in the master that completely separated the two sides of the chip, and thus, the chip had to be water filled from two different sides. The cut chip was placed in the plasma oven for activation, which turns the chip hydrophilic, which allows for filling the chip by capillary forces. The chip was activated for 30 seconds, and fresh Milli-Q water from an autoclaved beaker was placed on the coverslip by the chip entrance and water entered the chip through capillary forces.

Preparing chips for transportation (Lund)
The Milli-Q water-filled chip was placed in a fresh Petri dish. The previously cut PDMS corner was placed back in position to act as a lid, minimizing the risk of the chip drying out during transportation. Tape (Scotch Magic™ Tape) was used to keep both the PDMS corner and the chip in place in the Petri dish. The Petri dish lid was sealed with Parafilm along all edges, and tape

was again added to ensure the lid not opening. The Petri dish with the ready chip was placed under UV-light for 30 minutes to ensure sterility prior to being placed in a bump resistant box for transportation. See Figure 3a below for a photo of the chip ready for transportation.

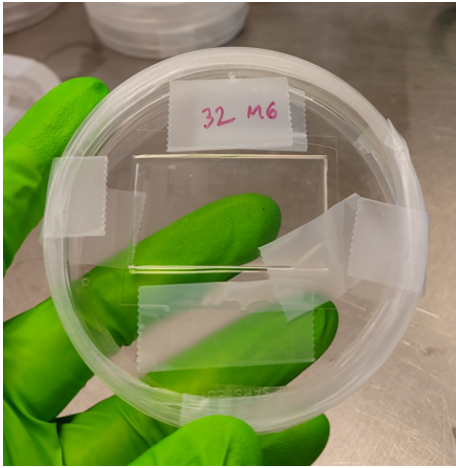
Diffusion of malt extract solution into chips (Nyabeda)

All chips were filled with malt extract in order to attract the microbial community and accelerate chip colonization. This was done through diffusion of a prepared malt extract solution into the Milli-Q water-filled chips. This was completed as close in time to chip installment as possible to minimize the time of any potential contaminating bacteria populating the nutrient-rich chips, while still allowing time for diffusion to occur.

The parafilm covering the Petri dish sides was cut open using a scalpel. The Petri dish lid was then opened as little as possible to allow the scalpel to enter. The chip entrance was cut open and the extra PDMS was moved to the side. Approximately 300 μL of mixed malt extract solution (10 g/L) was added evenly along the chip entrance using a polymer pipette. The Petri dish was then closed and sealed with parafilm again, and the chip was left to stand on bench for a minimum of 4 hours before installment. Ethanol was used to clean all surfaces and gloves after each chip preparation. Tools were cleaned by dipping in ethanol after each step. All lids were opened as little as possible to avoid contamination.

Finalize chip construction with support structures (Nyabeda)

To minimize the risk of the chip breaking after installment due to physical pressure from the surrounding soil and water, the chip was placed in support structure. The support structure comprised of a PMMA bottom (4x50x60 mm) to which the chip was placed on top of. Tape (Scotch Magic™ Tape) was placed along all coverslip edges to keep chip in place and avoid moisture entering in between the PMMA slide and the chip. Bubble wrap was then attached on top of the chip with tape to alleviate pressure from the soil above away from the chip, and to avoid any unforeseen physical shocks from above to be directly targeted at the chip. The chip entrance was left open to allow access for the soil. Finally, a string with a metal ring attached at the other end was attached to the bottom PMMA structure for marking the chip installment location for chip harvesting. All support structure pieces were prepared before-hand, but the final construction took place on the field immediately before chip installment. The malt extract-filled chips were transported to the field in the sealed Petri dishes. Figure 3b illustrates the final chip design with support structures.



(a) Semi-finalized chip prepared for transportation. Tape is seen holding both the chip and PDMS corner slab in place. Petri dish is sealed with parafilm.

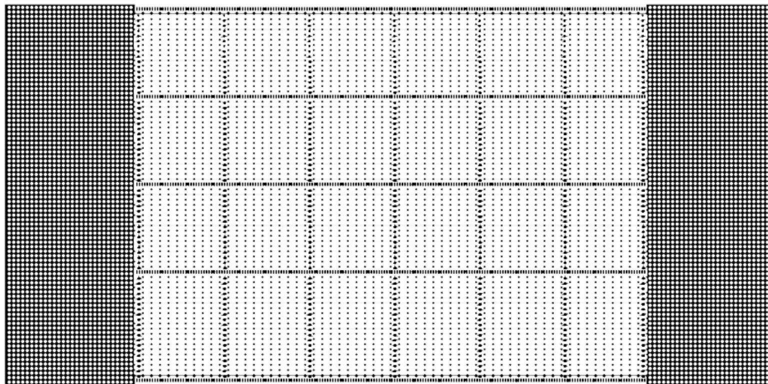
(b) Finalized chip with support structures ready to be installed in the field. The exposed chip entrance is facing the camera Support PMMA bottom, bubble wrap, and string with metal ring can be seen.

Figure 3: In-situ chip preparation and design.

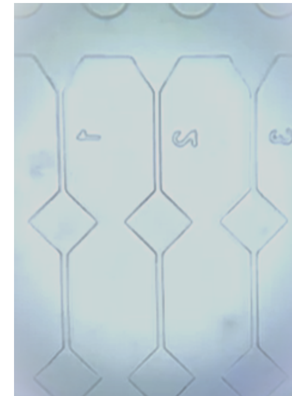
2.1.3 In-lab chips

2.1.3.1 Chip design

For the in-lab experiments, a different chip design was used compared to the in-situ experiments. The in-lab chips comprised of two chip entrances and two supporting pillar systems, at opposite sides of the chip, with one type of geometrical structure in between the entrances, namely channels with boxes. A schematic of the chip design can be found in Figure 4 below.



(a) In-lab chip schematic.



(b) Channels with boxes.

Figure 4: In-lab chip design. Figure 4a shows a schematic over the full chip, with pillar systems in the darker areas to the left and right. Figure 4b highlights the channels and structures connecting the two pillar systems.

2.1.3.2 Chip production

The production of the in-lab chips followed a similar methodology as the in-situ chips. When identical, it is referred to previous sections. Differences in the procedure are mentioned in the text.

PDMS slab production

The PDMS slab production followed the same methodology as the in-situ chips, described in section 2.1.2.2. For the in-lab chips, two masters were used in total, in contrast to four masters used for the in-situ chips.

Bonding

The bonding procedure followed the same methodology as the in-situ chips, described in 2.1.2.2. However, coverslips were cut to the dimensions 25x54 mm to match the chip dimensions. The PDMS slab was bonded to allow for soil to be placed on the coverslip next to the chip entrance.

2.1.3.3 Preparation of solutions and chips

As the in-lab chips had both an entrance and exit for fluids, the chips could be filled with liquids on-site in Kenya, without the need for a plasma oven. A polymer tubing with 1 mm in diameter was attached to one of the pillar systems, and the other pillar system was cut open across the full length with a scalpel.

Preparation of solvents (Lund)

Malt extract was mixed with Milli-Q water to a final concentration of 10g /100mL. The solution was stirred extensively with a magnet stirrer to ensure the malt extract to be fully dissolved.

To allow the biochar to be introduced inside the chips, it had to be crushed down to particle sizes smaller than the microfluidic channel height of 10 μm. This was done through the use of mortar and pestle, and a combination of different sized sieves, specifically 250μm, 125μm, 50μm, 20μm. The final 20 μm particles were placed on top a 2.7 μm filter in a funnel, onto which Milli-Q water was poured to push through biochar particles. The biochar particle-Milli-Q solution was collected in a Falcon tube under the filter. Milli-Q water was continuously added until a satisfactory amount of biochar particles had passed the filter and entered the Falcon tube. The concentration of biochar particles could not be determined using this methodology. Both the malt extract solution and the biochar particle solution were autoclaved before placed in bags for travel.

Filling the chips with solvents (Nairobi)

The chips were carefully removed from the sealed Petri dish and placed in new, individual Petri dishes, one per chip. Initially, ethanol was pushed through the chips to remove any potential contamination. Then, the specific solvents were introduced to the chips through the polymer tubing by syringe. The solvent was carefully pushed through the channels until it flowed through the exit channel.

2.2 Chip installation

2.2.1 In-situ experiments

2.2.1.1 Experimental plots design and description

The experimental plots were located in Nyabeda, Siaya County in Western Kenya by the Lake Victoria basin. The site consists of a complete randomized block experiment of three soil treatments: bare fallow, unfertilized crop, and fertilized crop. Each treatment is divided into two equal sized parts, one with biochar-enrichment, one without biochar-enrichment. Metal sheets surrounded each plot to avoid cross-contamination of treatments, and the full site was surrounded by fencing. The area experiences bi-annual rain seasons, one in October-December, one in March-May. Maize (*Zea mays*) and soybeans (*Glycine max*) are grown rotations and planted in sync with the rain seasons. Maize was grown during the time of this study.

Biochar produced locally by gasifer ovens[19] from *Acacia* trees was applied in two rounds during 2006 and 2007. Prior to the application, the biochar was crushed down to pieces smaller than 1 *cm*. Fertilization was done with the inorganic fertilizer 'Mavuno' with NPK 10:26:10. Fertilizer was added manually during crop planting. For a fully detailed description of the experimental plots and its management, see [20]. For a schematic overview over the experimental plot design, see Appendix A.

(a) Plots during chip installation.

(b) Plots during chip harvest and analysis.

Figure 5: Experimental plots. Metal sheets can be seen installed around each plot. Fence with barbed wire was surrounding the full field. Differences in crop growth between plots can be seen in plot 5b.

2.2.1.2 Chip placement selection

The chip placement was determined using a semi-randomized setup. Chips were grouped based on the master that had been used to produce them, from which a random pair selection was made between chip and plot number. A total of eight plots, two per plot type, had full representation of one chip per master, while the remaining four plots had chips produced from master number 1 and 2 only, due to more chips being produced from these masters.

2.2.1.3 Chip installation and plot details

A total of 44 chips were installed in the field, $n = 3$ or 4 chips per plot, $n = 11$ chips per plot type. Before installing the chips, three consecutive days with at least one hour of rain fall was waited upon to avoid soil being too dry and to indicate that the long rainy season was incoming. All chips were installed within the same five hour period, the 17th of March 2022. The chips were installed at a depth of 10-20 cm, and 10-20 cm away from the crop row, chip entrance facing the crop row. Soil was placed in contact with the soil entrance before refilling the holes with soil again, see Figure 6 Maize was planted in plant rows seven days after chip installation, the 24th of March. Fully detailed description of the plot design and previous treatments and crop yields can be found in [20].

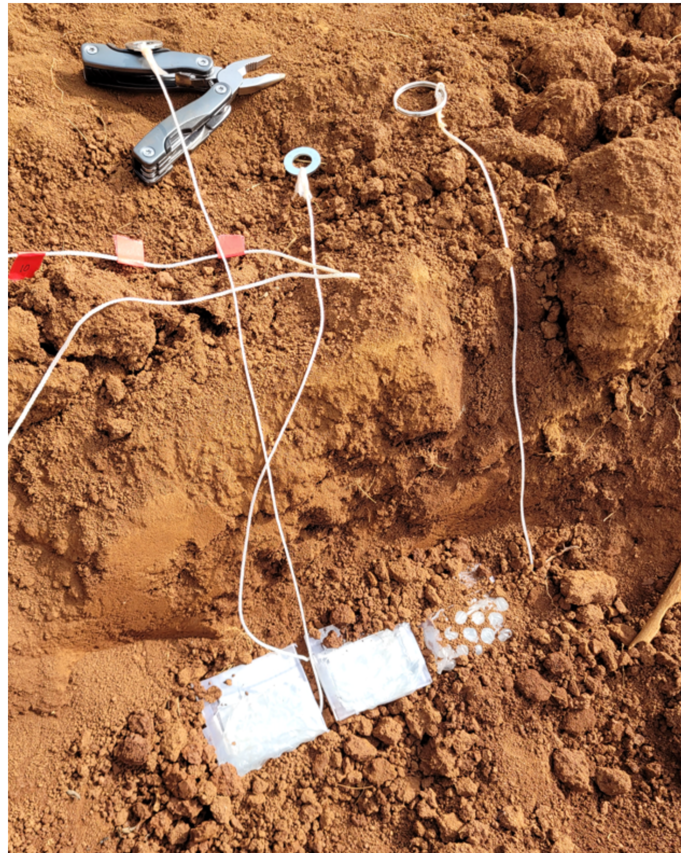


Figure 6: In-situ chip installment. Soil can be seen being placed in contact with the chip entrance. The bubble wrap from the support structure can be seen covered in soil. Strings with metal rings are guided to the surface for chip localization for harvest.

2.2.2 In-lab experiments

2.2.2.1 Inoculation of soil

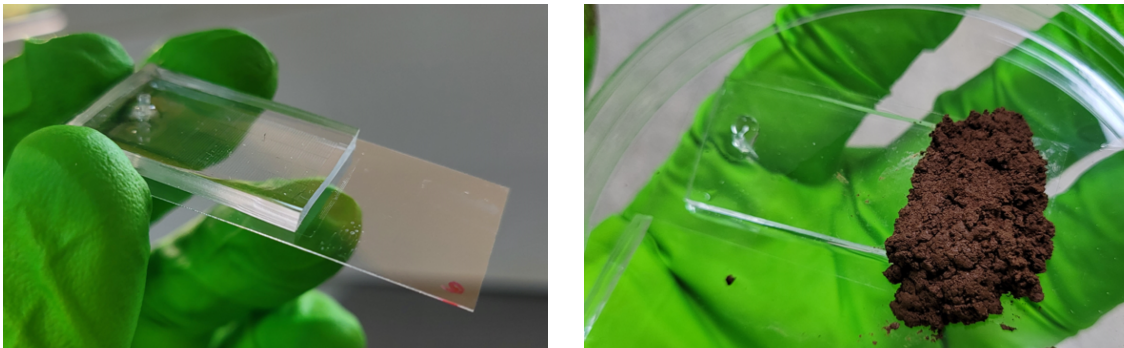
Soil was harvested from the fields in Nyabeda on the 17th of March. Approximately 500 grams of soil per plot type was harvested and placed into air-tight plastic zip lock bags. The soil was

transported by bus to Nairobi in a backpack at ambient temperatures. A total of three different experimental setups were conducted, see Table 1 below.

Test name	Chip treatment	Soil treatment		n chips
Control (A)	Malt solution ($5g/100mL$)	C+F		$n = 2$
Chip BC (B)	Malt solution ($5g/100mL$) + biochar solution	C+F		$n = 2$
Chip+Soil BC (C)	Malt solution ($5g/100mL$) + biochar solution	C+BC+F		$n = 2$

Table 1: Summary of in-lab experiments.

Inoculation of chips took place the 21st of March in lab setting in Nairobi. Before inoculation, the chips were fully prepared as described above in 2.1.3.3. 20 g of soil was weighed and mixed with 2 g of distilled water. The soil-water mix was mixed until no large soil aggregates remained, and the soil-water mix deemed homogeneous. 3 g was collected from the soil-water mix, and placed in connection to the chip entrance. The soil was gently pressed to form a cohesive shape and to ensure that the full entrance was covered by soil. The inoculated chip in the Petri dish was placed in a 27°C incubator, where it stayed during the full experiment duration. It was taken out of the incubator and placed in room temperature during chip analysis.



(a) Ready in-lab chip. Syringe was attached to tubing seen on the left-hand side.

(b) In-lab chip inoculated with soil.

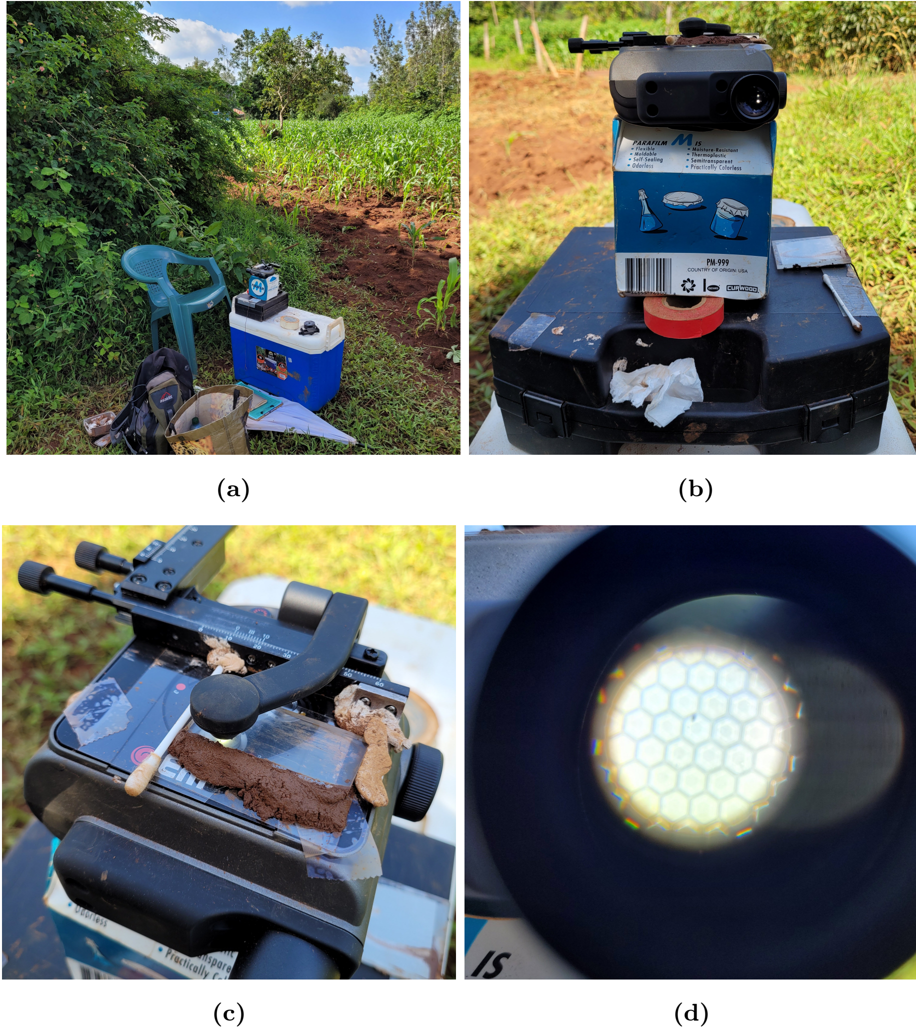
Figure 7: In-lab chip, pre and post inoculation of soil.

2.3 Data collection and analysis

2.3.1 In-situ experiments

2.3.1.1 Data description and collection

The chips were installed in the soil for a total of 43-56 days. Upon harvesting, the chip was immediately prepared for microscopy and analyzed within the same hour. Preparation for microscopy included to remove the chip from its support structure and clean the chip from any soil to improve image quality. The surrounding soil in close proximity to the chip entrance was left intact to both avoid altering the microbial abundance by, for example, pulling fungal hyphae out from the chip structure, and to avoid the chip drying out during analysis. The prepared chip was attached to the controllable XY-axes of the microscope using white tac, attaching the stage with the chip.



(a) (b) (c) (d)

Figure 8: Data collection setup. Figure 8a shows analysis station with a chair and a cooler box as a table on which the microscope is placed. A maize field in the background. Figure 8b shows microscopy setup, with the microscope placed on a height to allow space for the phone. Figure 8c shows chip attached to the top of the microscope, and Figure 8d shows microscopy image through the microscope lens.

The data collected for the in-situ experiments consisted fully of image data gathered from microscopy of the chips. During chip microscopy, data was collected from three different chip depths: at the beginning of the structures (pillar-structure interface), mid-way through structures (5 mm deep from pillar-structure interface), and finally at the end of the structures (18 mm deep from pillar-structure interface). All nematodes, protists, and cysts encountered along a parallel line with 100 μm in width were counted. Fungal hyphae data was collected by counting the number of hyphae crossings in the centre of the longitudinal line. Bacterial data was collected by capturing images of predetermined locations of the chip, also corresponding to the three chip depths, six images per chip depth. The number of bacteria was later determined using a neural network and image analysis. The neural network was trained and validated with data comprising of both historical data from previous experiments, and data from this thesis work. The data gathered for nematodes, protists, cysts, and fungal hyphae were counted simultaneously as the chip analysis took place on-site .

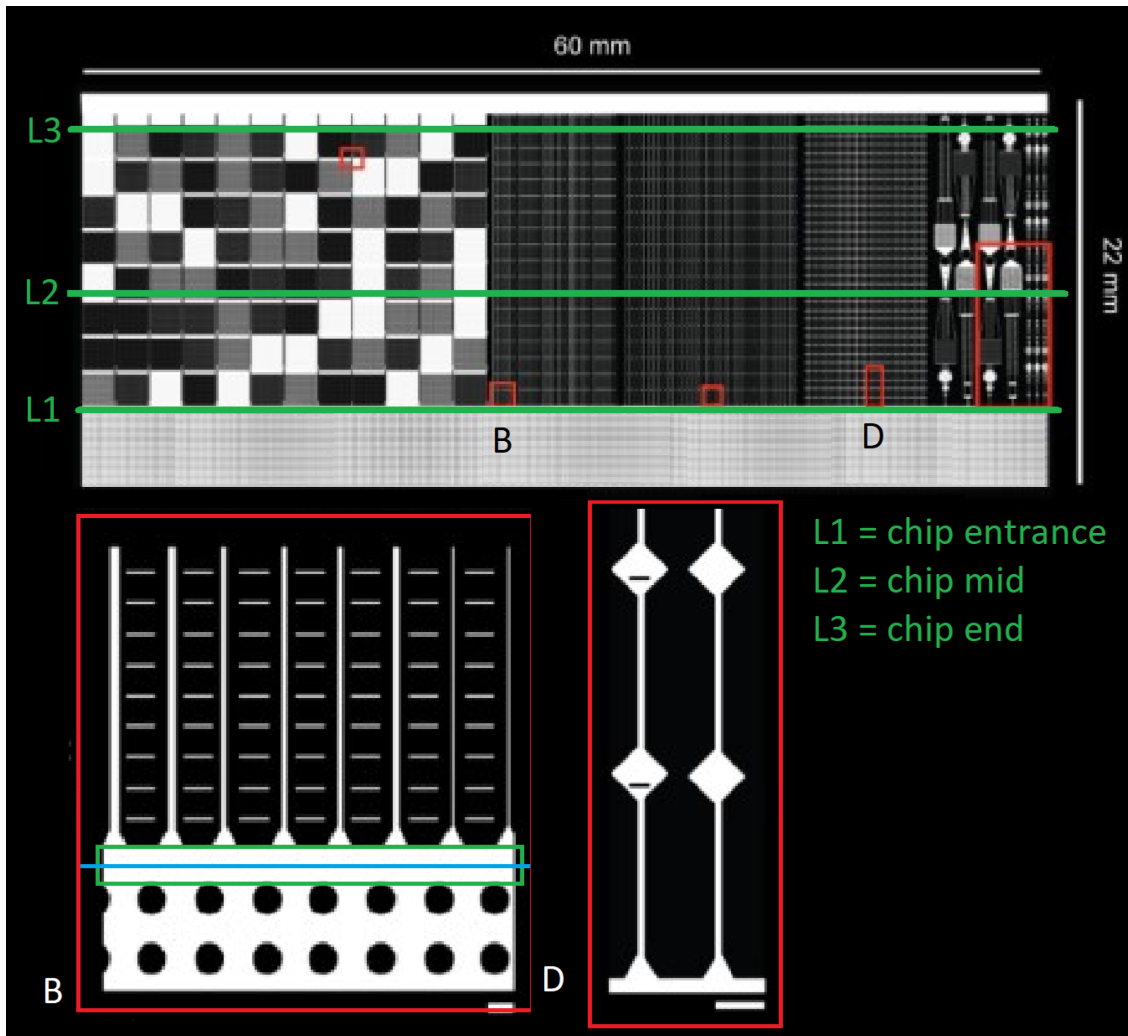


Figure 9: Schematic over the chip design and the data collection method. Green lines show where data was gathered. The green rectangle in red box B highlights the position and size of the area from which protists, nematodes, and cysts were counted from for L1, equal size was used for L2 and L3. The blue line in the green rectangle highlights where fungal hyphae crossing were counted from. Bacteria was counted from boxes from channels in red box D.

2.3.1.2 Measurement description

Based on the gathered data described in 2.3.1.1, two different types of quantitative measurements were compiled; microbe abundance and chip colonization depth ratio (CCDR).

Microbe abundance

The microbe abundance reflects the total number of counted organisms from the species of interest, see equation (1). Abundance was calculated separately for each chip depth.

$$Abundance = \sum counted\ organisms \tag{1}$$

Chip colonization depth ratio (CCDR)

To investigate whether soil amendment affects soil microbial growing capabilities, the chip colonization depth ratio (CCDR) measurement was developed. The purpose of the CCDR measurement is to quantify soil microbes' tendency to grow deeper into the chip once entered. The intent is to measure the microbes' ability to search for, and find, nutrients in complex soil systems. Variation from initial soil conditions is reduced from standardisation based on the microbe abundance in the chip entrance. See equation (2) and (3) below for the definition of the CCDR measurements used in this thesis.

$$CCDR(Mid) \equiv \frac{Abundance(Mid)}{Abundance(Entrance)} \quad (2) \qquad CCDR(End) \equiv \frac{Abundance(End)}{Abundance(Entrance)} \quad (3)$$

2.3.1.3 Statistics and data visualisation

The data was analyzed in JMP version 16.2.0. Significance was determined through full-factorial ANOVA tests. Significant results were defined as: $p < 0.05$, marked * in the results. Marginal significant results were defined as: $p < 0.1$, marked with (*) in the results. The data was compiled and visualized in Microsoft Excel and JMP.

2.3.2 In-lab experiments

2.3.2.1 Data description and collection

For the in-lab experiments, data could be gathered in a time-resolved manner. This allows us to study the dynamics of microbe chip colonization, as compared to the in-situ experiments where we only gather data at one single event. As with the in-situ experiments, the data collected consists fully of microscopy images from the chips. Data was gathered at day 1, day 3, day 8, day 23, and day 37. For each data gathering moment, data was collected at two chip depths: chip entrance and chip end. Images were also taken for counting bacterial data, but could unfortunately not be calculated due to inconsistent data as chips dried out, more about this in section 4.3.

2.3.2.2 Measurements description

Based on the gathered data described in 2.3.2.1, three quantitative measurements were compiled; fungal hyphae channel colonization, furthest fungal hyphae growth, and fungal hyphae chip colonization depth ratio (CCDR).

Fungal hyphae channel colonization

The fungal hyphae channel colonization measurement describes the number of channels populated by fungal hyphae. This measurement was gathered from two chip depths; chip entrance and chip end.

Furthest fungal hyphae growth
The furthest fungal hyphae growth measures the length in millimeters of the fungal hyphae that has reached the furthest in the chip.

Fungal hyphae chip colonization depth ratio (CCDR)
Identical to the CCDR measurement in the in-situ experiments, see 2.3.1.2.

2.3.2.3 Statistics and data visualisation

No statistical analysis was conducted for the in-lab experiments. Data was compiled and visualised in Microsoft Excel.

2.4 Questionnaire

The questionnaire was designed to investigate whether the visualization of the soil microbial ecosystem could affect people's perception and evaluation of soil ecosystems and soil management. The questionnaire was designed to consist of three different parts: two sets of questions, with a set of videos gained from the SoilChip to watch in between. The questions were designed as statements to which the respondents had a scalar answer response with full freedom to reply anywhere on the scale. Answers were later quantified into a scale of 0-15 by measuring the placement of their response. The full questionnaire design and the videos shown to the respondents can be found in Appendix B.

2.5 Supplementary methodological information

Microscopy All microscopy was conducted with the Em1 400 Portable Field Microscope, x10 N/A 0.25 and x40 N/A 0.65. Photos were captured using a Samsung S20 FE 5G SM-G781B phone, with the following camera settings: 12.2MP, 3024x4032, f/1.8, 1/1936, 5.4mm, ISO40. The microscope is seen clearly in Figure 8c.

3 Results

The results presented below are sectioned into three different main sections; in-situ results, in-lab results, and questionnaire results. The in-situ results are based on the results gained from the chips installed out in the field in Nyabeda, the in-lab results are the results gathered from the time-resolved lab trials in Nairobi. The questionnaire results are based on both questionnaire answers and discussions with the respondents. Statistical analysis was carried out for the in-situ experiment, and qualitative results were gathered from the in-lab experiment and questionnaire.

3.1 In-situ experiments

3.1.1 Qualitative results

Crop growth
Upon returning to the field 6 weeks after chip installment, clear differences could be visually observed in crop growth depending on soil treatment. Crops in fertilized plots were significant bigger in size, and crops in biochar-enriched plots were slight, but not clearly, bigger in size. Uncropped plots had, as expected, no crop growth. See Figure 5b for images. Differences in the soil between the different plots could also observed. When harvesting the chips, the soil from fertilized plots was experienced as more difficult to dig in, presumably due to more intense fungi growth. Biochar could also be visually observed in the soil with biochar-enrichment.

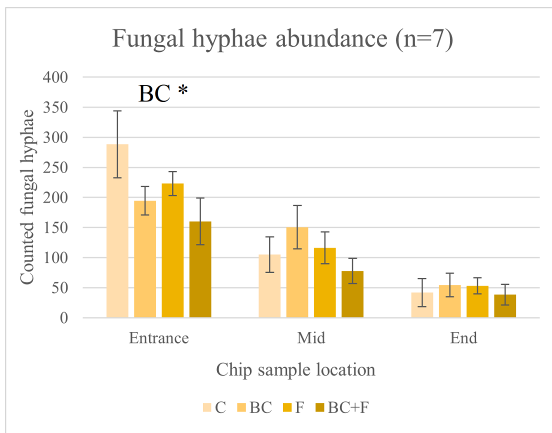
Chip colonization
All members of the microbial community were found to populate the chips: fungi, nematodes, protists, cysts, and bacteria. All 32 analyzed chips saw colonization of fungi, protists, cysts, and bacteria, and all chips except three chips (one from control plot, one from biochar-enriched plot, and one from fertilized and biochar-enriched plot) saw colonization of nematodes. Many different species of fungi, nematodes and protists could be found in the chips. No specific species identification was made for this study, but the based on the image quality reached by the experimental setup in this study, it is possible to complete visual identification of different species.

3.1.2 Quantitative results

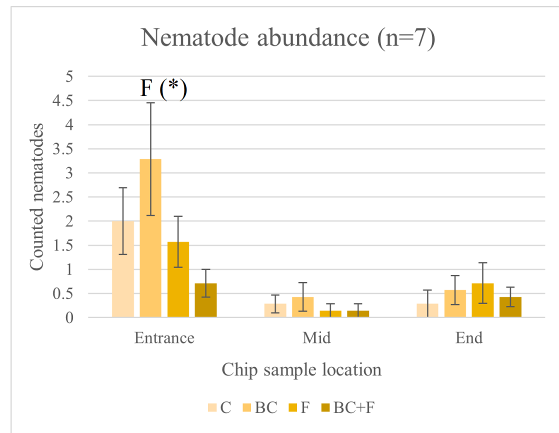
As described in section 2.3.1.2, two measurements were compiled for the in-situ experiments, microbial abundance and chip colonization depth ratio (CCDR). Microbial abundance reflects the total number of individual organisms found in the chip, and the CCDR measurement reflects the microbes tendency to grow deep into the chip once entered. A total of 32 individual chips were harvested and analyzed, from which 28 chips were used in the compilation of the results. The remaining four chips were used to test and define the methodology, and were thus excluded from the results.

3.1.2.1 Microbial abundance

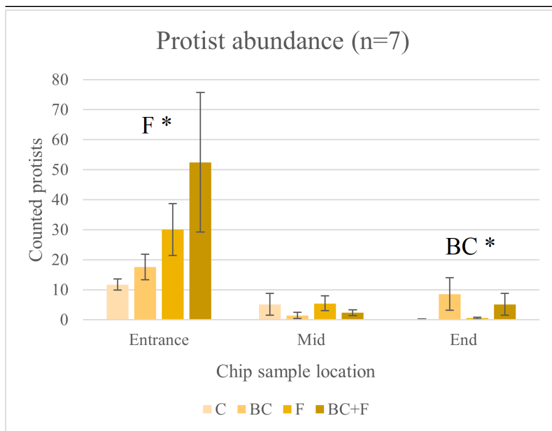
Below in Figure 10, the abundance of different species groups per plot type and chip depths is presented. The results show significant impact of soil amendment for fungal, protist, and bacterial abundance, and weakly significant impact of soil amendment on nematode abundance. Biochar indicates to lower the number of fungal hyphae growing close to the chip entrance, $p = 0.0457$, Figure 10a. Weak, positive, significant impact was found for fertilizer on nematode abundance by the chip entrance, Figure 10b, $p = 0.0546$. Both fertilizer and biochar indicate to increase protist abundance at the chip entrance and chip end, respectively, Figure 10c $p = 0.0181$ and $p = 0.0198$. Fertilizer indicate to increase bacterial abundance in the chip entrance, Figure 10e, $p = 0.0434$. No significant impact was found from either soil amendment on the cyst abundance, Figure 10d, nor the total abundance of microbes, Figure 10f. All p-values and F-ratios can be found in Appendix D.



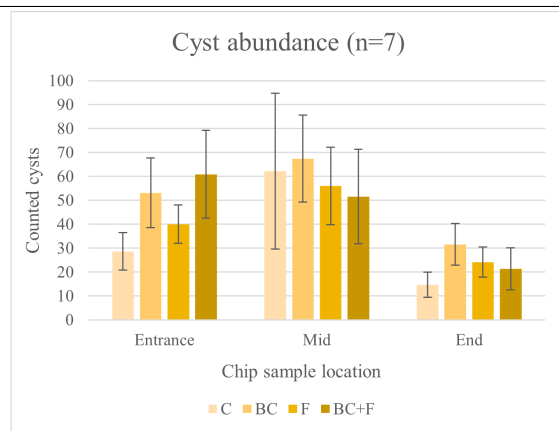
(a) Fungal hyphae abundance.



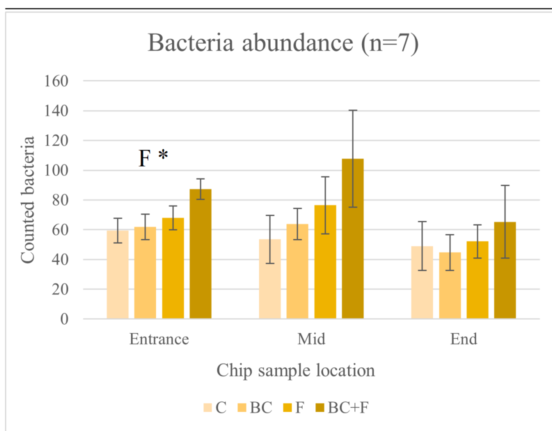
(b) Nematode abundance.



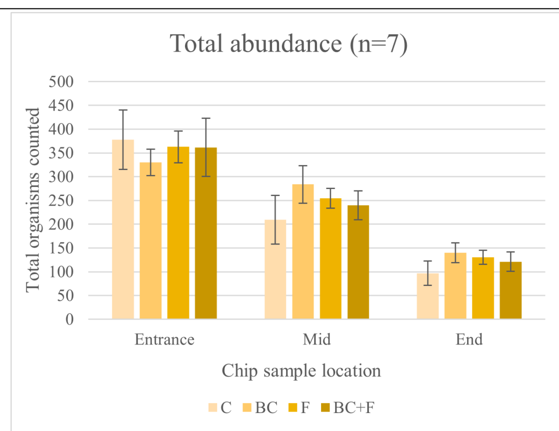
(c) Protist abundance.



(d) Cyst abundance.



(e) Bacteria abundance.



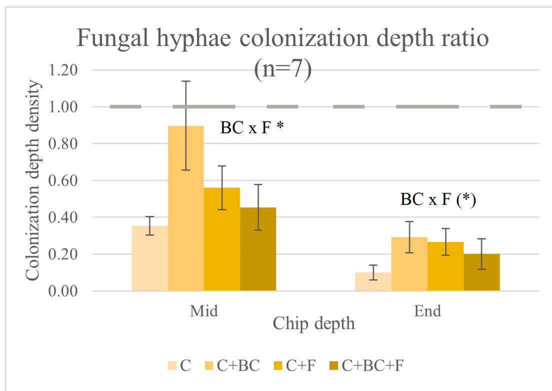
(f) Total abundance.

Figure 10: Average microbe abundance per plot type and chip sample location. Plot 10a to 10e shows different species, plot 10f shows the sum of all species. C = control, BC = biochar-enriched, F = fertilized. The data is collected from n=7 chips from each plot type. Error bars indicate ±1 standard error. A single asterisk indicate significance ($p < 0.05$), an asterisk in parenthesis indicate weak significance ($p < 0.10$) of that plot treatment.

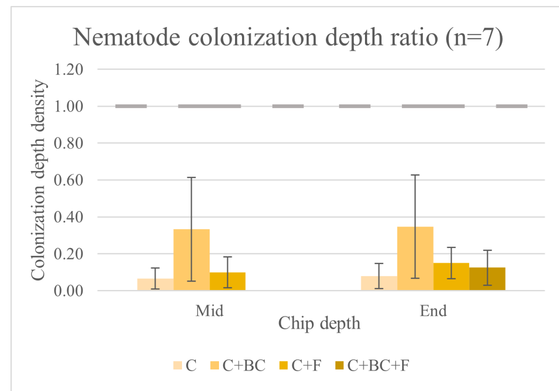
3.1.2.2 Chip colonization depth ratio (CCDR)

As described in 2.3.1.2, the CCDR measurement investigates soil microbes' tendency to grow deeper into the chip once entered. A compilation of the CCDR results can be find below in Figure 11, where each Figure represents a different set of microbe specie, and the different sets of columns represent different chip depths. The dotted line at $y = 1$ represents the microbe abundance at the chip entrance, and higher values denote thus a stronger fungal growth in the middle or in the end of the chips relative to the colonization of the entry parts, respectively.

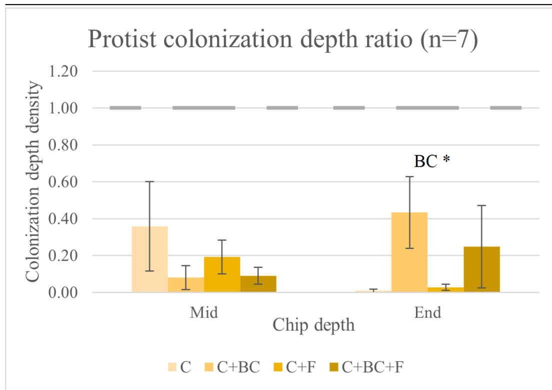
The results show that significant positive impact was found for the combination of biochar-enrichment and fertilization on fungal hyphae CCDR at both chip depth mid (significant, $p = 0.0407$) and end (weakly significant, $p = 0.0883$, Figure 11a). Positive significant impact was found for biochar-enrichment on protist CCDR, Figure 11c $p = 0,0396$. No significant impact of soil amendment was found for nematode, cyst, bacterial, or total CCDR. All p-values and F-ratios can be found in Appendix D.



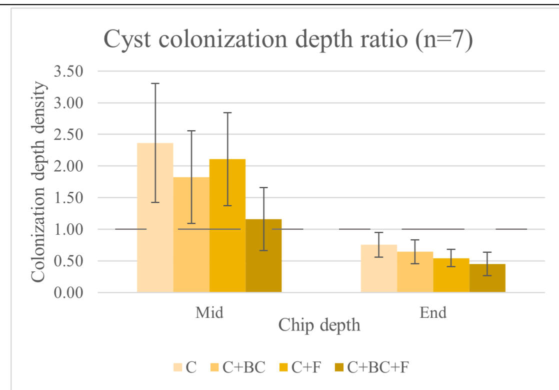
(a) Fungal hyphae CCDR.



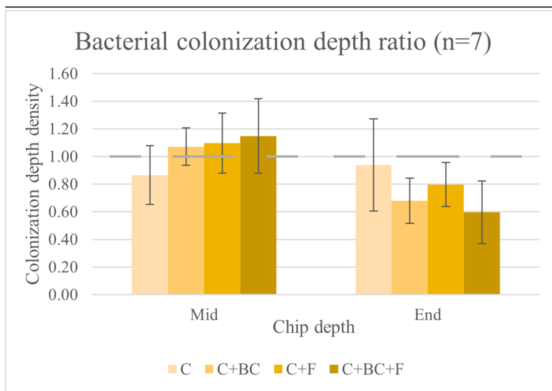
(b) Nematode CCDR.



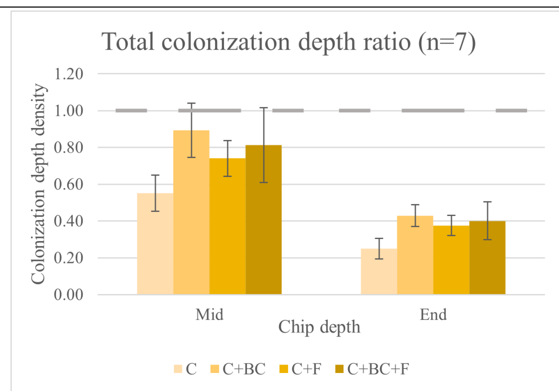
(c) Protist CCDR.



(d) Cyst CCDR.



(e) Bacterial CCDR.



(f) Total CCDR.

Figure 11: Average chip colonization depth ratio (CCDR) per plot type and chip depth. Plot 11a to 11e shows different species, plot 11f shows the sum of all species. The data is collected from n=7 chips from each plot type. Error bars indicate ±1 standard error. A single asterisk indicate significance ($p < 0.05$), an asterisk in parenthesis indicate weak significance ($p < 0.10$) of that plot treatment.

3.1.2.3 Fungal to bacteria ratio

The fungal to bacterial ratio was also calculated. The total counted number of bacteria and fungal hyphae per chip was plotted against each other, separated by plot type, see Figure 12 below. Significant negative dependence could be found for plot type BC+F, with $p = 0.0259$, $R^2 = 0.662$. p-values and R^2-values for all plot types are plotted in the figure.

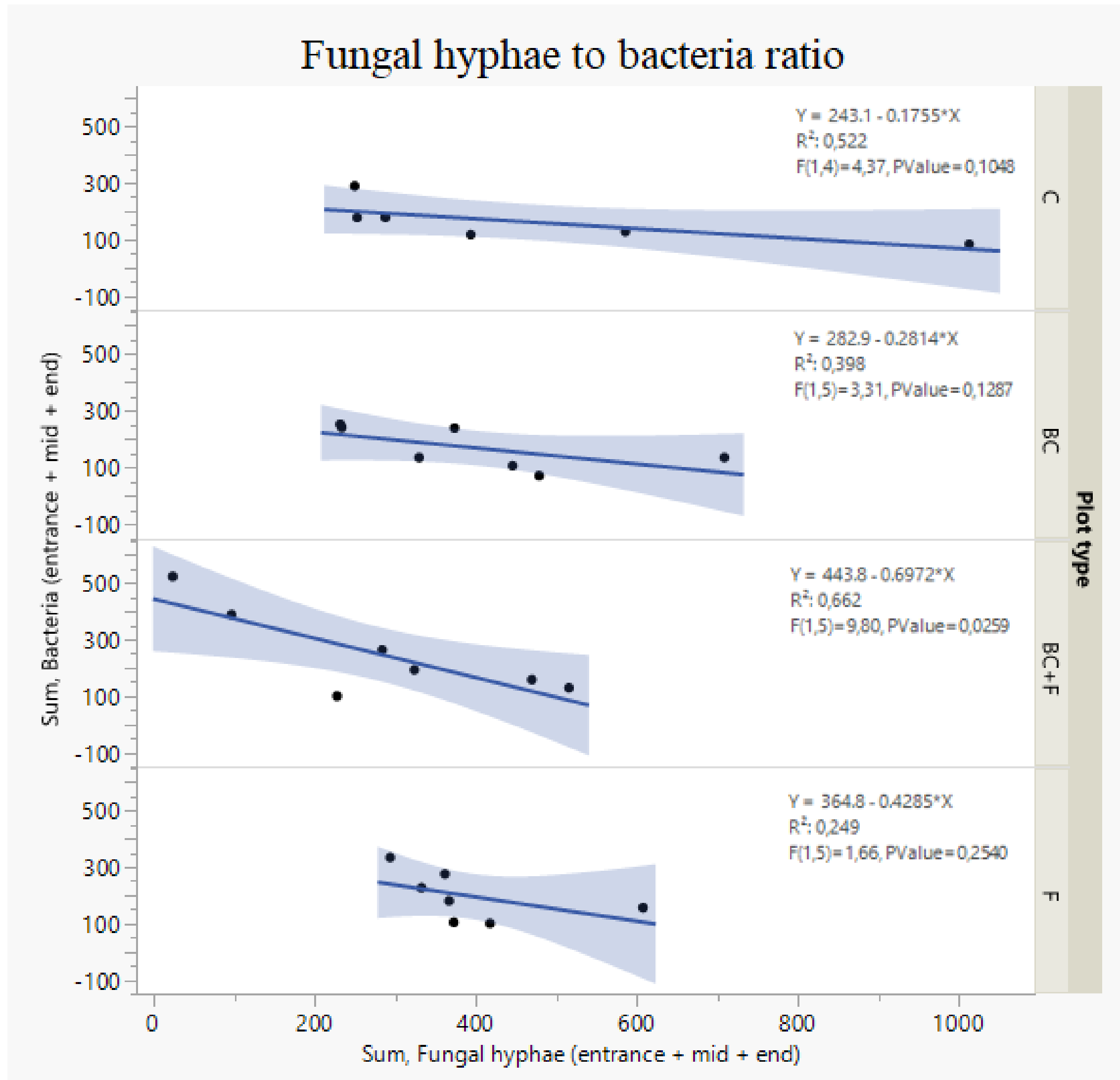


Figure 12: Fungal to bacterial ratio for each plot type. Shaded area shows 95% fit for statistical model. R^2-values and p-values are stated in the plots.

3.2 In-lab experiments

The in-lab experiments allowed us to gather time-resolved data, as the chips were inoculated and stored in lab-environment with harvested soil from the fields. Data was gathered at day 1, day 3, day 8, day 23, and day 37 after inoculation. A total of three different experimental setups were designed, with $n = 2$ chip per setup, as described in 2.3.2.1. Below is a table summarizing the experiments conducted, Table 2.

Test name	Chip treatment	Soil treatment	n chips
Control (A)	Malt solution ($5g/100mL$)	C+F	$n = 2$
Chip BC (B)	Malt solution ($5g/100mL$) + biochar solution	C+F	$n = 2$
Chip+Soil BC (C)	Malt solution ($5g/100mL$) + biochar solution	C+BC+F	$n = 2$

Table 2: Summary of in-lab experiments.

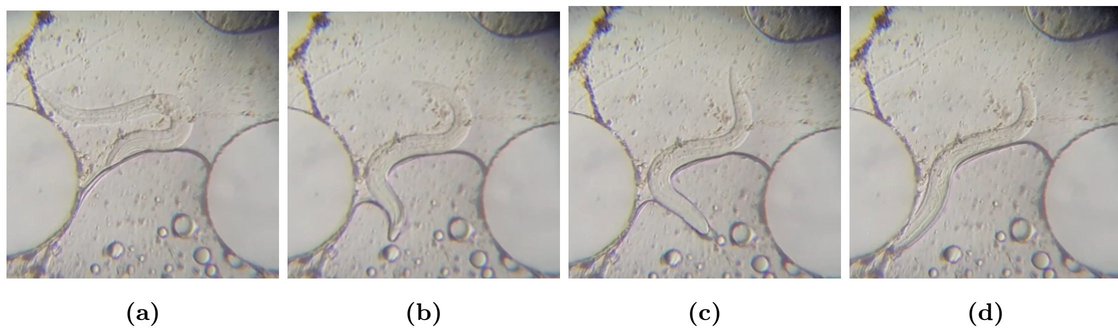
3.2.1 Qualitative results

All in-lab chips saw colonization of fungi and bacteria, while nematode and protist colonization could only be observed in a few chips. No cyst population could be found.

Fungal hyphae growth was strongest in test Chip BC, which saw early fungal growth in both chips. During chip analysis with microscope, fungal hyphae growth could be observed to occur in different focal points, showing that fungi grew at different chip heights, confirming that chip offers a 3-dimensional environment for the microbes to grow in.

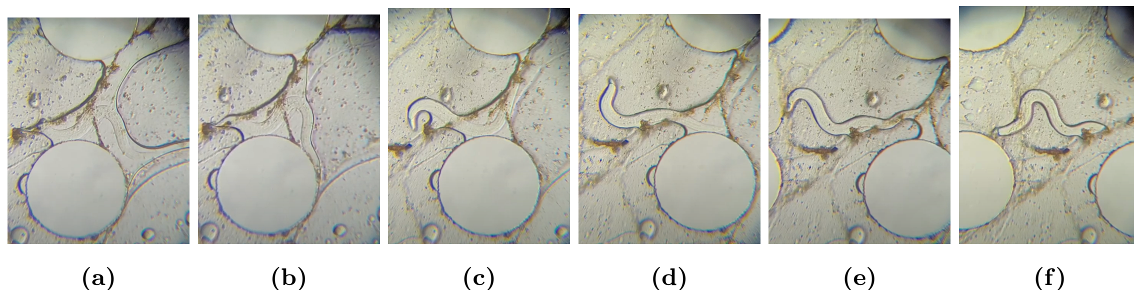
Bacteria colonization was seen at day 1 for both chips in Control test, while for the other chips it took either until day 3 (Chip BC 1 2, Soil + Chip BC 2) or even until day 8 (Soil + Chip BC 1) until bacteria could be seen populating the chips. Once bacteria had populated the chips, it was usually in high magnitude, sometimes covering large areas of the chip, sometimes only in a few concentrated areas. Bacteria colonization reached the other end of the chip in all six chips.

All chips were seen drying out, which was observed both upon inspection with microscope, and the actual soil inoculating the chips drying out. Chip drying out could also be observed in real time as the water front could be observed moving. Organisms could be seen pushed by the force of the water front moving, and nematodes could be observed interacting with the water front. Some nematodes were observed pushing the water front, presumably in search for food, see Figure 13. One nematode could be seen being trapped by surrounding air as the chip dried out. Once completely engulfed by air, the nematode could still manage to move around in the chip with a small volume of water attached around it, see 14. Some chips were also found with fungal hyphae growth under the PDMS structures, indicating that the bonding process during chip production might have been unsuccessful.



(a) (b) (c) (d)

Figure 13: Time series of nematode pushing the water front.



(a) (b) (c) (d) (e) (f)

Figure 14: Time series of nematode getting surrounded by air as chip is drying out.

3.2.2 Quantitative results

Three different quantitative measurements were compiled for the in-lab experiments: number of channels populated by fungal hyphae, furthest fungal hyphae growth, and fungal hyphae CCDR. Data for all measurements were gathered for each data gathering moment, resulting in time-resolved results for these measurements.

Below in Figure 15 and Figure 16, the number of channels populated by fungal hyphae, and the furthest fungi growth is plotted, respectively. Fungal growth was both quicker and more wide spread in Chip BC tests, as almost all possible channels were colonized already at day 8, and showing fungal hyphae growth almost to the end of the chip in both chips. Control and Soil+Chip BC showed similar results in both fungal channel colonization and furthest fungal hyphae growth, which both were slower and weaker than Chip BC .

The fungal hyphae CCDR was also calculated for the in-lab experiments, see Figure 17. The results are in-line with the fungal channel colonization results shown in Figure 15, with test Chip BC showing stronger fungal hyphae growth.

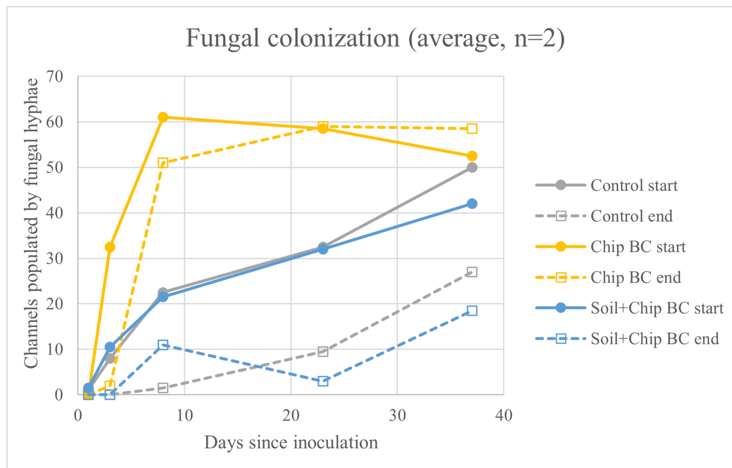


Figure 15: Average number of channels colonized by fungi per day, treatment, and sampling location.

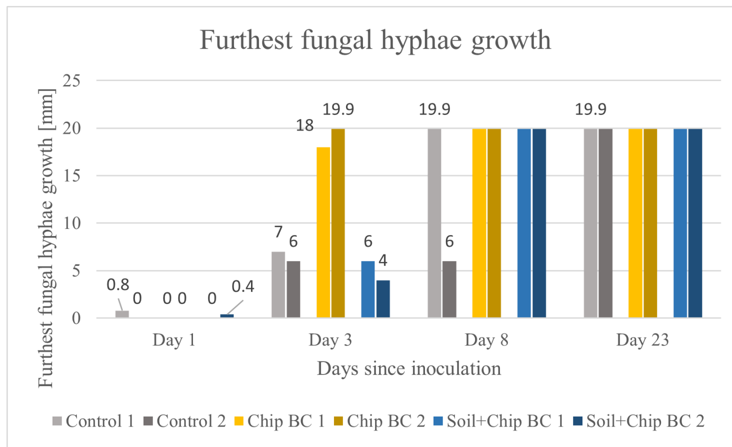


Figure 16: Furthest fungi growth measured in mm per day and chip. The full chip length was 19.9 mm.

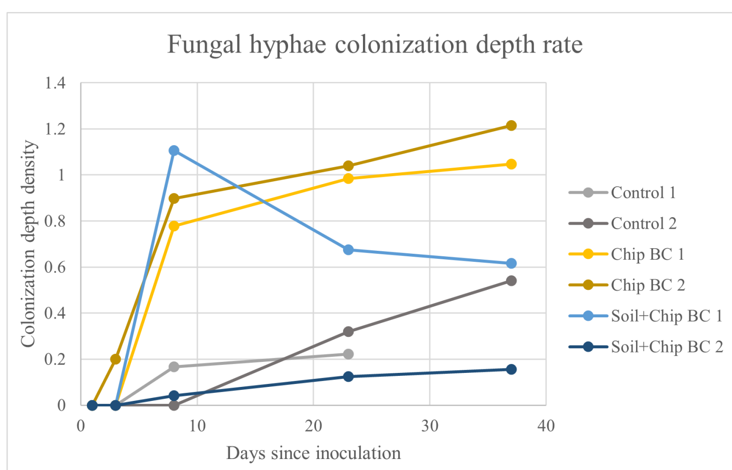


Figure 17: CCDR, all individual chips plotted. Data from Control 1, day 37, is missing.

3.3 Questionnaire

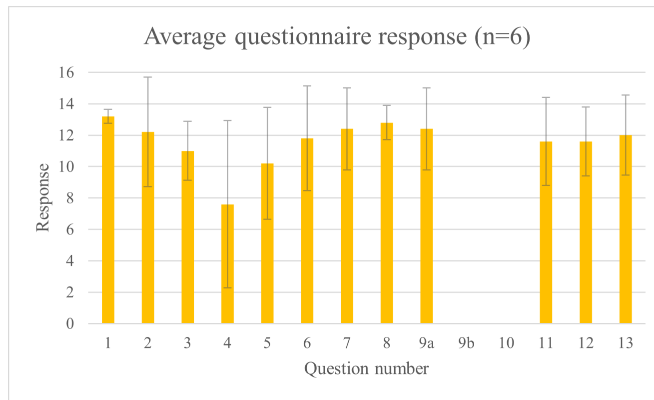
3.3.1 Quantitative results

A total of five households were visited, from which a total of seven respondents participated in the questionnaire. Respondent 1 consisted of two individuals who made joint answers on the questionnaire after a brief discussion. Respondent 2 and 3 were family members from the same household. All respondents were briefed on the setup for the questionnaire by the auther and the field assistant prior to starting filling the questionnaire. All respondents received assistance from the field assistant in interpretation and translation from English to Swahili. A compilation of all the results can be found in Table 3 below. Figure 18a shows the average response from the respondents, and Figure 18b shows the impact that the videos and following discussion had on soil microbe perception and the respondent's willingness to invest more in their fields. These impacts measurements were calculated by subtracting the values gathered from Q12-Q5, and Q13-Q8, respectively.

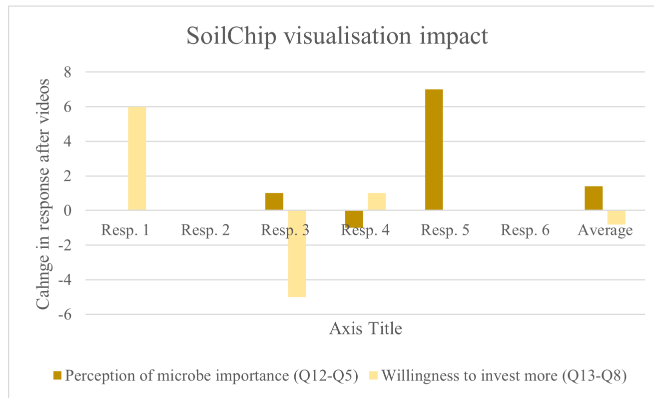
First set of questions - Before showing videos								
Q	**Resp. 1**	**Resp. 2**	**Resp. 3**	**Resp. 4**	**Resp. 5**	**Resp. 6**	**Average**	**std**
Q1	How important is agriculture for you?							
	14	13	14	13	13	13	13.2	0.4
Q2	How often do you work directly with soil?							
	5	14	14	6	13	14	12.2	3.5
Q3	How often do you worry about how much crop you will get from your agriculture?							
	10	10	9	10	13	13	11.0	1.9
Q4	How often do you think about the smallest living things in the soil?							
	0	5	14	6	1	12	7.6	5.3
Q5	How important are the very smallest living things in the soil for you?							
	13	13	7	14	6	11	10.2	3.6
Q6	How often do you think of different methods to keep your soil good for agriculture?							
	13	14	14	6	13	12	11.8	3.3
Q7	How do you feel about biochar application in your field?							
	13	8	14	14	14	12	12.4	2.6
Q8	Do you want to invest more or less money and time to apply biochar to your fields?							
	7	14	13	13	13	11	12.8	1.1

Second set of questions - After showing videos								
Q9a	How do you feel about the microbes we saw in the video?							
	14	14	8	14	14	12	12.4	2.6
Q9a	Other emotions?							
	Happy	-	A little bit scary	Happy	Happy	Happy		
Q10	Before seeing this video, have you ever seen or heard about microbes in the soil?							
	No	No	No	Yes	Yes	No		
Q11	Do you want to see more or fewer microbes in your soil?							
	13	14	7	13	13	11	11.6	2.8
Q12	Do you think the microbes in the soil are important for your agriculture?							
	13	13	8	13	13	11	11.6	2.2
Q13	Do you want to invest more or less money and time to make your soil a good home for the microbes?							
	13	14	8	14	13	11	12.0	2.5

Table 3: Compilation of the questionnaire responses. The original questionnaire design can be found in Appendix B. The results were quantified on a scale between 0-15. The higher the number, the more positive/often the response.



(a) Average questionnaire response. Error bars represent ±1 standard deviation.



(b) SoilChip vidoes impact.

Figure 18: Average questionnaire response and impact of watching videos gathered from the SoilChip.

3.3.2 Qualitative observations

In general, the respondents were perceived to have positive reactions to watching the videos and to take part in the study. All respondents said that it was exciting to see the videos, or that the videos were very helpful as a educational tool to explain the microbial soil ecosystem. Respondent 2 and 3 both said that without the videos, it would have been difficult to gain the same understanding, comparing to only on explaining orally.

Based on the questionnaire results, we can see that respondent 3, who expressed that the microbes looked "a little bit scary", seems to also have lost willingness to invest in soil, see 18b. Both respondent 1 and 5 showed positive impact from watching the videos, on perception on microbe importance and willingness to invest more, respectively. Both respondent 2 and 6 showed no measurable impact from watching the videos.

In addition to the questionnaire, insights were also gathered based on discussions together with the respondents and my field assistant. Respondent 3 and household members of Respondent 4 expressed worry regarding increasing price of fertilizers, especially in the last three months as prices has increased heavily due to the Russian invasion of Ukraine. Both respondents said that they were happy they had access to biochar as well as biochar was more easily available than fertilizers.

4 Discussion

4.1 Measurements - microbial abundance and CCDR

To discuss the effects of biochar on soil microbial communities, we first need to discuss the two main measurements used in this thesis; microbial abundance and the chip colonization depth ratio (CCDR), and how these were collected. Firstly, an important point to make is that the microbial abundance we look at is the abundance in the chip, not the actual soil, and that these might not be identical. It is probable that we experience some type of selection effect for the chip colonization, such as size exclusion, or exclusion on other biases. It is also possible that we can see a higher concentration of microbes populating the chips compared to the soil. It can thus be difficult to draw conclusions on absolute microbial abundance of the different plot types, but we can make relative comparisons between the plot types if we assume that the microbial communities of the different plot types behave identically.

Further, as described in section 2.3.1.2, the microbial abundance data was collected by scanning the chip at three different chip depths; entrance, mid, and end. It is important to take notice that these three areas only covers a small portion, approximately 2%, of the total chip area. This means that there were many microbes that colonised the chips that were not a part of the analysis. There were instances where big differences in microbe populations could be seen over small distances, and thus, big differences could be obtained in the abundance data based on chance as to where the population was localized. To minimize the effect of this, the data collection methodology remained unchanged throughout the data collection, with the predetermined data collection areas. Large populations of microbes were ignored if they were located outside of the counting areas. Additionally, as many chips as possible were analyzed, to attempt to minimize the effect of any outliers.

Another point of notice regarding the abundance data collection, is that the entrance of the chip covered more total open area from which the organisms were counted, due to more solid PDMS structures covering the measurement locations of mid and end chip. This naturally increases the probability of finding microbes by the chip entrance, which should be reflected in the results. Looking at the total abundance for each chip depth, see figure 10f, we see a gradual decline in abundance as we go deeper into the chip. The decline between mid and end should be close to the actual difference, but due to the higher open area covered for the chip entrance, it is difficult to determine if this comes from actual microbe colonization behavior, or from the increased probability of finding microbes by the chip entrance due to the data collection method. This is true for looking at individual species as well. For future studies, the data collection method should cover equivalent areas to make these analyses possible. However, again, we should be able to conduct relative comparisons between different plot types, as the data collection methodology was identical for all chips and thus, all plot types.

The chip colonization depth ratio (CCDR) was calculated based on the microbe abundance data, and the measurement was developed with the intent to quantify microbes' capability to find nutrients in complex soil systems, i.e. their foraging capability. The hypothesis is that biochar-enrichment might assist microbes in this aspect, and thus make nutrients in the soil more available for plants, which could explain why we can see increased crop yields from this soil amendment.

The CCDR measurement is especially interesting for fungal hyphae growth, as their growth can be tracked in a way that is not possible with the other microbes. Then, the CCDR can really measure the proportion of hyphae that continue to grow deep into the chip, whereas for the other microbes, the CCDR measurement becomes more of a comparison to where the microbe population is located in the moment of analysis. Based on the discussion above regarding the increased probability of finding microbes by the chip entrance, it is reasonable to see that most of the CCDR measurements are below 1.

4.2 Biochar and its effect on microbial communities

Now, after discussing the potential limitations and drawbacks of the measurements used in the study, we can continue to discuss the actual results gathered. As this study aims to investigate the impact of biochar-enrichment, significant results from fertilization will not be discussed. The findings show a few significant results based on biochar-enriched soil, namely:

- Fungal hyphae
  - Biochar-enriched plots showed significantly lower abundance of fungal hyphae by the chip
    entrance.
  - Plots with the combination of biochar-enrichment and fertilization showed significantly higher CCDR values for fungal hyphae.
- Protists
  - Biochar-enriched plots showed significantly higher abundance of protists by the chip ending.
  - Biochar-enriched plots showed significantly higher CCDR values for protists by the chip ending.

The significantly higher CCDR values from biochar-enriched, fertilized combination plots are very interesting. As discussed earlier, the intent of the CCDR measurement was to quantify microbe foraging capabilities, and the above results show that fungal hyphae in plots with biochar and fertilizer could be better foragers as the grow in higher proportion deep into the chips. At the same time, looking at the abundance data, we see a high abundance of fungal hyphae in the control plot. The results show that we have higher abundance of fungal hyphae colonizing the chips in the control plot, but few of them successfully colonize deeper into the chips.

One valid question to ask is if the significant results for both fungal hyphae CCDR are due to the soil amendment affecting the characteristics of the existing fungi community, assisting them to grow further distances, or if soil amendment affects the composition of the fungi community itself, favoring fungi with higher growing capability. This would induce a selection-bias that would affect our results. It is likely that both aspects, that soil amendments affecting both the composition and characteristics of the microbial community, play a role in explaining the results gained from this study, and to further study this, species identification needs to also take place to identify the proportion of different species in the community.

Looking closer into the protist abundance results, we can see a few chips with very high abundance in relation to the other chips. On first glance, they seem to be outliers which heavily corrupts the results, but even after removing outliers based on Grubbs' test ($p < 0.05$), we still see a significant difference in the protist abundance. That begs the question, how come we only see a significant difference in the very end of the chip, and not the two other chip depths? One observation that might explain this relatively high abundance in cysts all throughout the chip. The sharp decline in protist abundance, for all plot types, might be explained by the protists colonizing the chip quickly enter its dormant cyst state as they travel into the chip. The same type of selection-bias as discussed above could occur here, it could be that the protist composition of biochar-enriched plots favor protists that can endure the environment in the chip, and would thus not enter its dormant cyst state, explaining the significant results for protists.

To further build on the argument above, the composition of the microbial community could affect the microbe counts, especially for fungi, as we can gain different characteristics of different species. As the fungal hyphae abundance was defined as number of hyphae crossings over an imaginary line, the result could be affected due to the characteristics of the fungi populating the chips. If fungi with high level of branching colonizes the chip, that will result in a high fungal hyphae count as there will be many individual hyphae crossing the counting line, even though they all might stem from the same individual fungi. In the other extreme, if a fungi that do not branch at all enter the chip, it might only be counted once. These types of observations were made during chip analysis, but as no detailed species identification was made during this study, no further analysis was made of this. Previous research has found that fungi species that branch frequently do not grow as far nor fast as fungi that branch more seldomly [36].

The results we observed in fungal to bacteria ratio in section 3.1.2.3, a negative correlation of the abundance of bacteria to the abundance of fungi, indicate that the results gained from our experimental setup is coherent with the expected results based on previous studies[37]. The relationship of the two major microbial groups is generally assumed to be competitive, and this has recently been shown to be the case even at a micrometer-resolved spatial scale [35]. The results indicate a competitive spatial niche partioning relationship between fungi and bacteria.

From the in-lab experiments we could see that biochar-filled chips in combination with non-biochar-enriched soil heavily outgrew the other tests in fungal hyphae growth, both in terms of distance and density. It is interesting that we do not observe the same behavior for the chip and soil with both biochar enrichment. The results seem to indicate that it is the difference in biochar concentration that is of importance, as biochar-enriched soil did not see the same advancement of fungi growth into the chip. It is difficult to draw any conclusions from the data, but it indicates that biochar environments might be favorable for fungi that previously have not grown in biochar-environments. Further research on this topic would be most interesting.

4.3 Methodological approach and potential improvements

For this study, we designed both in-situ and in-lab experiments, which both comes with its advantages and disadvantages. With the in-situ experiments, we can study the microbial community in its natural environment, with the actual temperatures, rainfall, and all other natural conditions that the soil and the soil microbes experience. This gives clear advantages as we come as close as possible to our analysis targets. However, we lose the ability to gain time-resolved results as we

cannot access the chip as it is burrowed in the soil. For the in-lab experiments, we have continuous access to analyze the chips, and can thus gather time-resolved data. But then we lose the natural environment, which could affect the results we are gathering. With the SoilChip technology the time-resolved data is especially interesting, as we gain the possibility to really investigate the microbe dynamics and interactions, which other soil microbe analysis tools lack. An ideal option would be to gain the possibility to gather time-resolved data in-situ, perhaps with a minuscule microscope installed in connection with the chips.

No visual inspection was made of the chip state regarding contamination or chip fluid filling before installing them in the field. This was due to our inability to establish sterile environments out on the field, and in order to minimize the risk of microbial contamination into the chips, they were prepared without any prior quality control. It is thus unknown if all the chips managed to stay liquid-filled or if some, or all, dried out. From qualitative observations during chip preparation, liquid was found still in the chips when cutting open the chip entrance, but no further inspection or notation was made. For the analysis of the results, it was assumed that all chips behaved identically, and that all chips had equal opportunity to be colonized by microbes. It is likely that there were variation in chip performance, but this is for us unknown. The high number of repeats should assist in removing this potential chip variation.

Variation in chip colonization could also stem from chip positioning in the field. Although we aimed for equivalent positioning of the chip installment in regards to both depth and position relative to crop row, we could observe differences upon harvesting. Some of the chips were located directly under the crop row, while some were located at the intended plan of 10-20 cm away from the crop row. This difference in chip position could impact the microbe colonization, if we expect heterogeneous microbe populations in the soil, and if this heterogeneity is affected by crops and their roots. If a completely homogeneous microbe population is expected, chip positioning should not impact the colonization rate.

A neural network was used for the quantification of bacterial abundance, which adds a level of uncertainty of the results. The neural network has primarily been trained with data sets with images gathered from another microscopy, which also adds uncertainty in the precision. Upon inspection of a stick sample of 10 images, an accuracy of around 70% could be obtained. It was clear that the neural network missed counting a few bacteria, but this error was also seemed consistent over the different plot types. It was also evident that counting bacteria by hand would also bring bias, as the counting and identification of bacteria can be ambiguous. Therefore, it is assumed that the neural network produced the most reliable results, and that relative comparisons can be made accurately. However, we need to keep in mind that total abundance results might be misguiding. This also holds true for also other organisms, as the chip might induce size selection or selection based on other biases, as previously discussed.

For the in-lab chips we encountered problems with both the chip and soil drying out. We had planned for an irrigation scheme where we added 5-10 drops of water on the soil twice per week. This was scheme was followed, but we still saw the chip drying out. This made it difficult to study other microbe populations than fungi, as nematodes, protists, and bacteria all are aquatic-living organisms. This is probably also the reason to why we could not see any colonization of protists nor nematodes until late in the study. The fact that the tube for the syringe was remained open probably also had an impact to the chips drying out. An improved irrigation scheme and a bigger

soil volume could also be beneficial.

Continuing with the in-lab chips, we had initially planned for using more than only n=2 chips per test. We had fabricated in total 15 chips, but upon the final preparation steps of filling the chips with liquids, we encountered problems as the bonding of the PDMS and the glass coverslip broke. The filling method of using a syringe created high, localized, pressures as the fluid was pushed through the channels, and as the PDMS was hydrophobic without any prior plasma activation. Maybe the bonding was not completed optimally, or maybe a design with wider channels is preferred if trying to fill them without plasma activation. We also observed fungal hyphae growing in between the PDMS and coverslip, further indicating that the bonding process was not ideal.

4.4 SoilChip as an analysis tool

One of the key challenges in this thesis work was to develop an experimental methodology that would allow us to employ SoilChip technology in distant, rural, areas where no ordinary lab or analysis equipment is available. Based on the results and experiences gained from this thesis work, we can confidently show that SoilChip technology is a viable option for studies like these. We show that we can fabricate chips in Lund, Sweden, and transport them in a robust and sterile manner to employ in rural areas. The chips became successfully colonized by all categorizes of the microbial community, and we could successfully analyze the chips directly on the field using a portable field microscope. This is the first time this experimental methodology is employed.

With the results and insights based from the questionnaire and discussions with the local farmers, we also have reason to believe that the SoilChip technology can offer an educational advantage when explaining soil microbial results. Almost all respondents showed positive reactions to watching the SoilChip videos, and two of the respondents directly expressed that the videos made it easier to understand the research. This indicates that the SoilChip technology can also advance our research communication, and make soil research more accessible and interesting for a larger audience.

How does the SoilChip technology compare to other, traditional, methods for analyzing the microbial soil community? It definitely serves its niche of offering in-situ, direct, visualisation of the microbial community in their natural environment, which no other method can do. To utilize SoilChip technology to measure microbe abundance, as done in this study, might be inefficient to conventional techniques. But relative soil comparisons should be feasible, and with the perk of being able to conduct the full data gathering in-situ in the field, with no need to enter a lab for analysis, the SoilChip technology might be preferred. Further, with this study we show that SoilChip technology can be deployed intercontinentally, which allows for analysis costs to substantially decrease as chip fabrication can be centralised. With further technical advancements with the SoilChip technology, such as improvements in the neural network image analysis, or adding in-situ microscopy or spectroscopy, clearly offers an exciting future where we can further advance our understanding in sustainable and efficient agriculture, making food more accessible for all.

5 Conclusion

5.1 Conclusions

The aim of this thesis was to use microfluidic SoilChips to analyze the microbial ecosystem in biochar-enriched soil used for agriculture in Kenya. Both in-situ and in-lab experiments were successfully conducted, where microbial abundance and foraging capabilities were investigated. The chip colonization depth ratio (CCDR) measurement was developed for this thesis with the intent to quantify the microbe foraging capability. A minor social study was also conducted, where the impact of visualizing the microbial soil community was analyzed.

We show, for the first time ever, that we can successfully employ SoilChip technology to perform fieldwork studies and analysis in this manner without close-by lab equipment available. The experimental methodology and the logistical plan to transport semi-finalized chips from Sweden to Kenya was successful. In addition to this, we found a few significant effects on the microbial community based on soil amendment. We could see how fungal hyphal abundance by the chip entrance was significantly lower from biochar-enriched soils, and the results show that fungal hyphae CCDR is significantly larger from soil with the combination of fertilization and biochar-enrichment. We also saw significant results in protist abundance, where biochar-enriched soils saw high protist abundance and CCDR. The lab experiments also indicate that biochar-rich environments supports quick and dense fungal hyphae growth, although more research is required to draw any conclusions. The social study shows that the SoilChip technology can be an effective tool for communication and educational purposes within soil microbiology.

5.2 Future outlook

As the experimental methodology for this study was proven sound, it opens up the possibility to conduct more studies designed in this manner. This allows for studies taking place globally for a relative low cost as the chip fabrication process can be centralised. Future technical advancements with the SoilChip, through image analysis and implementing new functions, such as in-situ, live, monitoring, will allow for improved data gathering and new discoveries of the microbial soil community. With a cost-effective solution, SoilChip technology could potentially be employed globally to map microbial abundance in real-time, or used by farmers to specify individual soil management plans based on their specific soil conditions and microbial communities. SoilChip technology shows high potential in providing new insights to soil health, and contributing to finding new, efficient and sustainable, agricultural practices.

References

[1] World Food Programme. *Ending Hunger* (2022). Available at: https://www.wfp.org/ending-hunger. (Accessed: 12 June 2022).

[2] Sonja J Vermeulen, Bruce M Campbell, John SI Ingram, et al. Climate change and food systems. *Annual review of environment and resources*, 37(1):195–222, 2012.

[3] Serpil Savci. An agricultural pollutant: chemical fertilizer. *International Journal of Environmental Science and Development*, 3(1):73, 2012.

[4] Ameeta Sharma and Ronak Chetani. A review on the effect of organic and chemical fertilizers on plants. *International Journal for Research in Applied Science & Engineering Technology (IJRASET)*, 5(2):677–680, 2017.

[5] FSIN and Global Network Against Food Crises. *2022 Global Report on Food Crises* (2022). Available at: https://www.wfp.org/publications/global-report-food-crises-2022. (Accessed: 6 June 2022).

[6] UNICEF et al. The state of food security and nutrition in the world 2021. 2021.

[7] World Food Programme. *13 million people facing severe hunger as drought grips the Horn of Africa* (2022). Available at: https://tinyurl.com/4wcuemy3. (Accessed: 6 June 2022).

[8] United Nations. *Horn of Africa braces for 'explosion of child deaths' as hunger crisis deepens* (2022). Available at: https://news.un.org/en/story/2022/06/1119862. (Accessed: 6 June 2022).

[9] World Health Organization. *Stunting in a nutshell* (2015). Available at: https://www.who.int/news/item/19-11-2015-stunting-in-a-nutshell. (Accessed: 6 June 2022).

[10] Development Initiatives. *2021 Global Nutrition Report: The state of global nutrition.* (2021). Available at: https://tinyurl.com/y9hkwu63. (Accessed: 6 June 2022).

[11] UNICEF. *Malnutrition* (2021). Available at: https://tinyurl.com/3rsmjpbk. (Accessed: 6 June 2022).

[12] UNICEF. *Situation Analysis of Children and Women in Kenya, 2017* (2018). Available at: https://tinyurl.com/5dy682yk. (Accessed: 6 June 2022).

[13] Vidya Diwakar and Andrew Shepherd. Understanding poverty in kenya: A multidimensional analysis, 2018.

[14] FAO. *Kenya at a glance* (2021). Available at: https://tinyurl.com/3rn6b36j. (Accessed: 6 June 2022).

[15] USAID. *AGRICULTURE AND FOOD SECURITY, KENYA* (2022). Available at: https://www.usaid.gov/kenya/agriculture-and-food-security. (Accessed: 12 June 2022).

[16] Simon Jeffery, Frank GA Verheijen, Marijn van der Velde, and Ana Catarina Bastos. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, ecosystems & environment*, 144(1):175–187, 2011.

[17] Johannes Lehmann, Matthias C Rillig, Janice Thies, Caroline A Masiello, William C Hockaday, and David Crowley. Biochar effects on soil biota–a review. *Soil biology and biochemistry*, 43(9):1812–1836, 2011.

[18] Christopher J Atkinson, Jean D Fitzgerald, and Neil A Hipps. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and soil*, 337(1):1–18, 2010.

[19] Mary Njenga, Yahia Mahmoud, Ruth Mendum, Muyiki Iiyama, Ramni Jamnadass, Kristina Roing De Nowina, and Cecilia Sundberg. Quality of charcoal produced using micro gasification and how the new cook stove works in rural kenya. *Environmental Research Letters*, 12(9):095001, 2017.

[20] Thomas Kätterer, Dries Roobroeck, Olof Andrén, Geoffrey Kimutai, Erik Karltun, Holger Kirchmann, Gert Nyberg, Bernard Vanlauwe, and Kristina Röing de Nowina. Biochar addition persistently increased soil fertility and yields in maize-soybean rotations over 10 years in subhumid regions of kenya. *Field Crops Research*, 235:18–26, 2019.

[21] John PE Anderson. Soil respiration. *Methods of soil analysis: part 2 chemical and microbiological properties*, 9:831–871, 1983.

[22] Ellen Kandeler. Physiological and biochemical methods for studying soil biota and their function. In *Soil microbiology, ecology and biochemistry*, pages 53–83. Elsevier, 2007.

[23] Noah Fierer, Mark A Bradford, and Robert B Jackson. Toward an ecological classification of soil bacteria. *Ecology*, 88(6):1354–1364, 2007.

[24] Johannes Lehmann, Dawit Solomon, James Kinyangi, Lena Dathe, Sue Wirick, and Chris Jacobsen. Spatial complexity of soil organic matter forms at nanometre scales. *Nature Geoscience*, 1(4):238–242, 2008.

[25] George M Whitesides. The origins and the future of microfluidics. *nature*, 442(7101):368–373, 2006.

[26] Eric K Sackmann, Anna L Fulton, and David J Beebe. The present and future role of microfluidics in biomedical research. *Nature*, 507(7491):181–189, 2014.

[27] Wing Cheung Mak, Valerio Beni, and Anthony PF Turner. Lateral-flow technology: From visual to instrumental. *TrAC Trends in Analytical Chemistry*, 79:297–305, 2016.

[28] Younan Xia and George M Whitesides. Soft lithography. *Angewandte Chemie International Edition*, 37(5):550–575, 1998.

[29] J Cooper McDonald, David C Duffy, Janelle R Anderson, Daniel T Chiu, Hongkai Wu, Olivier JA Schueller, and George M Whitesides. Fabrication of microfluidic systems in poly (dimethylsiloxane). *ELECTROPHORESIS: An International Journal*, 21(1):27–40, 2000.

[30] Claire E Stanley, Guido Grossmann, Xavier Casadevall i Solvas, and Andrew J deMello. Soil-on-a-chip: microfluidic platforms for environmental organismal studies. *Lab on a Chip*, 16(2):228–241, 2016.

[31] Kristin Aleklett, E Toby Kiers, Pelle Ohlsson, Thomas S Shimizu, Victor EA Caldas, and Edith C Hammer. Build your own soil: exploring microfluidics to create microbial habitat structures. *The ISME journal*, 12(2):312–319, 2018.

[32] Paola Micaela Mafla-Endara, Carlos Arellano-Caicedo, Kristin Aleklett, Milda Pucetaite, Pelle Ohlsson, and Edith C Hammer. Microfluidic chips provide visual access to in situ soil ecology. *Communications Biology*, 4(1):1–12, 2021.

[33] Edith C Hammer. *The SoilChip* (2022). Available at: `https://soilchip.wixsite.com/soilchip`. (Accessed: 12 June 2022).

[34] Carlos Arellano-Caicedo, Pelle Ohlsson, Martin Bengtsson, Jason P Beech, and Edith C Hammer. Habitat geometry in artificial microstructure affects bacterial and fungal growth, interactions, and substrate degradation. *Communications biology*, 4(1):1–11, 2021.

[35] Carlos Arellano-Caicedo, Pelle Ohlsson, and Edith C Hammer. The effect of habitat complexity on microbial processes. In *EGU General Assembly Conference Abstracts*, pages EGU21–12264, 2021.

[36] Kristin Aleklett, Pelle Ohlsson, Martin Bengtsson, and Edith C Hammer. Fungal foraging behaviour and hyphal space exploration in micro-structured soil chips. *The ISME journal*, 15(6):1782–1793, 2021.

[37] Johannes Rousk, Louise Aldén Demoling, Adam Bahr, and Erland Bååth. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. *FEMS microbiology ecology*, 63(3):350–358, 2008.

A Experimental plot design

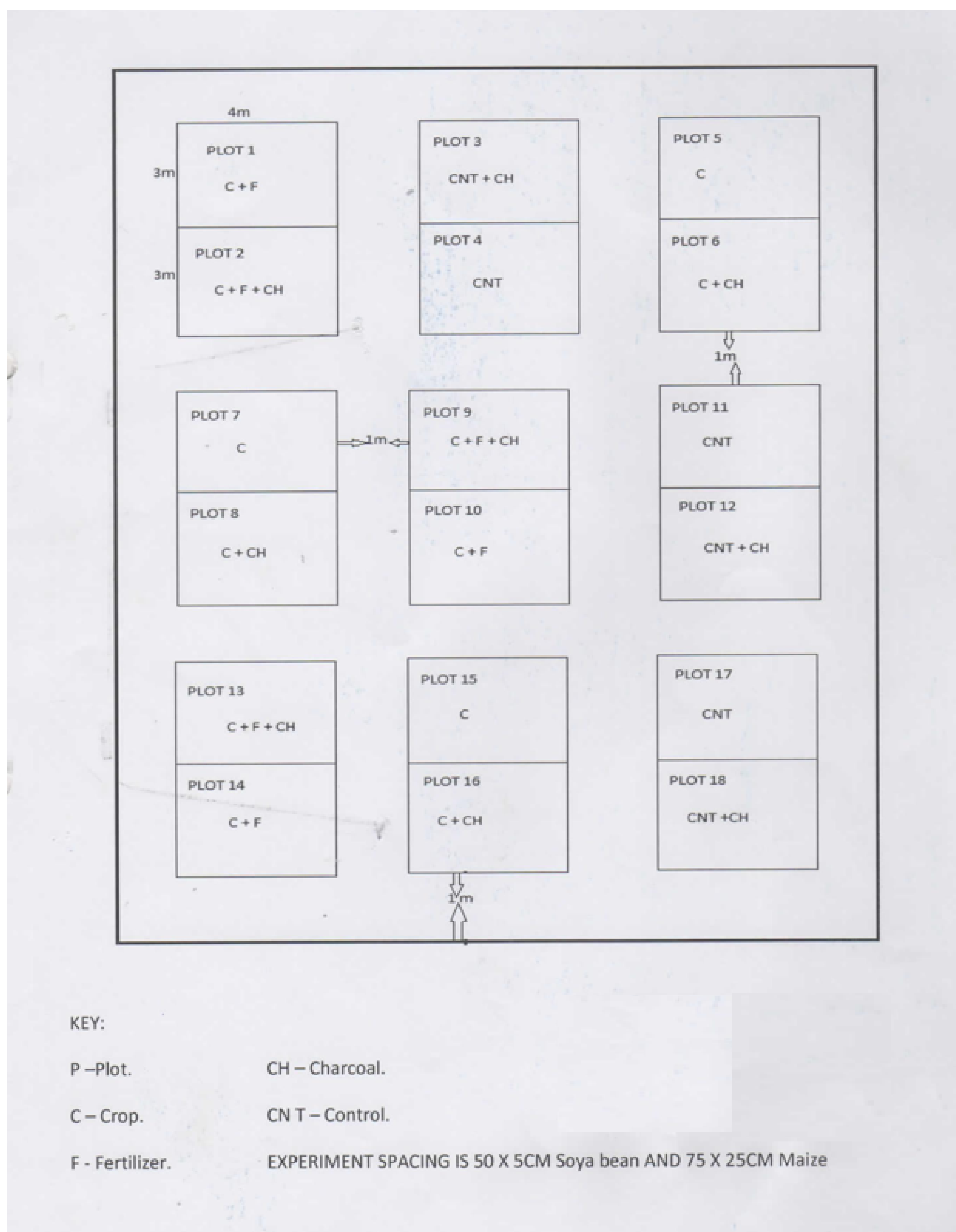


Figure 19: Schematic over experimental plot design. Maize was grown at the time of this study. Bare fallow control plots (plots 3, 4, 11, 12, 17, and 18) were not investigated in this study.

B Questionnaire

Name:

1. How important is agriculture for you?

Not important at all	Somewhat important	Most important of all

2. How often do you work directly with soil?

Never	A few times per month	A few times per week	Several times a day

3. How often do you worry about how much crop you will get from your agriculture?

Never	A few times per month	A few times per week	Several times a day

4. How often do you think about the very smallest living things in the soil?

Never	A few times per month	A few times per week	Several times a day

5. How important are the very smallest living things in the soil for you?

Not important at all	Somewhat important	Most important of all

6. How often do you think of different methods to keep your soil good for agriculture?

Never	A few times per month	A few times per week	Several times a day

7. How do you feel about biochar application in your field?

Very negative	Not negative nor positive	Very positive

8. Do you want to invest more or less money and time to apply biochar to your fields?

Less than today	The same as today	More than today

Figure 20: Questionnaire part 1. These eight questions were given to the respondents prior to watching the SoilChip videos. Respondents had full freedom to use the full scale for their response. Responses were later quantified into a 0-15 scale.

Name:

---

9. How do you feel about the microbes we saw in the video?

Very negative | Not negative nor positive | Very positive

|——————————————|——————————————|

Other emotions: ______________________________

10. Before seeing this video, have you ever seen or heard about microbes in soil?

Yes _________ No _________

11. Do you want to see more or fewer microbes in your soil?

Less than today | The same as today | More than today

|——————————————|——————————————|

12. Do you think the microbes in the soil are important for your agriculture?

Not important at all | Somewhat important | Most important of all

|——————————————|——————————————|

13. Do you want to invest more or less money and time to make your soil a good home for the microbes?

Less than today | The same as today | More than today

|——————————————|——————————————|

Figure 21: Questionnaire part 2. These five questions were given to the respondents after to watching the SoilChip videos. Respondents had full freedom to use the full scale for their response. Responses were later quantified into a 0-15 scale.

C In-situ raw data

	Plot no.	Plot type	Chip	Harvest date	Plant	Days plant	Fungi			Nematodes			Protists			Cysts		
							Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Row 1			1	2022-05-09	2022-	53	354	170	61	5	0	2	17	4	1	44	235	43
			2	2022-05-10	2022-	54	182	81	5	3	1	0	12	0	0	7	3	10
			3															
	Plot 5	C	4															
			1	2022-04-30	2022-	44	141	170	128	3			42	4				
			2	2022-05-06	2022-	50	168	128	76	2	0	0	42	18	1	44	15	21
			3	2022-05-11	2022-	55	235	146	36	0	0	0	16	10	2	37	51	21
	Plot 1	C+F	4															
			1	2022-04-30	2022-	44	77	53	24	6	0	0	11	2	0			
			2	2022-05-06	2022-	50	191	181	73	3	0	0	27	1	40	11	2	4
			3	2022-05-11	2022-	55	199	234	45	10	0	1	15	7	9	29	160	36
	Plot 6	C+BC	4															
			1	2022-05-06	2022-	50	308	135	26	1	0	0	17	5	27	12	38	11
			2	2022-05-10	2022-	54	170	50	7	1	0	0	48	1	2	38	16	12
			3	2022-05-12	2022-	56	141	110	72	0	0	0	38	0	1	52	156	69
	Plot 2	C+F+BC	4															

	Plot no.	Plot type	Chip	Harvest date	Plant	Days plant	Fungi			Nematodes			Protists			Cysts		
							Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Row 2			1	2022-05-06	2022-	50	305	52	36	0	1	0	15	3	0	8	46	9
			2	2022-05-08	2022-	52	238	46	3	2	0	0	5	1	0	46	41	8
			3	2022-05-11	2022-	55	583	254	175	1	0	0	13	1	0	52	30	8
	Plot 7	C	4															
			1	2022-05-07	2022-	51	199	122	40	1	0	0	2	0	0	2	12	0
			2	2022-05-09	2022-	53	145	113	74	4	0	1	22	4	1	35	91	27
			3	2022-05-12	2022-	56	265	235	107	2	0	1	54	4	0	73	46	22
	Plot 10	C+F	4															
			1	2022-05-07	2022-	51	259	55	15	3	0	1	2	0	1	19	64	1
			2	2022-05-09	2022-	53	227	95	51	3	1	0	6	0	1	93	76	24
			3	2022-05-12	2022-	56	176	50	5	1	0	0	20	0	1	35	55	48
	Plot 8	C+BC	4															
			1	2022-05-07	2022-	51	276	113	126	1	0	0	187	0	3	142	79	21
			2	2022-05-11	2022-	55	123	123	37	0	1	1	48	4	3	77	43	30
			3															
	Plot 9	C+F+BC	4															

	Plot no.	Plot type	Chip	Harvest date	Plant	Days plant	Fungi			Nematodes			Protists			Cysts		
							Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3	Line 1	Line 2	Line 3
Row 3			1	2022-04-29	2022-	43	92	39	19	1			1					
			2	2022-05-05	2022-	49	166	78	5	3	0	0	15	27	0			
			3	2022-05-09	2022-	53	191	53	8	0	0	0	5	0	0	15	18	10
	Plot 15	C	4															
			1	2022-05-08	2022-	52	278	15	0	0	0	3	12	0	0	40	44	22
			2	2022-05-10	2022-	54	272	55	39	2	1	0	62	2	0	49	133	56
			3															
	Plot 14	C+F	4															
			1	2022-05-09	2022-	53	241	306	161	1	2	2	34	2	8	105	41	45
			2	2022-05-10	2022-	54	68	134	31	2	0	0	19	0	0	80	74	63
			3															
	Plot 16	C+BC	4	not found	2022-	#VALUE!												
			1	2022-04-30	2022-	44	190	54	15	4			6					
			2	2022-05-05	2022-	49	21	2	0	0	0	1	3	0	0	7	1	0
			3	2022-05-08	2022-	52	82	13	1	2	0	1	26	6	0	98	28	6
	Plot 13	C+F+BC	4															

Figure 22: In-situ experiments raw data, organized by plots.

plot type	BC	Fertilizer	n	plot no.	Fungi line 1	Fungi line 2	Fungi line 3	Nematodes lin	Nematodes lin	Nematodes lin	Protists line 1	Protists line 2	Protists line 3	Cysts line 1	Cysts line 2	Cysts line 3	Bacteria line 1	Bacteria line 2	Bacteria line 3
C	n	n	1	5	354	170	61	5	0	2	17	4	1	44	235	43	44	58	25
C	n	n	2	5	182	81	5	3	1	0	12	0	0	7	3	10			
C	n	n	3	5															
C	n	n	4	5															
C	n	n	5	7	305	52	36	0	1	0	15	3	0	8	46	9	31	17	69
C	n	n	6	7	238	46	3	2	0	0	5	1	0	46	41	8	81	94	3
C	n	n	7	7	583	254	175	1	0	0	13	1	0	52	30	8	51	12	20
C	n	n	8	7															
C	n	n	9	15															
C	n	n	10	15	166	78	5	3	0	0	15	27	0				71	106	112
C	n	n	11	15	191	53	8	0	0	0	5	0	0	15	18	10	78	34	65
C	n	n	12	15															
C+BC	y	n	1	6															
C+BC	y	n	2	6	191	181	73	3	0	0	27	1	40	11	2	4	51	42	15
C+BC	y	n	3	6	199	234	45	10	0	1	15	7	9	29	160	36	33	37	3
C+BC	y	n	4	6															
C+BC	y	n	5	8	259	55	15	3	0	1	2	0	1	19	64	1	39	52	46
C+BC	y	n	6	8	227	95	51	3	1	0	6	0	1	93	76	24	72	99	70
C+BC	y	n	7	8	176	50	5	1	0	0	20	0	1	35	55	48	100	69	85
C+BC	y	n	8	8															
C+BC	y	n	9	16	241	306	161	1	2	2	34	2	8	105	41	45	70	44	23
C+BC	y	n	10	16	68	134	31	2	0	0	19	0	0	80	74	63	68	104	71
C+BC	y	n	11	16															
C+BC	y	n	12	16															
C+F	n	y	1	1															
C+F	n	y	2	1	168	128	76	2	0	0	42	18	1	44	15	21	32	32	40
C+F	n	y	3	1	235	146	36	0	0	0	16	10	2	37	51	21	61	32	7
C+F	n	y	4	1															
C+F	n	y	5	10	199	122	40	1	0	0	2	0	0	2	12	0	78	139	59
C+F	n	y	6	10	145	113	74	4	0	1	22	4	1	35	91	27	58	105	63
C+F	n	y	7	10	265	235	107	2	0	1	54	4	0	73	46	22	77	53	26
C+F	n	y	8	10															
C+F	n	y	9	14	278	15	0	0	0	3	12	0	0	40	44	22	100	142	92
C+F	n	y	10	14	272	55	39	2	1	0	62	2	0	49	133	56	70	32	78
C+F	n	y	11	14															
C+F	n	y	12	14															
C+BC+F	y	y	1	2	308	135	26	1	0	0	17	5	27	12	38	11	100	46	12
C+BC+F	y	y	2	2	170	50	7	1	0	0	48	1	2	38	16	12	67	33	0
C+BC+F	y	y	3	2	141	110	72	0	0	0	38	0	1	52	156	69	86	97	10
C+BC+F	y	y	4	2															
C+BC+F	y	y	5	9	276	113	126	1	0	0	187	0	3	142	79	21	68	35	26
C+BC+F	y	y	6	9	123	123	37	0	1	1	48	4	3	77	43	30	82	112	70
C+BC+F	y	y	7	9															
C+BC+F	y	y	8	9															
C+BC+F	y	y	9	13															
C+BC+F	y	y	10	13	21	2	0	0	0	1	3	0	0	7	1	0	119	272	133
C+BC+F	y	y	11	13	82	13	1	2	0	1	26	6	0	98	28	6	89	159	141
C+BC+F	y	y	12	13															

Figure 23: In-situ experiments raw data, organized for data analysis. Bacterial data included.

D p-values and F-ratios, in-situ experiments

Abundance								
	p-value					F-ratio		
Fungi	BC	F	BC+F		Fungi	BC	F	BC+F
Entrance	***0,0457***	0,1939	0,681		Entrance	4,4419	1,7866	0,1732
Mid	0,8963	0,2971	0,1563		Mid	0,0173	1,136	2,1414
End	0,9553	0,9018	0,4774		End	0,0032	0,0156	0,521
Nematodes	BC	F	BC+F		Nematodes	BC	F	BC+F
Entrance	0,7753	**0,0546**	0,1618		Entrance	0,0833	4,0833	2,0833
Mid	0,7268	0,2994	0,7268		Mid	0,125	1,125	0,125
End	1	0,6505	0,3679		End	0	0,2105	0,8421
Protists	BC	F	BC+F		Protists	BC	F	BC+F
Entrance	0,6321	***0,0181***	0,6321		Entrance	0,2355	6,4755	0,2355
Mid	0,1528	0,8077	0,9031		Mid	2,1804	0,0606	0,0151
End	***0,0198***	0,4005	0,1813		End	6,3201	0,7351	1,9058
Cysts	BC	F	BC+F		Cysts	BC	F	BC+F
Entrance	0,1045	0,4847	0,8938		Entrance	2,8572	0,5044	0,0182
Mid	0,9854	0,6292	0,8314		Mid	0,0003	0,2396	0,0464
End	0,368	0,9583	0,2093		End	0,8432	0,0028	1,6687
Bacteria	BC	F	BC+F		Bacteria	BC	F	BC+F
Entrance	0,1848	***0,0434***	0,3043		Entrance	1,869	4,5678	1,104
Mid	0,346	0,1365	0,6333		Mid	0,9256	2,3808	0,2338
End	0,9896	0,6617	0,8247		End	0,0002	0,1965	0,0626
Total	BC	F	BC+F		Total	BC	F	BC+F
Entrance	0,3995	0,5878	0,413		Entrance	0,7346	0,3016	0,6932
Mid	0,7685	0,6624	0,5195		Mid	0,0885	0,1952	0,5195
End	0,1314	0,5451	0,62		End	0,72	0,4672	0,4385

CCDR								
	p-value					F-ratio		
	BC	F	BC+F			BC	F	BC+F
Fungi mid	0,158	0,4385	***0,0407***		Fungi mid	2,1234	0,6207	4,6815
Fungi end	0,3922	0,6096	**0,0883**		Fungi end	0,7593	0,2678	3,1565
Nematodes mid	0,6902	0,4754	0,3848		Nematodes mid	0,1644	0,5327	0,7958
Nematodes end	0,5715	0,7211	0,4959		Nematodes end	0,333	0,1317	0,4843
Protists mid	0,1746	0,5673	0,5219		Protists mid	1,9571	0,3365	0,4225
Protists end	***0,0396***	0,5803	0,4961		Protists end	4,7343	0,3142	0,4776
Cysts mid	0,3306	0,5454	0,7879		Cysts mid	0,988	0,3766	0,0741
Cysts end	0,5797	0,28	0,9616		Cysts end	0,3156	1,2243	0,0024
Bacteria mid	0,558	0,4821	0,7205		Bacteria mid	0,3534	0,5106	0,1312
Bacteria end	0,3136	0,6181	0,8971		Bacteria end	1,0615	0,2554	0,0171
Total mid	0,1617	0,7089	0,3552		Total mid	2,0845	0,1427	0,8888
Total end	0,1648	0,4996	0,286		Total end	2,053	0,47	1,191

Figure 24: p-values and F-ratios from in-situ experiments.

E In-lab data

#Channels populated				channels populated		
	Fungi, chip entrance (Pillars-Channels)					
test	chip	day 1	day 3	day 8	day 23	day 37
A	F19	2	8	18	18	*no data*
	F20	0	8	27	47	50
B	F13	0	45	63	67	63
	F15	0	20	59	50	42
C	F16	1	11	19	40	52
	F17	2	10	24	24	32
	Fungi, chip end (pi-ch)					
test	chip	day 1	day 3	day 8	day 23	day 37
A	F19	0	0	3	4	*no data*
	F20	0	0	0	15	27
B	F13	0	0	49	*66*	*66*
	F15	0	4	53	52	51
C	F16	0	0	21	*no data*	32
	F17	0	0	1	3	5
	furthest fungi growth (marking)					
test	chip	day 1	day 3	day 8	day 23	day 37
A	F19	0	0.8	7	19.9	19.9
	F20	0	0	6	6	19.9
B	F13	0	0	18	19.9	19.9
	F15	0	0	19.9	19.9	19.9
C	F16	0	0	6	19.9	19.9
	F17	0	0.4	4	19.9	19.9

Figure 25: Raw data from lab experiments.