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Impacts of pesticide cocktails and fertilizers on the soil microbial community and soil functioning

GEO 511 Master's Thesis

Author
Celina Steffani
16-707-358

Supervised by
Dr. Florian Walder (florian.walder@agroscope.admin.ch)

Faculty representative
PD Dr. Guido Lars Bruno Wiesenberg

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Department of Geography, University of Zurich

Abstract

Agricultural intensification led to a multiplication of pesticide and fertilizer usage, which raised concerns about negative environmental impacts, especially the adverse effects on non-target soil organisms. The soil microbial community is involved in essential soil processes and performs a number of functions that improve soil fertility which is an important ecosystem service of soil. Therefore, changes in their composition and structure can impact the system's productivity and sustainability. To date, many controversial effects of agrochemicals on soil organisms have been found, and mainly the impact of single active substances and the separate application of pesticides and fertilizers were studied. However, it is common agricultural practice to combine pesticide cocktails and fertilizers in today's agriculture. Therefore, this thesis aims to assess the impact of synthetic and biological pesticide cocktails as well as organic and inorganic fertilizers applied as individual or combined treatments in a pot experiment with leaf lettuce in the greenhouse. The abundance and composition of the soil microbial community, especially arbuscular mycorrhizal fungi (AMF), and soil functioning (litter decomposition, plant growth, nutrient content) were monitored for 60 days.

Fertilizers influenced the soil microbial community composition and structure more than pesticide cocktails. However, the interaction between pesticide cocktails and fertilizers led to an even higher impact. Treatments with synthetic and biological pesticide cocktails as well as organic fertilizers did not show a significant effect on the abundances of the soil microbial groups. Only inorganic fertilizer application resulted in a significant increase in the abundance of all microbial groups (Gram+ and Gram- bacteria, and saprotrophic and arbuscular mycorrhizal fungi) when applied without pesticides. When inorganic fertilizers were combined with one of the two pesticide cocktails, this stimulatory effect of inorganic fertilization was reduced and no longer significant. Furthermore, due to the low tolerance of leaf lettuce to the synthetic pesticide cocktail, its growth was strongly reduced, which further negatively influenced AMF root colonization and increased litter decomposition. As an impact on the soil microbial community and soil functioning was found by pesticides and fertilizers, further research is needed to understand the interaction of both substances in the context of agriculture.

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
C	Carbon
C_{org}	Organic carbon
$CHCl_3$	Chloroform
$D_{39}C_{20}$	Deuterated eicosanoic acid
DCM	Dichloromethane
DM	Dry matter
DT_{50}	Degeneration time to reduce substance concentration by 50 %
FID	Flame ionization detector
FRAC	Fungicide Resistance Action Committee
GC	Gas chromatography
GLFA	Glycolipid fatty acids
HRAC	Herbicide Resistance Action Committee
IRAC	Insecticide Resistance Action Committee
K	Potassium
K_{foc}	Organic carbon normalized Freundlich distribution coefficient
KOH	Potassium hydroxide
MMI	Multimode inlet
MeOH	Methanol
N	Nitrogen
Na_2SO_4	Anhydrous sodium sulfate
NLFA	Neutral lipid fatty acids
$(NH_4)H_2PO_4$	Ammonium dihydrogen phosphate
NH_4NO_3	Ammonium nitrate
OM	Organic matter
P	Phosphor
PC19:0	1,2-dinonadecanoyl-sn-glycero-3-phosphocholine
PLFA	Phospholipid fatty acid
RCBD	Randomized complete block design
S	Sulfur
Vol	Volume
Vol/ Vol	Volume percentage or ratio
WHC	Water holding capacity
Wt	Weight
Wt/ vol	Weight per volume

1 Introduction

In the 1950ties, due to a technical revolution, an industrial intensification of agriculture could take place (Guleria and Tiku, 2009). This intensification resulted in an agricultural land use expansion and increased food production, which enabled the growth of the world population and led to an even higher food demand (Tilman *et al.*, 2002). Therefore, different farming strategies have evolved to maximize yield. Until recently, **conventional farming** methods were predominately used for crop and vegetable production (Chausali and Saxena, 2021). Unfortunately, the over-application of synthetic pesticides and inorganic fertilizers resulted in detrimental environmental impacts to soil, surface water, and groundwater (Corwin and Scudiero, 2019) and deteriorated soil fertility and health. For these reasons, alternative systems have been established to reduce the dependence on external sources and rely more on natural processes (Mäder *et al.*, 2002). One of these is **organic farming** which gained increasing attention in the last decade as a low-input system. By using sustainable farming methods as well as biopesticides and biofertilizers, it is achieved to protect nature and the soil, preserve ecosystem functions, and increase natural soil fertility (Grimm *et al.*, 2016).

1.1 Role of microorganisms in soil fertility

Soil plays a crucial role in ecosystem functioning, and agricultural ecosystems depend on soil's ecosystem services (Power, 2010). One of these services is soil fertility (Zhang *et al.*, 2007) which is the ability of soil to supply nutrients to plants and sustain plant growth (FAO, 2022). As microorganisms are involved in important ecological processes in the soil, such as nutrient cycling and nutrient acquisition through organic matter decomposition and mineralization, they are essential for soil fertility and plant growth (Van Der Heijden *et al.*, 2008; Bünemann *et al.*, 2018). Their abundance, composition, and activity will largely determine the sustainability of a soil system (Van Der Heijden *et al.*, 2008) and changes in their structure can have a significant impact on the system's productivity (Bender, Wagg and van der Heijden, 2016). Due to their high sensitivity to environmental changes, they are an important indicator for soil health (Dong *et al.*, 2021) which is the capacity of a soil to function as a living ecosystem (Lehmann *et al.*, 2020).

The soil microbial community comprises five major taxonomic groups: algae, bacteria (including actinomycetes), fungi, protists, and viruses. Microorganisms produce enzymes that catalyze essential reactions necessary for soil life and processes and degrade pesticides and hydrolyze fertilizers (Bakshi and Varma, 2010). Especially **arbuscular mycorrhizal fungi** (AMF) are a very important component of the soil microbial community, as they are obligate biotrophic symbionts and can form plant root symbioses with the vast majority of vascular plants (Rivera-Becerril *et al.*, 2017; Hage-Ahmed *et al.*, 2019), including agricultural and horticultural crops (Rivera-Becerril *et al.*, 2017). AMF play a key role in terrestrial ecosystems as they improve plant nutrition (Johnson *et al.*, 2005) by making nutrients available to plants (predominantly phosphorus; Liu *et al.*, 2016). The root colonization with AMF involves subtle signaling between the plant and the fungi (Smith and Smith, 2011). The fungi's spores germinate in the soil and infect the root system of a host plant (Parniske, 2008). Hyphae grow into the root cells and form tree-shaped subcellular structures for nutrient exchange (arbuscules; Parniske, 2008) as well as spherical structures for storage and propagation (vesicles; Biermann and Linderman, 1983). Due to the formation of an extensive mycorrhizal network around the root system, AMF can take up nutrients and water from a large resource area and deliver them to the plant (Parniske, 2008). In exchange, plants provide the fungi with organic carbon (Smith and Smith, 2011). Furthermore, AMF can increase the abiotic (e.g., drought) and biotic (e.g., pests) stress tolerance (Smith *et al.*, 2010) and enhance the resistance to soil-borne pests and diseases (Gosling *et al.*, 2006). This symbiosis thus makes a major contribution to sustainable agriculture (Rivera-Becerril *et al.*, 2017), and due to its susceptibility to perturbations, it can be used as a potential indicator for soil fertility (Bender *et al.*, 2016) and ecosystem health (Kaur *et al.*, 2005).

1.2 Pesticides and fertilizers in agriculture

Agricultural intensification induced a multiplication of chemical inputs (FAO, 2021a, 2021b), especially in horticulture (Moeskops *et al.*, 2010). The use of agrochemicals such as pesticides and fertilizers ensure food security by controlling pests, diseases, and weeds and providing crops with nutrients (Imfeld and Vuilleumier, 2012; Álvarez-Martín *et al.*, 2016). **Pesticides** are organic and inorganic chemicals of synthetic or natural origin that target a specific organism (Bünemann *et al.*, 2006). The molecular complexity of pesticides is enormous. They include a wide range of different groups according to their primary target, i.e., herbicides, insecticides, and fungicides (more information in the Appendix 6.3), and differ in their modes of action, which can affect specific or general processes in organisms (FRAC, 2021; HRAC, 2021; IRAC, 2021). An ideal pesticide acts specifically on target organisms, is biodegradable, and does not leach into the groundwater (Johnsen *et al.*, 2001). In agricultural systems, multiple pesticides are usually mixed and applied simultaneously as a cocktail or sequentially over the cropping season (Topping, *et al.*, 2020). Therefore, a mix of different pesticides at different concentrations is found in the soil (Riedo *et al.*, 2021). **Synthetic pesticides** are substances or mixtures of substances produced synthetically that interact with organisms or kill them (EPA, 2021c). With their various modes of action (FRAC, 2021; HRAC, 2021; IRAC, 2021), they aim to prevent, destroy, repel or mitigate pests and can be used as a plant regulator, defoliant, or desiccant (EPA, 2021d). Plant protection is essential for crop production, and alternatives for sustainable agriculture are needed (McDougall, 2018). **Biopesticides**, as a more environmentally friendly alternative, have emerged over the last decades (Baker, *et al.*, 2020; Manda, *et al.*, 2020) and are promoted by international (EU, 2019) and Swiss politics (BK, 2021). They are derived from natural sources from animals, plants, bacteria, and certain minerals (EPA, 2021c) and are supposed to be quickly biodegradable, have a low environmental risk, and affect non-target organisms only slightly (Guleria and Tiku, 2009). According to Syngenta (2021a), biopesticides can be divided into microbial (bacteria, fungi, viruses), macrobiotic (parasites, predatory mites, nematodes), and biological (botanicals; plant or algae extracts, metabolites of microorganisms) substances. Due to their complex chemical composition, the exact mechanisms of action, degradation behavior, and possible interactions with humans and the environment are much more difficult to characterize (Syngenta, 2021a).

In addition to pesticides, **fertilizers** are applied to ensure a sufficient nutrient supply for plant growth to maintain the current structure and output of agriculture. Nitrogen (N), phosphorus (P), and potassium (K) are applied in large quantities as they constitute main plant components (Isherwood, 2000). With the invention of the Haber-Bosch process at the beginning of the 20th century, it was possible to synthesize ammonia from atmospheric nitrogen and hydrogen and produce **inorganic fertilizers**, which enabled a great advance in agricultural productivity (Paull, 2009). Inorganic fertilizers are one of the most important sources of supplied nutrients for crops (Dudaš *et al.*, 2016) as they are cheap, immediately available to plants, and target applicable (Neuweiler and Krauss, 2017). However, even before the advent of inorganic fertilizers, organic fertilizers were predominantly used for fertilization for quite some time (Chausali and Saxena, 2021). **Organic fertilizers** stem from organic sources and can be divided into farmyard (manure and slurry) and recycling (compost and liquid/ solid digestate) fertilizers. In recent years, compost amendments have become increasingly important, especially in horticulture (Neuweiler and Krauss, 2017). Compost needs to be mineralized to release inorganic nitrogen that can be absorbed by plants (Liu *et al.*, 2014). This results in delayed availability but longer effectiveness, and the time of nutrient release can only be insufficiently estimated (Neuweiler and Krauss, 2017). In addition to the recycled fertilizers, there are **organic commercial fertilizers** produced from by-products of the processing of animal or plant products used as a complement in organic horticulture (Neuweiler and Krauss, 2017; Speiser *et al.*, 2021), e.g., Biorga Quick. Due to the fine granulation of Biorga Quick (horn meal, feather meal, meat bone meal), nutrients are quickly available and are thus administered at the start of vegetation for rapid nutrient supply when demand is high (Hauert, 2021).

1.3 Impacts of pesticides and fertilizers on the soil microbial community

Besides the many benefits of applying pesticides and fertilizers in agriculture, their use can also lead to soil contaminations and thus adverse effects on non-target organisms threatening soil fertility (Rodríguez-Eugenio *et al*, 2018). The effects of agrochemicals on the soil microbiome are complex and diverse (Bünemann *et al.*, 2006; Zhong *et al.*, 2010; Imfeld and Vuilleumier, 2012; Lazcano *et al.*, 2013) and still not fully studied. Even though pesticides are designed to act on a specific target site of pests and diseases, and their risk is carefully evaluated before coming onto the market, their impact on non-target organisms has become a serious concern (Bünemann *et al.*, 2006; Verbruggen *et al.*, 2010; Bender *et al.*, 2016). **Synthetic pesticides** can either interfere with the active site of enzymes or be used as a nutrient source through degradation, which can shift the community balance (Riah *et al.*, 2014). The review of Puglisi (2017) showed that synthetic pesticides can influence the activity, abundance, and structure of the microbial community and depending on the substance, the microbial community can be influenced negatively, not at all, or even positively (Bünemann *et al.*, 2006). Interference of synthetic pesticides at a particular developmental stage of AMF can be detrimental to the establishment of arbuscular mycorrhiza and the fungi's survival (Helander *et al.*, 2018).

The potential impacts of **biological pesticides** on the soil microbial community are not yet well studied. But despite the natural origin, there were mixed reports about the impact of biological pesticides on non-target organisms (Amichot *et al.*, 2018). When applied at the recommended dose, biological pesticides mainly did not alter the community structure (Spyrou *et al.*, 2009) or diversity (Fournier *et al.*, 2020). However, only a few studies looked at the effect on soil microorganisms, as they were mainly about the impact on higher organisms, e.g., earthworms (Ponsankar *et al.*, 2016), spiders (Cunha Pereira *et al.*, 2020), and bees (Cunha Pereira *et al.*, 2020). Furthermore, the effect of biological pesticides on AMF has hardly been tested. But some found that they did not affect the colonization ability and diversity of AMF even under intensive application schemes (Ipsilantis *et al.*, 2012).

Until now, primarily the effects of a single active substance or product were studied (e.g., Guo *et al.*, 2015), even though this is not recommended due to the possible formation of resistances (FRAC, 2021; HRAC, 2021; IRAC, 2021). With **pesticide cocktail** application, exposure to multiple pesticides increases the toxicity in the soil and can lead to pesticide accumulations (Topping *et al.*, 2020). Interpretations about the effect of pesticide cocktails can be difficult as their application can cause interactions of the chemical compounds as well as synergisms or antagonism are possible (Thompson and Wilkins, 2003). Therefore, the impact of pesticide cocktails can be unpredictable (Hernández *et al.*, 2017), and risk assessments with only single substances underestimate the impact on microorganisms, as higher exposure concentrations can shift impacts from sublethal to lethal (Laetz *et al.*, 2009).

Depending on the fertilizer type, the effects on the soil microbial community can vary widely (Lazcano *et al.*, 2013). An increasing nutrient input can generally enhance the soil microbial biomass and activity, leading to potential alterations in the community composition (Zhong *et al.*, 2010; Lazcano *et al.*, 2013; Geisseler and Scow, 2014). **Inorganic fertilizers** are applied in vast amounts and can lead to environmental pollution. Improper use of inorganic fertilizers can have a range of serious adverse effects on the environment through nitrate and phosphorus leaching and eutrophication of water bodies, but also through emissions of the greenhouse gas nitrous oxide (Hagedorn *et al.*, 2018). In the long-term, inorganic fertilizer use can result in soil acidification and soil organic carbon depletion, which contributes to the deterioration of soil fertility (Liu *et al.*, 2019). Results of short-term inorganic fertilization are controversial as different biome types with various soils, N application rates, and types, and especially experimental duration are looked at (Lv *et al.*, 2017; Ma *et al.*, 2021; Yayi *et al.*, 2021). The addition of essential nutrients such as N and P can counteract nutrient limitations for microbial growth (Griffiths, Spilles and Bonkowski, 2012). However, a higher direct nutrient availability with inorganic fertilizers in soil can decrease plants' dependency on the AMF symbiosis and root colonization with AMF as well as reduce AMF abundance in the soil (Gosling *et al.*, 2006).

Organic fertilizers are used to maintain and improve soil fertility in the long-term (Mäder *et al.*, 2002). As soil microorganisms are important in compost decomposition, they are stimulated by the amendment whereby AMF colonization was found to not be suppressed (Gosling *et al.*, 2006). Additionally, compost amendments are shown to have the potential to mitigate the negative impacts of pesticides on the soil system (called buffer effect; Álvarez-Martín *et al.*, 2016). Due to the high sorption capacity of organic matter (OM) in compost, pesticides' persistence, dynamics, and dissipation can be altered due to immobilization (Marín-Benito *et al.*, 2014). Thus the bioavailability and concentrations in the soil solution can be reduced and the impact on soil organisms mitigated (Álvarez-Martín *et al.*, 2016; Carpio *et al.*, 2020). This buffer effect can further be provoked by increased microbial activity after compost amendment which accelerates the degradation of the substances (Carpio *et al.*, 2020).

The interpretation of microbial community dynamics is challenging as it integrates interwoven relationships between microbial groups (Kaviya *et al.*, 2019). However, since the microbial community is sensitive to environmental changes (Yin *et al.*, 2010), it can be taken as an indicative marker for studying environmental impacts by agricultural management on soil quality and health (Sharma *et al.*, 2016). Thereby, the usage of the culture-independent technique of **phospholipid fatty acid (PLFA) analysis** can give insights into the relationship between microbial communities and their environments at the time of sampling (Willers *et al.*, 2015). It is a rapid, inexpensive, and sensitive method used to identify microbial groups by individual biomarkers (Frostegård *et al.*, 2011) and allows the detection of treatment effects on the soil microbial community composition (Ramsey *et al.*, 2006).

In summary, applications of pesticides and fertilizers can interfere with soil microorganisms and therefore pose a threat to soil ecosystem services, especially soil fertility (Johnsen *et al.*, 2001; Imfeld and Vuilleumier, 2012; Stanley and Preetha, 2016). Nevertheless, little is known about the response of the soil microbial community following the combined application of pesticides and fertilizers. Huang *et al.* (2021) found that pesticides and fertilizers can influence each other and thus induce inhibitory and promoting effects on microbial processes. Furthermore, Rillig *et al.* (2019) revealed that **multiple stressors** can increase the impact on soil life, making further studies on the impact of pesticide cocktails in combination with fertilizers even more urgent.

1.4 Aim of this thesis and research questions

As ecosystem functioning and stability are closely related to the abundance and activity of soil microorganisms (Yang *et al.*, 2018), it is essential to study the impacts of pesticide cocktails and fertilizers on the soil microbial community and soil functioning. Therefore, this thesis aims to compare a conventional and organic horticulture system through a manipulative greenhouse experiment with leaf lettuce. The goal is to find out how the application of synthetic and biological pesticide cocktails, inorganic and organic fertilizers, as well as pesticide cocktails in combination with fertilizers affect the soil microbial community, especially AMF. Furthermore, the assessment of the above-ground biomass and nutrient content of the plant as well as the litter decomposition are taken as a proxy for soil functioning to observe the influence of changes in the microbial abundance and activity.

Based on the four main research questions (R), the following hypotheses (H) are formulated:

- R1. What impact does the application of **synthetic or biological pesticide cocktails** have on the soil microbial community?
    - H1.1 The synthetic pesticide cocktail is assumed to decrease the microbial biomass, especially AMF, and cause a shift in the soil microbial community composition.
    - H1.2 The biological pesticide cocktail is assumed to have a minor impact on the soil microbial community and AMF, and cause only a slight shift in the soil microbial community composition.

- R2. What impact does the application of **inorganic or organic fertilizers** have on the soil microbial community?
    - H2.1 The inorganic fertilizer is assumed to stimulate the overall microbial biomass growth but decrease AMF, and lead to a shift in the soil microbial community composition.
    - H2.2 The organic fertilizer is assumed to stimulate the overall microbial biomass growth and affect AMF less, which causes a smaller shift in the soil microbial community composition.

- R3. What impact on the soil microbial community can be found if **pesticide cocktails** are applied **in combination** with **fertilizers**?
    - H3.1 The simultaneous application of the inorganic fertilizer in combination with the pesticide cocktails is assumed to increase their impact on the soil microbial community composition.
    - H3.2 The simultaneous application of the organic fertilizer in combination with the pesticide cocktails is assumed to mitigate their impact on the soil microbial community composition, especially with synthetic pesticides.

2 Materials and Methods

2.1 Experimental set-up

A pot experiment in the greenhouse was conducted in 1.5-liter pots (14.2 cm upper diameter, 11 cm lower diameter, 11.9 cm height) filled with a clay loam from a grassland located near Agrocope Reckenholz (47°25'51.6"N 8°31'10.2"E). The field was previously cultivated by cover crops with legumes and cruciferous plants and lastly ploughed at the beginning of 2021. Before the experiment, the soil was sieved (< 5 mm) and thoroughly homogenized. 1300 g of equivalent dried soil was filled into each pot. Additionally, for the pots that belonged to the organic fertilizer treatment, 40 g of soil was replaced by compost (< 5 mm sieved) for a basal fertilization. The compost-soil mixture was thoroughly homogenized with a scoop before filling. At the beginning of the experiment, the soil without compost had a pH (H_2O) of 6.4, containing 2.4 mass-% of organic C (C_{org}), 0.3 mass-% of total N, 67.4 mg/ kg of total P, and 160.9 mg/ kg of total K, as well as 2.3 mg/ kg of available P, and 15.7 mg/ kg of available K. After compost addition, the physicochemical soil characteristics changed to a pH (H_2O) of 6.7, containing 2.6 mass-% of C_{org}, 0.3 mass-% of total N, 92.9 mg/ kg of total P, and 331.3 mg/ kg of total K, as well as 2.9 mg/ kg of available P and 45.0 mg/ kg of available K (see Table 8 in the Appendix). A gardening fleece was placed at the bottom, and a saucer was placed under each pot to prevent the loss of soil and catch any leachate (see Figure 1). The position of the pots in the greenhouse was defined by the "randomized complete block design" (RCBD; Hartung *et al.*, 2019), where nine replicates distributed over six blocks were rearranged every second week. The design was made in the R environment (version 3.6.1) with the package *agricolae* and *dplyr* and the function *design.rcbd* (R Core Team, 2021).

Figure 1 Experimental set-up in the greenhouse chamber at 22.04.2021 – The day of pesticide and fertilizer applications before the harvest.

2.1.1 Model plant

Due to its sound greenhouse cultivation, the possibility to repeatedly harvest, the fast growth, the responsiveness, and colonization with AMF, leaf lettuce (*Lactuca sativa var. crispa*) was used as a model plant. Within a pot, six seeds of leaf lettuce (Samen Mauser AG, 2021) were planted at a depth of 1 cm. The pot's surface was divided into six uniform circular sectors. Each seed was planted 3 cm from the edge in every sector. After seeding, the pots were watered to 80 %-WHC (water holding capacity). After three weeks of germination, the three least developed seedlings were pricked carefully from the pot with their entire root system. The three leaf lettuce plants continued to grow for another week until the experimental treatments were applied.

2.1.2 Water holding capacity (WHC)

The water holding capacity (WHC) is the maximum amount of water a soil can hold against gravity (Hendriks, 2010). To estimate the WHC in the experimental system, 12 pots (six with and six without compost) were water-saturated, placed on a grid, and weighed as soon as no more water dripped out at the bottom, representing the weight at 100 %-WHC. This procedure was repeated four times for better WHC estimations. Additionally, 12 pots (six with and six without compost) were dried at 105 °C for 48

hours until equilibrium weight to determine the dry weight at 0 %-WHC. The pots were watered with rainwater to maintain a WHC between 55 to 80 %.

2.1.3 Growth conditions

To create a suitable environment for the leaf lettuces, the greenhouse chamber was set to a daily cycle of 16 hours day and 8 hours night. Supplemental artificial lighting was switched on if the natural light level fell below 400 W/m^2. The temperature during the experimental period (end of March to end of June 2021) was kept at an average temperature of 21.9 ± 0.2 °C (mean ± SE) with a minimum and maximum of 13.4 and 35.6 °C, respectively. The relative humidity was on average 53.1 ± 0.6 % (mean ± SE).

2.1.4 Soil property analysis

To analyze the physiochemical properties of the soil, three soil samples, each with and without compost, were taken during the experimental set-up. The soil was dried at 60 °C for 24 h, sieved (< 2 mm), and for a subsample, roots were removed and was milled with a vibration mill (Frisch, Pulverisette 2) to analyze the C_{org} content. The analysis was done by the laboratory at Agroscope Reckenholz according to the Swiss reference methods of the Federal Agricultural Research Station (FAL, 1996). The pH value was determined in an aqueous soil suspension as the hydrogen ion activity. The organically bound carbon in the soil (C_{org}) was determined by the Walkley−Black method due to oxidation of the organic matter. By multiplying C_{org} by 1.725, values for humus contents [%] were calculated. The soil texture was analyzed by a pipetting method where the soil suspension is dispersed, sedimented, extracted from a defined depth, and dried. The grain size fraction is related to the fine soil and the texture defined by the particle diameters: < 2 µm as clay, 2-50 µm as silt, and > 50 µm as sand.
For the soil nutrient analysis, total nitrogen and carbon were determined by the Dumas method, whereby the soil sample was combusted, and the thermal conductivity of the resulting gas determines the nitrogen and carbon content. Potassium (K) was extracted with a 0.5 M ammonium acetate-EDTA solution (pH 4.65) and measured by emission spectrophotometry. Phosphorus (P) in the soil reacted with ammonium molybdate in an acidic solution to form phosphorus molybdenum blue. The resulting blue coloration is determined photometrically and determines the P content in the soil.

2.2 Experimental design

To compare a conventional and organic horticulture system, synthetic and biological pesticide cocktails and inorganic and organic fertilizers were applied in a full factorial design (see Figure 2), resulting in nine treatments. Each treatment was replicated six times (54 pots in total).

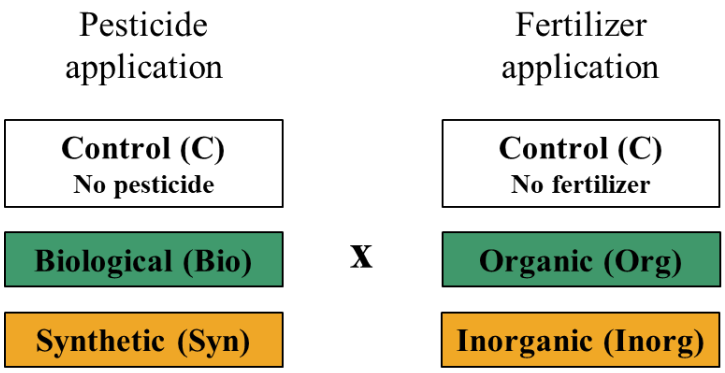

Figure 2 Nine treatments (C+C, C+Org, C+Inorg, Bio+C, Bio+Org, Bio+Inorg, Syn+C, Syn+Org, Syn+Inorg) resulting from different combinations of pesticide (no pesticides (pesticide control), biological and synthetic) and fertilizer (no fertilizers (fertilizer control), organic and inorganic) applications.

2.2.1 Pesticide and fertilizer dosage

For the synthetic and biological pesticide cocktails, a mixture of six different active substances with various modes of action were used in each (Table 1; for a more detailed description, see Chapter 6.3). The pesticides are only applied as a respective (synthetic or biological) pesticide cocktail and not individually. These are frequently used pesticides in horticulture and were applied at the recommended field rate. No biological herbicides were used, as they are not permitted in organic farming.

Table 1 Synthetic (Agroscope, 2021) and biological (Speiser et al., 2021) pesticide products, listed by their respective pesticide classification, active substances and its content, recommended field rate and product per pot according to the manufacturer.

	Pesticide Classification	Product name	Active substance	Content of active substance	Recommended field rate	Product per pot
synthetic	Herbicide	Stomp Aqua	Pendimethalin	38.9%; 455 g/l	3.5 l/ha	0.0056 ml
	Fungicide	Filan	Boscalid	50%	0.5 kg/ha	0.8 mg
	Fungicide	Switch	Cyprodinil Fludioxonil	37.5% 25%	0.8 kg/ha	1.28 mg
	Insecticide	Pirimor	Pirimicarb	50%	5 g/m^2	0.08 g
	Insecticide	Cypermethrin	Cypermethrin	11%; 100 g/l	0.25 l/ha	0.0004 ml
biological	Fungicide	Vacciplant	Laminarin	4.3%; 45 g/l	1 l/ha	0.0016 ml
	Fungicide	Armicarb	Potassium bicarbonate	85%; 850 g/kg	3 kg/ha	4.8 mg
	Fungicide	Curenox 50 WG	Copper hydroxide	50%	2 kg/ha	0.008 ml
	Fungicide	Netzschwefel Stulln	Sulfur	80%	1.5 kg/ha	2.4 mg
	Insecticide	NeemAzal-T/S	Azadirachtin A	1%; 9.8 g/l	3 l/ha	0.048 ml
	Insecticide	Audienz	Spinosad	44.2%; 480 g/l	0.3 l/ha	0.0048 ml

The fertilization rate for the inorganic fertilizer was according to the nutrient requirements of the Swiss recommendation for leaf lettuce in a greenhouse. A quantity of 50 kg N/ha, 4.4 kg P/ ha, and 41.5 kg K/ha (Table 1b in Neuweiler and Krauss, 2017) was applied as a self-mixed inorganic fertilizer with 26.14 mg of ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 219.5 mg ammonium nitrate (NH_4NO_3), and 95.3 mg potassium hydroxide (KOH) per pot (Table 2; for calculations see Appendix 6.4.1).

Table 2 Inorganic fertilizer composition, whereas the amount of fertilizer corresponds to a fertilizer rate of 50 kg N/ha, 4.4 kg P/ ha and 41.5 kg K/ha.

Chemical compound	Molecular formula	Amount [mg per pot]
Ammonium dihydrogen phosphate	$(NH_4)H_2PO_4$	26.1
Ammonium nitrate	NH_4NO_3	219.5
Potassium hydroxide	KOH	95.3

For the organic fertilization, compost and Biorga Quick were used. Compost was applied at the recommended rate of 25 t DM/ ha (40 g compost/ pot; Chapter 3.3 in Neuweiler and Krauss, 2017). For the basal fertilization at the beginning of the pot set-up, a mature compost from the company Biomassenhof AG in Winterthur was used and consisted of horticultural waste (mixture of green cuttings

and tree residues). As it is estimated that approximately 10% of N in the compost is available to plants (Table 8 in Neuweiler and Krauss, 2017), Biorga Quick was additionally applied to attain a comparable amount of available N as with the inorganic fertilization. Since only 70 % of N in Biorga Quick is plant available, an application rate of 74.41 g/m^2 was used (1.2 g Biorga Quick/ pot; calculation in Appendix 6.4.2; Hauert, 2021). The laboratory values of the compost and Biorga Quick are listed in Table 3 and 4, respectively.

Table 3 Chemical composition of Biorga Quick from Hauert

NO	OS	Cl
12 %	80 %	0 %

NOTE: "NO" = nitric oxide, "OS" = organic substance, "Cl" = chlorine

Table 4 Chemical composition of the mature compost from the Biomassenhof AG in Winterthur (further values in Appendix 6.4.2).

pH	C/N	Total-N [g/kg DM]	P (P_2O_5) [kg/t DM]	K (K_2O) [kg/t DM]
8.13	18.99	8.32	2 (4.58)	11.2 (13.44)

NOTE: "DM" = dry matter

2.2.2 Pesticide and fertilizer application

After four weeks of leaf lettuce establishment (day 0), the above-ground biomass was harvested as described in Chapter 2.4.4, and pesticides and fertilizers were applied afterward. On the day of application, the individual pesticides and the inorganic fertilizer were mixed separately with one liter of rainwater and stirred on a magnetic stirrer for five minutes (500 rpm) until fully dissolved. The water amount used for application was 400 l/ha (= 0.642 ml/ pot) and was conducted with the spraying chamber of "Schachtner Gerätetechnik" with the "Teejet 8002 EVS" nozzle by Lechler (spraying settings in Table 5; Teejet Technologies, 2021). This configuration allowed a uniform distribution of the substances over the pot's surface. After each substance, the spraying chamber was cleaned to avoid contaminations. To test whether the spraying chamber had a high variability within and between spraying, six test sprayings were carried out beforehand. For each spraying, six pots were placed in a row in the spraying chamber. The relative standard error over all sprayings and pots was below 1 %, which means that a low variability can be assumed.

Table 5 Spray chamber settings to achieve an application rate of 400 l/ ha (Lechler, 2021).

Speed [km/h]	Spraying pressure [bar]	Straying angle [°]	Spraying distance [cm]	Spraying width [cm]
2	2.1	80	28	15-18

As Biorga Quick is a granulate, it was first ground into a fine powder in a vibrating mill (Retsch MM400) and then spread evenly over the pot's surface using a mesh sieve (< 1 mm) with the same circumference as the pot. After all applications, each leaf lettuce plant was sprayed with rainwater with a hand sprayer, and all pots were watered to the upper level of the WHC range.

2.3 Soil sampling procedure

60 days after the pesticide and fertilizer applications and harvesting the plant biomass, soil samples were taken by pouring the soil out of the pot into a container. The root system for AMF colonization and the rooibos tea bag were retained. The soil was thoroughly mixed for 45 seconds, a subsample of ~100 g of

soil was retrieved, and remaining roots within the subsample were removed with tweezers for 10 minutes. The sample was freeze-dried (< 6.11 mbar, -12 °C), sieved (< 2 mm), and stored at -20 °C until further usage for PLFA analysis.

2.4 Determination of different biological and functional parameters

2.4.1 Phospholipid fatty acid extraction and analysis

The phospholipid fatty acid (PLFA) analysis was conducted to assess the microbial community composition and was performed using the method of Frostegård et al. (1991), based on the protocols by Waldrop and Firestone (2006) and Gunina *et al.* (2017) with some modifications of Zosso and Wiesenberg (2021; see Appendix 6.5). The methylation followed the procedure in Wiesenberg and Gocke (2017; see Appendix 6.6). The analysis was done according to the following steps (Figure 3): Lipid extraction by liquid-liquid extraction (1), lipid separation by solid-phase extraction (2), methylation of fatty acids in the phospholipid fraction (3), and PLFA identification and quantification using a GC-FID (4).

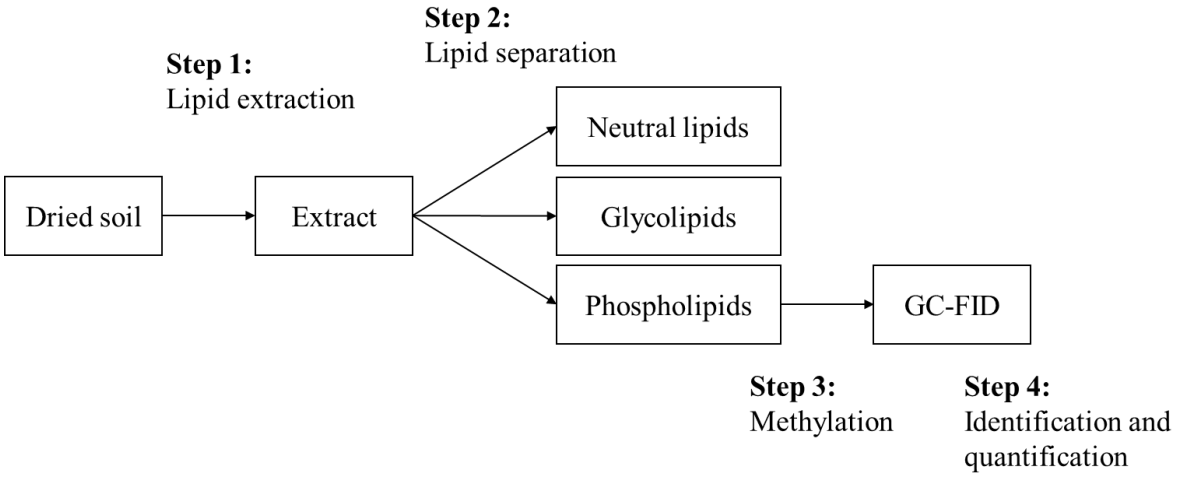

Figure 3 Experimental procedure of the PLFA analysis with lipid extraction from the soil sample (step 1), a subsequent lipid separation by solid-phase extraction (step 2) into three lipid fractions (neutral-, glyco- and phospholipids), followed by the methylation (step 3) of phospholipids and PLFA identification and quantification using a GC-FID (step 4; gas chromatograph (GC) equipped with a flame ionization detector (FID)).

Step 1: 5 g of freeze-dried and sieved soil (< 2 mm) was extracted by 4 ml of freshly prepared extraction solution (1 : 2 : 0.8 (vol/vol/vol) of chloroform ($CHCl_3$) : methanol (MeOH) : citric acid buffer (pH 4, 0.15 M) per gram soil with three extraction steps. 50 µl of the phospholipid C19:0 (PC19:0, 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine, Aventilipids, concentration: 1.055 mg/ ml) was added directly to the soil in the glass tube before extraction as an internal standard for the quantitative determination of PLFAs and quantification to nanomoles per gram of soil (nmol/ g DM). For the first extraction step, the closed glass tube with the liquid-soil mixture was placed on a horizontal shaker for 2 h (200 rpm). After 10 min of centrifuging (2500 rpm), the supernatant was transferred to a separation funnel. For the second extraction, 5 ml of extraction solution were added to the extraction residues (organics) in the glass tube, shaken for 30 min, centrifuged for 10 min, and the supernatant transferred to the separation funnel. The second extraction step was repeated once. After all the supernatants of a sample were combined in the separation funnel, chloroform and citric buffer were added in an amount of 0.34 times the total volume of added extraction solution. The separation funnels were shaken on a horizontal shaker for 15 min (70 rpm). After separation overnight, the lower organic phase was released into a flask. 10 ml chloroform were added to the separation funnel to perform a liquid-liquid extraction. The funnels were shaken for 15 min (70 rpm), and the lower phase was released into the flask. The liquid-liquid extraction was repeated twice. The flask with the organic phase was placed in a Multivapor

(Multivapor™ P-6, Büchi Labortechnik AG, Switzerland) to reduce the volume to 100 ul by applying an underpressure (500 mbar) and heat through a water bath (45°C).

Step 2: The previously volume-reduced organic phase was sequentially separated into neutral- (NLFA), glycol- (GLFA), and phospholipid fatty acids (PLFA) by solid-phase extraction using an activated silica column (Silica 60, Honeywell Fluka, USA; activated overnight at 110°C) containing a glass fiber filter. The samples were transferred to the column and eluted by sequentially adding 5 times 1 ml chloroform (NLFA), 4 times 5 ml acetone (GLFA), and 4 times 5 ml methanol (PLFA). Out of these fractions, only the PLFA fraction was retained and is presented in this study. The PLFA fraction was reduced to 100 ul in the Multivapor (pressure: 400 mbar, water bath: 50 °C), and afterwards the remaining water in the samples was removed over a column with anhydrous sodium sulfate (Na_2SO_4), and the samples were dried under a N_2 stream.

Step 3: If the dried PLFA fraction exceeded 2 mg, an aliquot was used for the further procedure as the methylation protocol is only valid for quantities below 2 mg. For the methylation, 300 µl dichloromethane (DCM), 50 µl control standard ($D_{39}C_{20}$ – deuterated eicosanoic acid, concentration: 0.101 mg/ ml), and 500 µl boron-trifluoride-methanol were added to the PLFA fraction in a vial. Sample vials were placed on a heating block at 60 °C for 18 min, 500 µl ultra-purified water was added after samples were cooled down to room temperature. The mixture was centrifuged for 3 min (2500 rpm), and the lower organic phase was transferred onto a column with anhydrous Na_2SO_4 and after filtration collected in an autosampler vial. A few drops of DCM were added to the remaining mixture, centrifuged, and again transferred to the column. The last step was repeated 5 – 8 times until the organic phase remained colorless to enable quantitative transfer of the sample. To ensure that no water was remaining in the samples, the samples were run a second time over a column with anhydrous Na_2SO_4 and reduced by evaporation so that the methylated fatty acids could be transferred to a micro inlet (Wiesenberg and Gocke, 2017).

Step 4: Identification and quantification of the individual PLFAs were done by using a gas chromatograph (GC; Agilent Technologies, Inc.; 7890 B) equipped with a multimode inlet (MMI) connected with a flame ionization detector (FID). To support the peak identification, one sample of each treatment and an external standard series of 27 fatty acids (Larodan, Inc., USA; Sigma Aldrich, Inc., USA; Avanti Polar Lipids, Inc., USA) were measured on a GC (Agilent Technologies, Inc.; 6890 N) coupled to a mass spectrometer (MS; Agilent Technologies, Inc.; 5973 N) and compared to the National Institute of Standards and Technology (NIST) and Wiley mass spectra library. Both used GC systems were equipped with a J&W DB-5MS column with 50 m length, 0.2 mm inner diameter, and 0.32 µm film thickness. The temperature programs for the GC oven and MMI are in the Appendix (6.7; Table 15).
For PLFA quantification, for each integrated peak per chromatogram the ratio to the internal standard (PC 19:0) was calculated [nmol/ g DM] using the following equation (Quideau *et al.*, 2016; for the derivation, see Appendix 6.8)

$$\text{PLFA content } \left(\frac{\text{nmol}}{\text{g DM}}\right) = \frac{\frac{\text{areaPLFA x C19:0 std added}}{\text{area C19:0}}}{\text{sample weight}} \text{ x } \frac{M_{C19:0}}{M_{PLFA}}$$

where areaPLFA is the peak area for each identified PLFA, $C_{19:0}$ std added is the amount of added $C_{19:0}$ [nmol] to each sample, area C19:0 is the peak area of the $C_{19:0}$, sample weight is the weight of the dried soil [g] added to the centrifuge tube before extraction, $M_{C19:0}$ is the molar weight of the $C_{19:0}$ and M_{PLFA} is the molar weight of the identified PLFA (Quideau *et al.*, 2016).

For the PLFA classification and assessment of the microbial community structure the functional groups of Willers *et al.* (2015) were used: Gram-positive bacteria (Gram+ ; $iC_{14:0}$, $aC_{14:0}$, $iC_{15:0}$, $aC_{15:0}$, $iC_{16:0}$, $aC_{16:0}$, $iC_{17:0}$, $aC_{17:0}$), Gram-negative bacteria (Gram- ; $C_{16:1\omega7c}$, $C_{16:1\omega9c}$, $C_{18:1\omega5c}$, $C_{18:1\omega11c}$, $cyC_{17:0}$, $cyC_{19:0}$), actinobacteria ($10MeC_{16:0}$, $10MeC_{18:0}$), saprotrophic fungi ($C_{18:2\omega6c,}$ $C_{18:2w9c,12c,}$ $C_{18:3w6c,9c,12c}$), and AMF ($C_{16:1\omega5c}$). For calculating the total abundance of microorganisms (nmol/ g DM), all these diagnostic PLFAs from the functional groups together with the saturated fatty acids ($C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, and $C_{18:0}$) were summed up. The saturated fatty acids were not included in the calculation of the relative abundance of functional microbial groups (percentage of the total mole amount; mol %) as they are not completely specific to any group and can partially be derived from plants (Willers *et al.*, 2015).

The assignment of the functional groups from Willers *et al.* (2015) to different main microbial groups was according to the paper by Joergensen (2021) and is visualized in Figure 4. Actinobacteria were included in the group of Gram+ bacteria and not considered on their own. Therefore, the analysis was done for the Gram+ and Gram- bacteria, saprotrophic and arbuscular mycorrhizal fungi, as well as for the main groups of bacteria (Gram+ and Gram-) and fungi (saprotrophic and arbuscular mycorrhizal). Bacteria and fungi were summed together to get the total microbial biomass. Some samples were repeatedly measured and extracted. The mean value of a sample was calculated for the evaluation, and data variability was discussed in the Appendix 6.9.

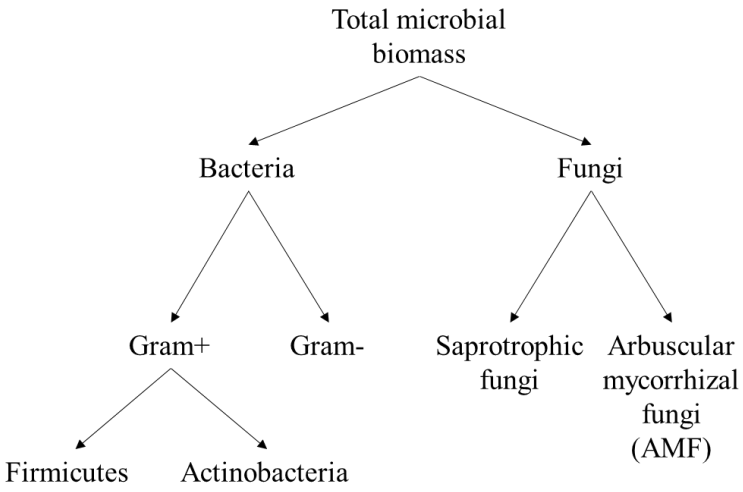

Figure 4 Assignment of PLFAs to different main microbial groups according to the paper by Joergensen (2021). Soil bacteria are composed of Gram+ bacteria and Gram- bacteria, whereas the latter is composed of Actinobacteria and Firmicutes. The soil fungi consist of arbuscular mycorrhizal and other mainly saprotrophic fungi.

2.4.2 Determination of AMF root colonization

To determine the root colonization by arbuscular mycorrhizal fungi (AMF), the sampled leaf lettuce roots from the harvest at day 60 were washed with tap water. The roots of all three leaf lettuces in one pot were combined, and the top and bottom 2 cm were cut off. The roots were cut into 2 cm long pieces and stored in 70 % ethanol (EtOH). After rinsing off the EtOH with deionized water, a solution with 10 % potassium hydroxide (KOH, wt/vol) was added to clear the roots by incubating them at 80 °C for 10 - 20 minutes. Afterward, the roots were again rinsed with deionized water, and a 5 % ink-vinegar solution (vol/ vol) was added to stain the roots during a further incubation at 80 °C for 20 - 30 min. After washing the roots with deionized water, they were stored for at least a week in a 50 % glycerol solution (Vierheilig *et al.*, 1998). To analyze the roots under the

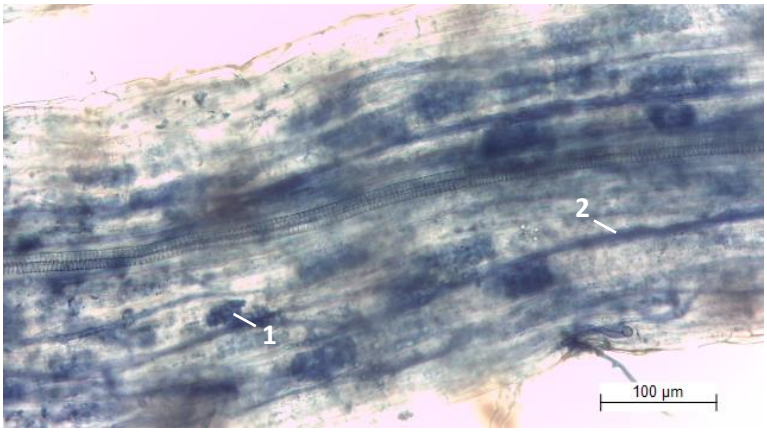

Figure 5 Arbuscules (1) and hyphae (2) of a leaf lettuce root seen under a transmitted light microscope with 200 x magnification.

microscope with 200 - fold magnification, 25 - 30 root pieces were placed in parallel on a microscope slide and mounted in a 50 % glycerol solution (vol/ vol). The slide was divided into sections with a spacing of 4 mm along the long axis. The field of view was moved perpendicular to the sectioning. 100 intersections were examined, and for each was decided whether at least one hypha, arbuscle, or vesicle (positive) or if none (negative) were seen at the intersection (see Figure 5). With this information, the total AMF colonization was determined by the total number of positive intersections (McGonigle *et al.*, 1990). To avoid biases, the analysis was conducted blindly and by the same person.

2.4.3 Litter decomposition

Tetrahedron-shaped synthetic tea bags from Lipton were used as a simplified litter bag. The commercially available green (*Camellia sinensis*, EAN: 87 22700 05552 5) and rooibos (*Aspalathus linearis*, EAN: 87 22700 18843 8) tea bags (5 cm sides) contained 1.71 ± 0.03 g and 1.92 ± 0.03 g (mean ± SD) tea leaves respectively and had some natural flavoring as supplements (Keuskamp *et al.*, 2013). With a mesh size of 0.25 mm, only microorganisms and mesofauna could enter the bags but excluded macrofauna (Setälä *et al.*, 1996). Furthermore, the ingrowth of fine roots was still possible, but roots were easy to remove. To determine the initial weight before the experiment, tea bags were oven-dried for 18 h at 70 °C to a constant weight. One tea bag per pot was buried in a hole of 3 cm diameter and 5 cm depth in the center of the pot. Although several teabags per replicate are recommended by Keuskamp *et al.* (2013), this could not be done due to the lack of space. Green tea bags were buried at day 0 before pesticide and fertilizer application. During the first harvest at day 20, the green tea bags were exchanged with the rooibos tea bags and left until day 60. After removing adhering soil particles and roots from the retrieved green and rooibos tea bags by hand, the tea bags were dried at 70 °C for 48 h. Afterward, the dry weight was measured, and due to the loss of material over time, the decomposition rate per pot could be determined.

2.4.4 Plant biomass and nutrient content

The plant biomass was harvested before the application of pesticides and fertilizers at day 0 and after 20, 30, 40, 50, and 60 days. The leaves of the lettuce longer than 6 cm were cut 0.5 cm above the leaf base whereas the cotyledons were not harvested. After the harvest, the plant biomass was dried in a paper bag at 65 °C for 48 h until equilibrium weight to determine the dry weight and to do a nutrient analysis. A day before and right after each biomass harvest, the pots were watered to 80 %-WHC.

The nutrient content of the leaf lettuce was determined for day 20 and 60, whereas for day 60 the dried biomass of the harvest of day 30, 40, 50, and 60 was combined. The dried biomass was milled for 4 minutes with the vibrating mill (Retsch MM400) into a fine powder (< 0.75 mm). Nitrogen (N), phosphorus (P), and potassium (K) contents were analyzed according to the Swiss reference method of the Federal Agricultural Research Station (FAL, 1996). For N analysis, 10 mg of milled plant sample was weighed into tin capsules and analyzed using an element analyzer (varioPyroCube) which works according to the Dumas method. The P and K content was determined in the undiluted digestion solution of the plant biomass sample (HCL ashing digestion) and measured by ICP-OES (inductively coupled plasma – optical emissions spectroscopy; FAL, 1996).

2.5 Statistical analysis

All data visualization and statistical analysis were performed in the R environment (version 3.6.1; R Core Team, 2021). The packages used for data visualization were *ggplot2* and *ggpubr* and are further mentioned in italics in parentheses. Data underwent two-way analysis of variance (ANOVA) to test for the effects of pesticide and fertilizer applications and their interaction on the response variables (total microbial biomass, absolute abundances of the functional microbial groups, tea decomposition rates, dry weights, nutrient contents). The ANOVA was followed by Tukey's honestly significant difference (Tukey HSD) post hoc test (*dplyr*) to reveal differences between treatments. Furthermore, the effect size (η^2) of the different variables in ANOVA was determined to measure the proportion of variance on the total variance by a given variable (*lsr* and *effectsize*). The data was checked for heteroscedasticity by Levene's test for homogeneity of variance and visual observation of the "Residuals vs. Fitted plot" (*car*). Normality was tested by the Shapiro-Wilk test and visual observation of the Q-Q-plot. If normality or homogeneity of the residuals was not given, a log or square-root transformation was applied. If, after data transformation, one of the two ANOVA test assumptions were not met, a nonparametric Kruskal-Wallis test was performed to test for the effects of pesticide and fertilizer applications. Differences between treatments were assessed using the Wilcox-Test as a post hoc test where multiple pairwise comparisons between treatments were performed (*dplyr*).

The PLFA mole percentage (mol %) data was square root-transformed before producing Bray-Curtis dissimilarity indices with the *vegdist* function (*vegan*). To visualize differences in the microbial community composition and show overall dispersion patterns a metric multidimensional scaling (MDS, also called principal coordinate analysis or PCoA) was done as an unconstrained ordination (*vegan*, *calibrate*, and *Biostrings*) based on the dissimilarity matrix. A two-way PERMANOVA analysis of the square-root transformed PLFA data was subjected to determine the influence on the microbial community structure by the pesticide and fertilizer applications and their interaction. A pairwise permutation MANOVA of the Bray-Curtis dissimilarity matrix was employed to test if the microbial communities were differing between the individual treatments. As an unconstrained ordination can sometimes mask real patterns of differences among groups, a constrained ordination was done to uncover these patterns and test a priori hypotheses (Anderson and Willis, 2003). Canonical Analysis of Principal Coordinates (CAP) allowed a constrained ordination of the dissimilarity matrix, which was calculated with the *capscale* function. A subsequent PERMANOVA to test for statistical significance of the ordinations with 9999 permutations via the *adonis* function was subjected (*vegan*).

A significance level of $\alpha = 0.05$ was chosen for all the statistical analysis.

3 Results

The figures were made according to the full factorial design with pesticide applications (naming scheme: “Control” = no pesticides, “Biological” = biological pesticide cocktail, “Synthetic” = synthetic pesticide cocktail) and fertilizer applications (naming scheme: “Control” = no fertilizers, “Organic” = organic fertilizer, “Inorganic” = inorganic fertilizer).

3.1 Soil biology

3.1.1 Determination of the soil microbial biomass and community composition

The **total microbial biomass** was expressed as the total abundance of all PLFAs (nmol / g DM), which varied between 395 and 1053 nmol/ g DM over all treatments. A small but significant main effect of the fertilizer application (η^2 value = 10 % - explaining the proportion of variance on the total variance by a given factor) was found as the organic fertilizer application increased the total abundance of PLFAs in comparison to the fertilizer control application. However, there was an interaction effect which was slightly higher (η^2 = 16 %). Only a significant increase in the total microbial biomass by inorganic fertilization was found when not combined with either of the two pesticide cocktails (C+Inorg; see Figure 6). Additional figures (Chapter 6.10) and all the statistical test results (Table 17) are listed in the Appendix.

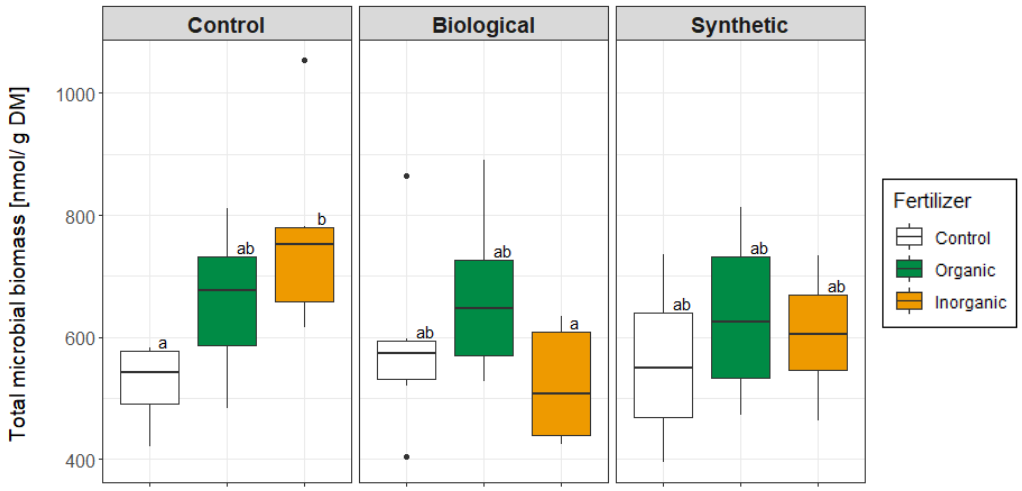

Figure 6 Total microbial biomass [nmol PLFA/ g DM] as total abundance for the different pesticide (Control = no pesticides, Biological, Synthetic) and fertilizer (Control = no fertilizer, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

On average, bacteria account for 86 % of the total microbial biomass, where Gram+ and Gram- bacteria account for 53 and 33 %, respectively. Fungi account for around 14 %, within which saprotrophic fungi and AMF make up 10 and 4 % of the total biomass.

Since **bacteria** (Gram+ and Gram-) are the most dominant source of PLFAs, it is not surprising that the absolute bacterial abundance shows the same pattern as the total biomass. The fertilizer application had a marginally main effect on the absolute bacterial abundance ($p < 0.1$, $\eta^2 = 8$ %) as well as a significant main effect was found for **Gram+** bacteria ($\eta^2 = 10$ %). With organic fertilizer application, a slight increase in bacterial biomass ($p < 0.1$) and a significantly higher amount of Gram+ bacteria compared to the fertilizer control application were found. In contrast, only a marginally main effect by the pesticide application ($\eta^2 = 8$ %) on the absolute abundance of **Gram-** bacteria was found, where there was a tendency to lower biomass with biological pesticide application ($p < 0.1$). However, for all bacterial groups, a significant interaction was found (bacteria: $\eta^2 = 18\%$, Gram+: $\eta^2 = 18\%$, Gram-: $\eta^2 = 16\%$) where only a significant increase in biomass with inorganic fertilization was found without the simultaneous application of either of the two pesticide cocktails for all three bacterial groups (see Figure 7 A to C). Compared to the control treatment (C+C), the inorganic fertilizer application without pesticides resulted in a 51 % higher bacterial biomass (C+Inorg).

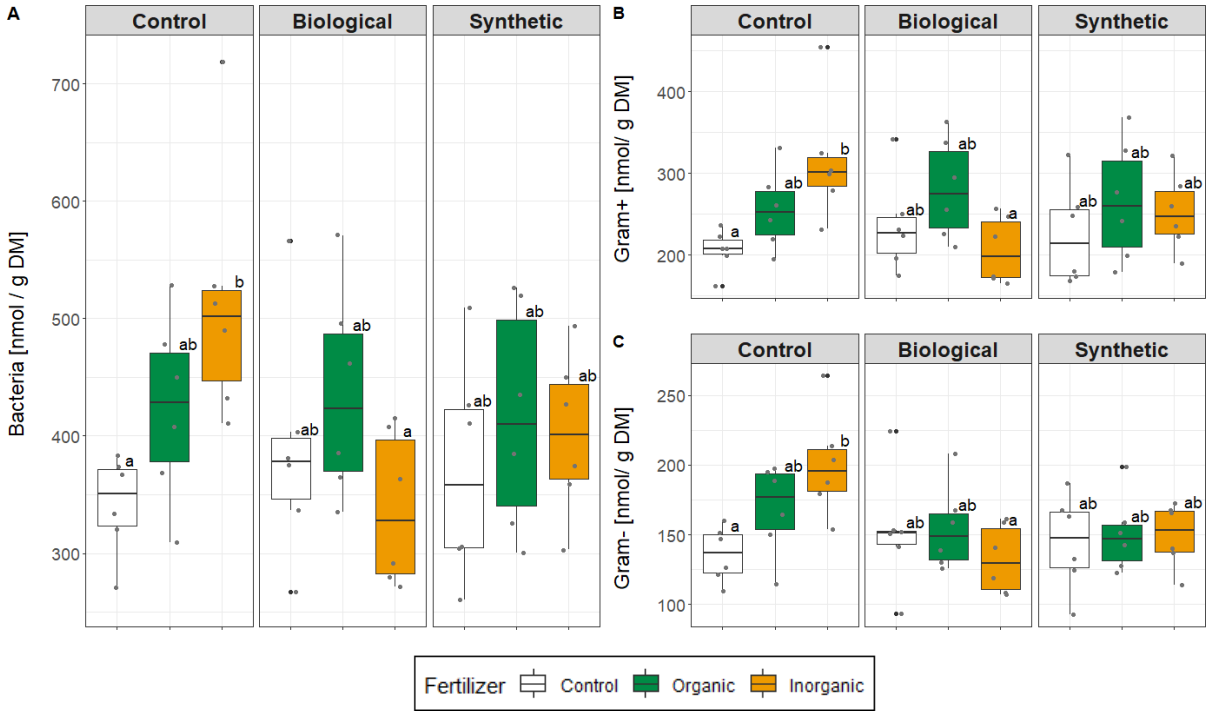

Figure 7 Absolute abundance [nmol/ g DM] of PLFAs specifically diagnostic to the bacteria group (A) with Gram+ (B), and Gram- (C) bacteria for the different pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

Fertilizer application (η^2 = 10 %) showed a significant main effect on the main group of **fungi** (saprotrophic and arbuscular mycorrhizal) as well as on the absolute abundance of **saprotrophic fungi** (η^2 = 11 %) and marginally on **AMF** ($p < 0.1$, η^2 = 3 %). As already with bacteria, organic fertilizer application increased the absolute abundance of fungi, saprotrophic fungi and led to a marginally higher AMF abundance ($p < 0.1$) compared to the fertilizer control application. As an exception, for the AMF biomass pesticide application (η^2 = 32 %) had a significant main effect, whereby synthetic pesticide application significantly decreased AMF abundance compared to the pesticide control. Nevertheless, for all fungal groups a significant interaction was found (fungi: η^2 = 20 %; saprotrophic fungi: η^2 = 15 %; AMF: η^2 = 18 %. The increasing effect of inorganic fertilization can only be seen without simultaneous application of either of the two pesticide cocktails for all three fungal groups (see Figure 8 A to C). Compared to the control treatment (C+C), the inorganic fertilizer application without pesticides resulted in a 53 % higher fungal biomass (C+Inorg).

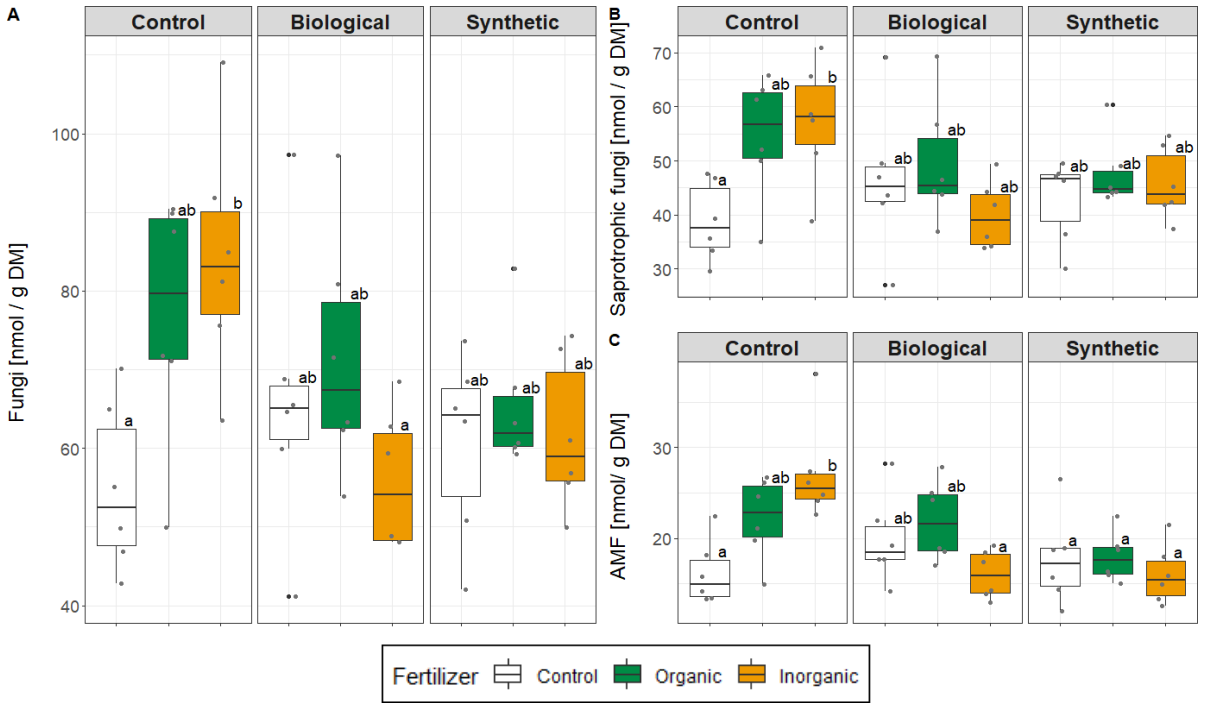

Figure 8 Absolute abundance [nmol/ g DM] of PLFAs specifically diagnostic to the fungi group (A) with saprotrophic (B), and mycorrhizal (C) fungi for the different pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

Looking at the **overall composition of the microbial community**, pesticide and fertilizer application were found to have a significant effect on the microbial community composition (see Table 18 in the Appendix). The influence of pesticide applications is less strong (η^2 = 6 %), than with fertilizer applications (η^2 = 9 %) but the effect was mainly driven by the interaction (η^2 = 13 %). There was a trend to a difference in Bio+Inorg compared to Bio+Org and C+Org as well as C+Org compared to Syn+Inorg treatments ($p < 0.1$). Nonetheless, no visual separation between applications and treatments of the unconstrained multidimensional statistical (MDS) analysis of PLFA profiles is possible (see Figure 22 in the Appendix).

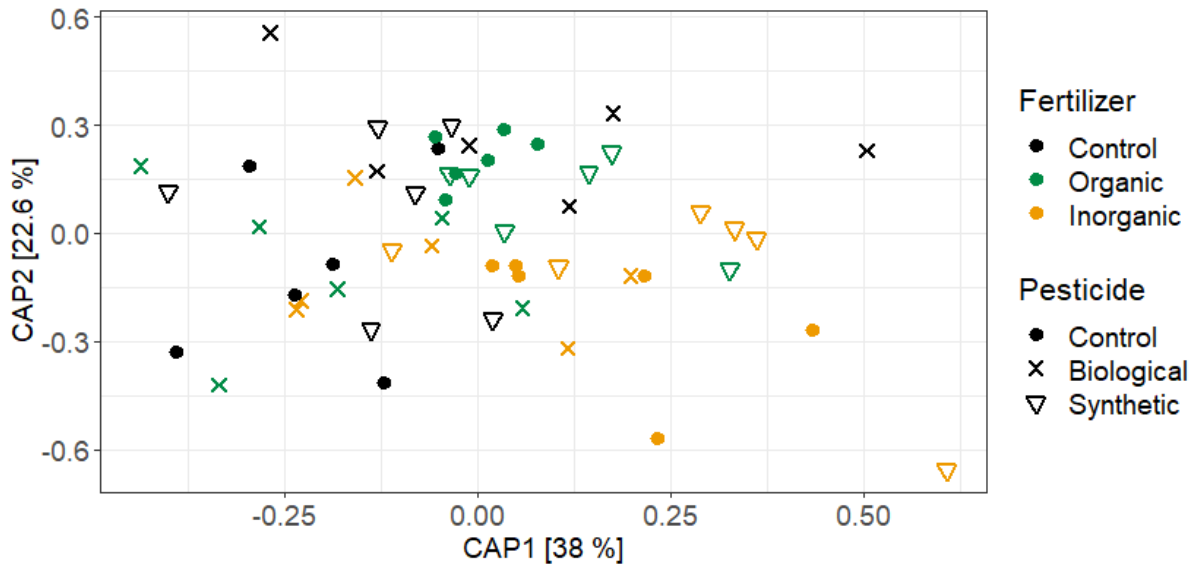

Figure 9 Canonical analysis of principal coordinates (CAP) plot constrained to the factors pesticide (forms) and fertilizer (color) application conditioned for block. PERMANOVA with 9999 permutations showed that pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) application explained 30 % of the total variance and the 95% confidence interval (CI) ranged between 20 and 44 %, with p = 0.002.

Canonical analysis of principal coordinates (CAP) constrained to the factors of pesticide and fertilizer application allowed to highlight factorial patterns that were masked in an unconstrained MDS ordination (see Figure 9). Samples with organic fertilizer application (green) mostly clustered together, and the samples with inorganic fertilizer application (yellow) were scattered and further away from the other two fertilizer applications. Thus, no distinct clusters were found, but a tendency to a shift in community composition with inorganic fertilizer application could be identified.

3.1.2 AMF root colonization

The total AMF colonization of the leaf lettuce roots varied a lot over all treatments (12 - 73%). All treatments showed a similar pattern, as arbuscules followed by hyphae contributed most to the total AMF root colonization. Vesicles were found only sporadically and did not contribute strongly to the total colonization (see Table 19 in the Appendix).

AMF root colonization was mainly influenced by the pesticide (η^2 = 69 %, Figure 10A) and only slightly but significantly by fertilizer application (η^2 = 4 %, Figure 10B), but no significant interaction was found. The synthetic pesticide application showed a significant reduction by 50 % regarding total AMF root colonization compared to the pesticide control and biological pesticide application.

Further, a difference between the fertilizer control and organic fertilizer application can be found, where with organic fertilization, the total AMF root colonization decreased significantly.

For the **arbuscular root colonization**, only a main effect of the pesticide application (η^2 = 43 %, Figure 10 C) was found. As before, the synthetic pesticide application significantly decreased arbuscular colonization compared to the pesticide control and biological pesticide application.

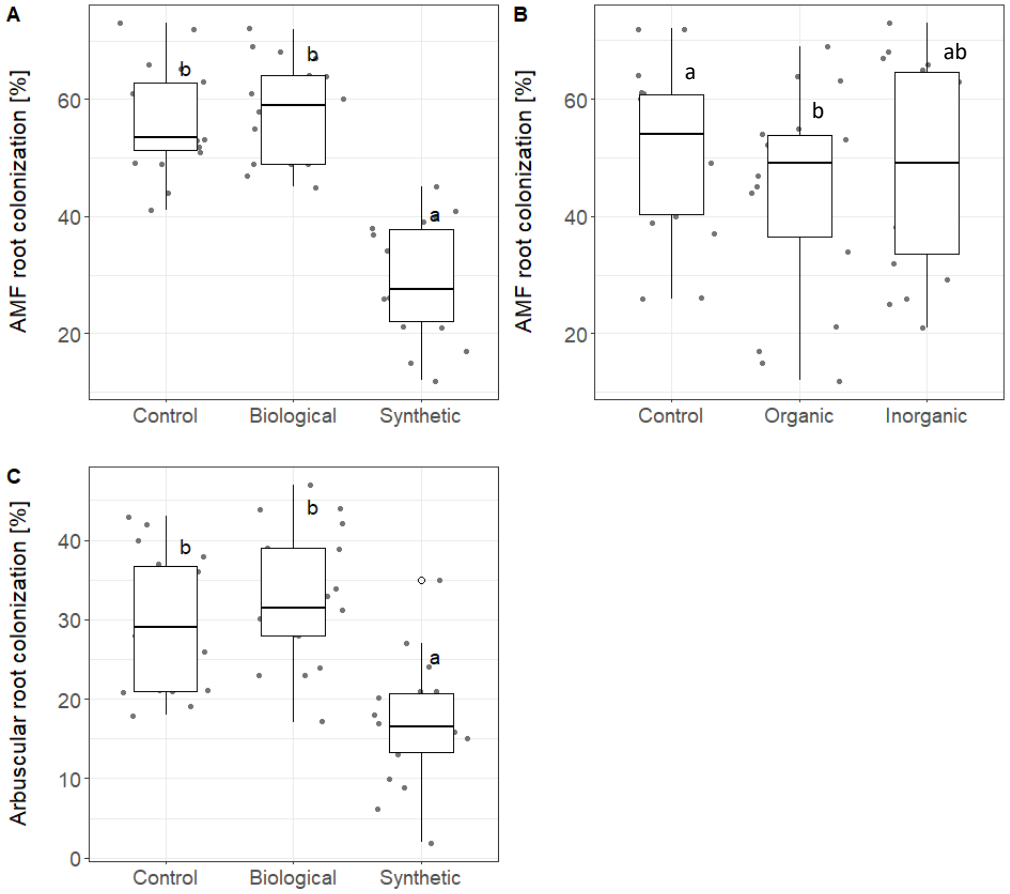

Figure 10 Total AMF (A,B) and arbuscular (C) root colonization of leaf lettuce roots in response to the different pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

3.2 Soil functioning

3.2.1 Litter decomposition

Across all treatments, 61.8 to 66.6 % of the green tea was decomposed and only around 28.6 to 44.0 % of the rooibos tea. There was only a significant main effect of the fertilizer application (η^2 = 25 %) on the **green tea decomposition**. A significantly lower green tea decomposition with organic and inorganic fertilizer application than the fertilizer control was found with a reduction of 1.8 and 1.5 %, respectively (see Figure 11).

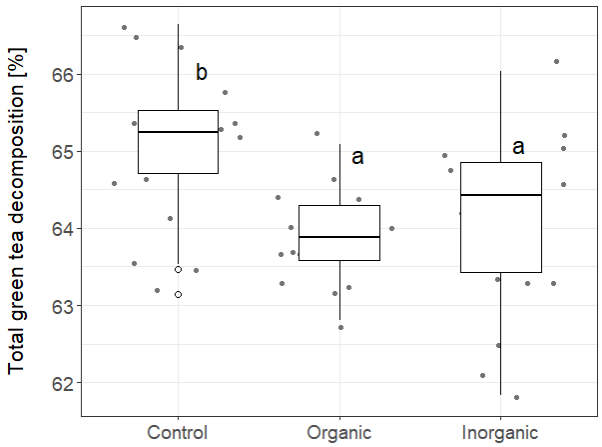

Figure 11 Total green tea decomposition with fertilizer applications (Control, Organic, Inorganic). Letters indicate differences based on Tukey pairwise comparison with p < 0.05.

For the **rooibos tea decomposition**, there was a marginally significant main effect by fertilizer application ($p < 0.1$, η^2 = 2 %) towards a higher rooibos tea decomposition with organic fertilizer application. However, the greatest main effect was caused by the pesticide application (η^2 = 80 %). There was a significant increase with synthetic pesticide application compared to the pesticide control (no pesticides) and biological pesticide application where a 28 and 23 % increase was found, respectively. Furthermore, a marginal increase with biological pesticide application was found compared to the pesticide control ($p < 0.1$, see Figure 12).

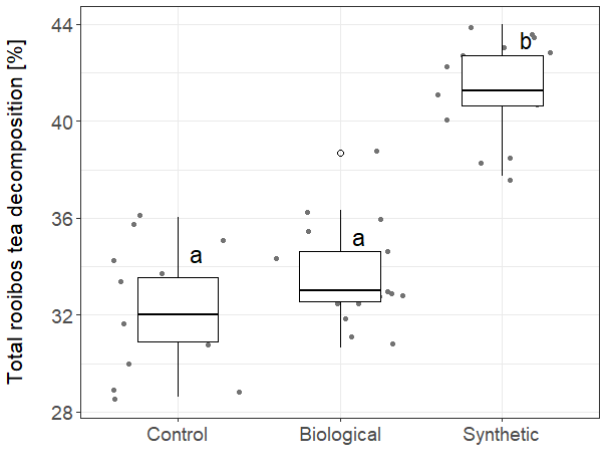

Figure 12 Total rooibos tea decomposition with pesticide applications (Control, Biological, Synthetic). Letters indicate differences based on Tukey pairwise comparison with p < 0.05.

3.2.2 Plant biomass and nutrient content

Pesticide application showed the most pronounced main effect on the leaf lettuce biomass after 20 and 60 days (20: η^2 = 78 %; 60: η^2 = 64 %). Mainly the synthetic pesticide application showed a reduced dry weight compared to the other two pesticide applications, while the main effect was smaller although significant for the fertilizer applications (20: η^2 = 16 %; 60: η^2 = 19 %). Between the fertilizer applications, organic fertilizer significantly increased the dry weight in both harvests. There was no effect of the inorganic fertilizer application compared to the fertilizer control application at day 20, but a trend to an increased above-ground biomass was found at day 60 ($p < 0.1$). However, an interaction between both factors was found which was smaller but still significant (20: η^2 = 2 %; 60: η^2 = 10 %).
The inorganic fertilization affected biomass growth only in combination with no or biological pesticides, but not with synthetic pesticides for both timepoints. The synthetic pesticide treatments (Syn+C, Syn+Org, Syn+Inorg) were comparable, and the mean of all was 18.2 % and 31.7 % lower than the treatment without fertilizers and pesticides (C+C) at day 20 and 60, respectively (see Figure 13 A and B). This growth reduction could also be seen visually, as the leaf lettuce was stunted after synthetic pesticide application. Therefore, an additional experiment was conducted to see which of the individual synthetic pesticides had the strongest influence on biomass growth (see Appendix 6.1).

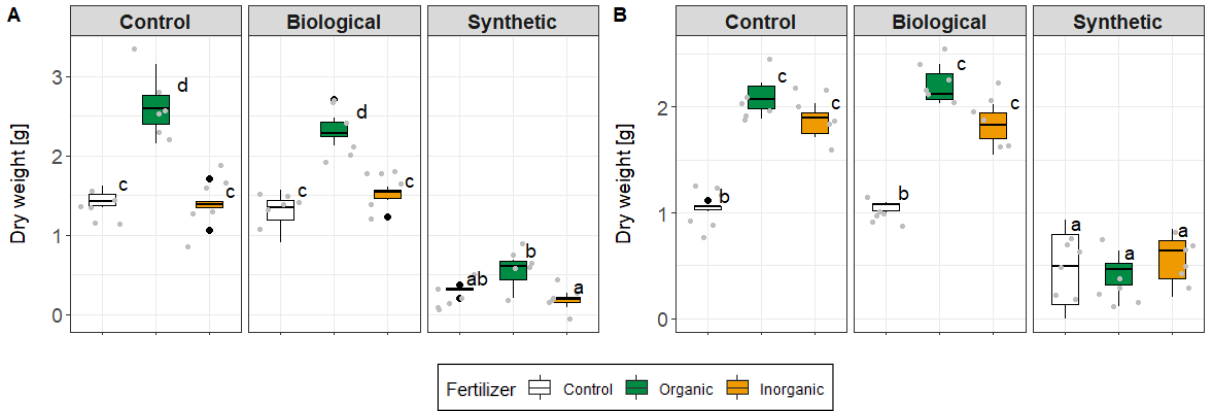

Figure 13 Dry weight [g] of the biomass harvests at day 20 (A) and day 60 (B) in response to the different pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

Similar results as for the dry weight were found for the nutrient contents. Pesticide application showed the most pronounced main effect on nutrient contents at day 20 (N: η^2 = 75 %; P: η^2 = 66 %; K: η^2 = 58 %) and 60 (N: η^2 = 31 %; P: η^2 = 75 %; K: η^2 = 50 %) whereby the synthetic pesticide application decreased the nutrient contents compared to the other two pesticide applications. For the fertilizer application a smaller but still significant main effect for all the nutrients was found for day 20 (N: η^2 = 12 %; P: η^2 = 23 %; K: η^2 = 36 %) and 60 (N: η^2 = 13.3 %; P: η^2 = 15 %; K: η^2 = 32 %) whereby the organic fertilizer application led to a higher nutrient content in the leaf lettuce compared to the fertilizer control and inorganic fertilizer application for both timepoints. With an exception for the N content at day 60, where both fertilizations resulted in increased but comparable contents (see Figure 27 and 28 in the Appendix). The interaction between both factors was almost negligible but still significant for both timepoints (further details on nutrient contents in Appendix 6.10.6).

4 Discussion

The soil microbial community is diverse and essential for the proper functioning of an agronomic system as they support a number of important ecosystem services such as soil fertility (Zhang et al., 2007; Van Der Heijden et al., 2008). Pesticide cocktails and fertilizers are increasingly used in agriculture (FAO, 2021a, 2021b) and were found to exert a major threat to non-target soil organisms (Karpouzas *et al.*, 2014). So far, studies mainly examined single active substances and looked at the separate application of pesticides and fertilizers. However, these studies do not correspond to the current agronomic practices where pesticide cocktails are applied in combination with different fertilizers. Therefore, this short-term greenhouse experiment with leaf lettuce intended to assess the impact of synthetic and biological pesticide cocktails as well as inorganic and organic fertilizers applied as individual and combined treatments on the soil microbial community, especially AMF, and soil functioning. It was expected that the synthetic pesticide cocktail and inorganic fertilizer influence the soil microbial community more negatively than the biological pesticide cocktail and organic fertilizers. Furthermore, the combination of the pesticide cocktails with the inorganic fertilizer was expected to increase the impact, while in combination with organic fertilizers, the impacts were expected to be mitigated. To answer the research questions, different biological and functional parameters were investigated to observe the influence of the different treatments on the composition and structure of the rhizosphere microbiome.

Impacts of pesticide and fertilizer applications…

4.1 … on plant growth and nutrient contents

The nutrient content of a plant can be used as a proxy for microbial activity and functionality (Jacoby *et al.*, 2017) as microorganisms and especially AMF can improve plant growth by increasing the bioavailability of nutrients (Schimel and Bennett, 2004) and mineral nutrition (Van Der Heijden *et al.*, 2008). However, as seen by this study's results, nutrient contents were strongly dependent on the plant biomass growth and were mainly influenced by synthetic pesticide application. While biological pesticides did not influence plant growth, synthetic pesticides resulted in stunted growth of the leaf lettuce and significantly reduced the above-ground biomass at both timepoints. The additional experiment (see Appendix 6.1) showed that leaf lettuce tolerated Stomp Aqua and Pirimor less than the other synthetic pesticides in the cocktail, which resulted in stunting of the plants with synthetic pesticide application. Therefore, synthetic pesticides directly inhibited plant growth resulting in a decrease in root biomass, strong influence on the rhizosphere and the soil system.

Besides the effect of pesticides on plant growth, fertilizers also had an impact, although relatively minor compared to those of synthetic pesticides. Both organic and inorganic fertilization increased the plant biomass and nutrient contents, indicating that the experimental system was working and microorganisms mineralized the compost which released inorganic N and P into the soil (Van Der Heijden *et al.*, 2008). Since only an effect on the plant biomass growth by inorganic fertilization was visible at day 60, one can assume that the inorganic fertilizer needed to be converted into available forms. For example, ammonium dihydrogen phosphate (one component of the mineral fertilizer) must first be separated into the two basic components ammonium (NH_4^+) and phosphate ($H_2PO_4^-$) to be absorbed by the plant (International Plant Nutrition Institution, 2010). Therefore, as seen in the nutrient contents, one can assume that P was limiting for plant growth with inorganic fertilization at day 20 (see Figure 27 in Appendix).

4.2 … on the soil microbial community

Pesticides and fertilizers were found to inhibit microbial processes, reduce specific microbial groups or change the overall composition and structure of the soil microbial community (Zhong *et al.*, 2010; Puglisi, 2017). In this study, it was found that fertilizers influenced the soil microbial community more than pesticides. However, the greatest effect on the soil microbial community composition and structure originated from the interaction of pesticides and fertilizers.

Pesticides are an integral part to sustain yields in today's agriculture. Especially synthetic pesticides were found to have non-target effects on the soil microbial community with a collateral impact on soil fertility and functioning (Fournier *et al.*, 2020). Unexpectedly, none of the microbial groups, not even AMF, were negatively affected by the synthetic pesticide cocktail in this study. Generally, pesticides can provoke a range of responses of the soil microorganisms, which can lead to alterations in their composition and function due to disturbances (Imfeld and Vuilleumier, 2012). However, as the PLFA analysis was only a snapshot at the time of sampling, it may be that there were only transient effects (Riah *et al.*, 2014; Shao and Zhang, 2017) that could not be depicted here. Rousidou *et al.* (2013) showed that pest management with a single synthetic pesticide at the recommended field rate in a field study had minor effects on the microbial community and was followed by fast community recovery after a few weeks. The same was found in a laboratory experiment with soil microcosm, where adverse effects have occurred shortly after pesticide application with single substances, but after two weeks were comparable to the non-treated samples (Shao and Zhang, 2017). Generally, soil microbial communities can respond differently to perturbation but are capable of maintaining their structure and returning to an initial state (Imfeld and Vuilleumier, 2012). Therefore, it can be assumed that an effect of the synthetic pesticide cocktail was found shortly after application, and the microbial community returned to its original state after a while.
In comparison to synthetic pesticides, biological pesticides are viewed as an environmentally friendly alternative with less harmful effects on the soil microbial community (Manda *et al.*, 2020). Indeed, no effect of the biological pesticide cocktail on any functional microbial group was found in this study. But despite the natural origin, there were mixed reports about the impact of biological pesticides on non-target organisms (Amichot *et al.*, 2018). On the one hand, Spyrou *et al.* (2009) showed that e.g., Azadirachtin (active substance used in the cocktail) at the recommended dose did not alter the structure of the soil microbial community in a laboratory and field study of 15 to 60 days. On the other hand, Singh *et al.* (2015) and Walvekar *et al.* (2017) found that Azadirachtin at the recommended dose negatively impacted the soil microbial population after 10 to 80 days in a pot experiment, and the effects were even similar to synthetic pesticides tested. In addition, Rousidou *et al.* (2013) and Shao and Zhang (2017) showed that 20 days after application of individual biopesticides, the effect on microorganisms was no longer visible and one of the tested biological pesticides even stimulated the growth of fungi and bacteria (Rousidou *et al.*, 2013). Therefore, as with synthetic pesticides, the biological pesticide cocktail may have also had a transient effect or did not affected the microbial community at all.

In addition to the effects caused by pesticides, fertilizers were also found to impact the microbial community, especially AMF (Lazcano *et al.*, 2013). Organic amendments can be beneficial for soil fertility by increasing nutrient availability and stimulating biological activities (Cozzolino *et al.*, 2016; Tayyab *et al.*, 2019) and therefore promote growth, diversity, and activity of specific microbial groups (Gosling *et al.*, 2006). In this study, organic fertilizers fostered microbial soil life indicated by a slight visual increase in the microbial biomass of all functional groups (bacteria and fungi) regardless of whether they were applied with or without either of the two pesticide cocktails. However, none of these increases were statistically significant. The stimulation of the microbial biomass by application of organic fertilizers was often associated with the increase in organic matter and soil fertility over the long-term (10 - 60 years; Diacono and Montemurro, 2011). However, Dinesh *et al.* (2010) discovered

that an increase in microbial biomass growth with organic manure could already be found after one growing season (7 months), indicating that biological promoting mechanisms exist even in the short-term. An overall increase in microbial biomass after green compost application was reported at laboratory (after 91 days; Pose Juan *et al.*, 2015) and field scale (after 2 years; Singh *et al*, 2016) and Álvarez-Martín *et al.* (2016) pointed out that the increase in the microbial biomass can also be attributed to introducing exogenous microorganisms to the soil which were existing in the compost. However, Lazcano *et al.* (2013) found no significant increase in microbial biomass with vermicompost applied at the standard dose (80:24:20 kg/ ha of N:P:K) after 3 months. All these studies indicate that only after a certain time an effect by the compost could be found. Therefore, it can be assumed that if this experiment had lasted longer, the increase would have been more pronounced. Overall, all the organic fertilizer treatments showed comparable values in the microbial biomass with the exception of the AMF abundance where this slight visual increase was not found when organic fertilizers were combined with the synthetic pesticide cocktail. An increase in AMF abundance without pesticides was also found by Palenzuela *et al.* (2002). They mentioned that it was evident after 8 months in a field study that the growth of hyphae and propagules of AMF in the soil was increased with organic matter addition as AMF can colonize compost patches. But in this study, the growth was inhibited when synthetic pesticides were additionally applied.

In comparison to organic fertilization, there are hardly any studies that look at the effects of inorganic fertilization on soil microorganisms over the short-term, especially not in laboratory or greenhouse experiments. Most are at field or ecosystem scale and last for several years to decades (Böhme *et al.*, 2005; Sun *et al.*, 2015; Khan *et al.*, 2018). In this study, the CAP analysis indicated the most distinct shift in the soil microbial community composition with inorganic fertilization but no clear cluster was found. Furthermore, inorganic fertilization only showed a significant stimulatory effect on all microbial abundances (bacteria and fungi) in the absence of both pesticide cocktails (C+Inorg) and even the AMF abundance increased. The increase in microbial biomass can be explained by the addition of N, which is often limiting for microbial processes and growth (Carnicer *et al.*, 2015). Interestingly, when inorganic fertilizers were combined with synthetic pesticides (Syn+Inorg), there was still a slight visual increase (but not statistically significant) in the abundances of most microbial groups compared to the treatment with no applications (C+C). Only for AMF, the positive effect of inorganic fertilization with synthetic pesticides was entirely eliminated. Surprisingly, the positive effect of the inorganic fertilizer was completely canceled out when combined with biological pesticides (Bio+Inorg), with the exception for saprotrophic fungi, where the microbial abundance was comparable but still lower to the treatment with only inorganic fertilization (C+Inorg). Therefore, both pesticide cocktails reduced the stimulatory effect on the soil microorganisms by inorganic fertilization, and somehow, the biological pesticides interacted even more with the inorganic fertilizers than the synthetic pesticides. The understanding of the effects of pesticide cocktails together with fertilizers is still in its early stages. Only recently, there have been studies showing how the combination of pesticides and fertilizers affects soil microorganisms (e.g., Khan *et al.*, 2012; Carpio *et al.*, 2020). Thereby, the problem is that usually, only one pesticide in combination with different organic amendments are tested (Álvarez-Martín *et al.*, 2016; García-Delgado *et al.*, 2018), and different laboratory methods are used (Carpio *et al.*, 2020; Huang *et al.*, 2021). Furthermore, the focus lies on long-term field studies with organic and conventional farming (e.g., Araújo *et al.*, 2008), which makes the comparability to this study even more difficult.

4.3 … on the AMF root colonization

AMF are of great interest in agroecosystems as their symbiosis with crops can promote nutrient uptake, plant growth, and crop production and are significant for low-input farming (Singh *et al.*, 2016). While the AMF abundance in the soil was not negatively affected by neither pesticide nor fertilizer applications, the AMF root colonization was significantly decreased by synthetic pesticides and organic

fertilizer applications. Even though the effect of pesticide cocktails on AMF has hardly been tested (Riedo *et al.*, 2021), the reduction in AMF root colonization with synthetic pesticides in this study corresponds with the findings of Riedo *et al.* (2021), where a decrease in AMF colonization was found with increasing pesticide residues. However, the results must be considered with caution, as the synthetic pesticides severely stunted the roots and hardly any root system has formed for colonization. With less root biomass, the likelihood of AMF coming into contact with the roots and colonizing them also decreases. As already for the AMF abundance in the soil, this study found no effect on the AMF root colonization by the biological pesticide cocktail. Ipsilantis *et al.* (2012) found that under conservative application schemes with single biological pesticides (Spinosad, pyrethrum, and terpenes), no effects on the AMF colonization were observed (Ipsilantis *et al.*, 2012). Nevertheless, not many studies investigated the effect of biological pesticides on AMF colonization.

Compared to the strong effect of the synthetic pesticide cocktail, AMF colonization decreased less with organic fertilizer application in this study. Limited studies and contrasting results exist about the effect of compost on AMF colonization depending on the type, origin, and regime of organic amendments (Sun *et al.*, 2016; Dong *et al.*, 2021). A reduction in AMF root colonization in this study could be explained by the increase (+ 26 %) in available P content in the soil with compost addition (see Appendix 6.2). As available P is the primary driver of AMF root colonization (Liu *et al.*, 2019), the enriched soil P contents can lead to a reduction in AMF colonization, as sufficient P is available for crop demand and the dependence on the symbiosis is reduced (Blanke *et al.*, 2011). This was also found by Cavagnaro (2014), where a reduction in AMF colonization with greenwaste compost application (20 - 30 t/ ha) after 55 days of a glasshouse experiment was evident. However, this reduction must also be treated with caution. One can consider this significant difference of root colonization with organic fertilization as an artifact due to the strong influence of synthetic pesticides (see Figure 25 in the Appendix).

Surprisingly, AMF root colonization was not significantly influenced by inorganic fertilization in this study. It was expected that AMF root colonization would decrease as AMF were found to be very sensitive to nutrient changes (Liu *et al.*, 2012). As inorganic fertilizers increase the nutrient level in the soil, plants are less reliant on the AMF symbiosis as they can directly absorb enough nutrients by themselves, which inhibits colonization (Liu *et al.*, 2019). However, it might be that the single application of 4.4 kg P/ ha was too little to exert a negative effect on AMF colonization (Liu *et al.*, 2012), and therefore, no significant effect of inorganic fertilizers was found in this study.

4.4 … on the litter decomposition

Litter decomposition is crucial for nutrient cycling and carbon dynamics (Mori *et al.*, 2021), as well as to maintain plant growth, soil fertility (Martínez-García *et al.*, 2021), and ecosystem functioning (Keuskamp *et al.*, 2013). An impact on the microbial biomass can reflect an impact on the functioning of the soil ecosystem, notably on the decomposition of organic matter (Fournier et al., 2020). Therefore, litter decomposition can be used as an indicator for soil microbial activity (Martínez-García *et al.*, 2021) and tea decomposition can provide information about the soil functioning at a local scale (Keuskamp *et al.*, 2013). In this study, green and rooibos tea decomposition were within the range of other laboratory studies (Keuskamp *et al.*, 2013; Duddigan *et al.*, 2020). Thereby, green tea decomposition (64 - 65 %) was higher than rooibos tea decomposition (31 - 42 %) because green tea consists of more labile compounds (carbohydrates, amino acids), which are faster degradable than rooibos tea which has more recalcitrant compounds (lignin, tannins, cuticular matrix; Keuskamp *et al.*, 2013; Suseela *et al.*, 2013). Unlike with the microbial community, pesticide cocktails and fertilizers did not show an interaction effect on litter decomposition.

It was expected that the decomposition would increase with organic and inorganic fertilization (Lazcano *et al.*, 2013). While the former stimulates microbial processes (Tayyab *et al.*, 2019), the later was found to increase soil respiration (Bünemann *et al.*, 2006). However, exactly the opposite was the case in this study. A decrease in green tea decomposition was found 20 days after the application of inorganic and

organic fertilizer. The same results were found by Spiegel *et al.* (2018), where after 28 days of inorganic (80 kg N/ ha) and organic (compost) fertilization in the field, litter decomposition was highest in the control. However, comparison with this study's results is difficult because the field has been fertilized and cultivated since 1991, and the organisms may have already adapted to the fertilization. This could also be the case for the soil used in this study, but the exact fertilizer use over the last years is unknown. Nevertheless, one explanation for the reduced litter decomposition with the inorganic fertilizer could be that inorganic fertilizers containing ammonium nitrate reduced microbial activity, as was found by Thirukkumaran and Parkinson (2000) found in a laboratory experiment (after 40 days). However, even though the activity and growth of microorganisms are stimulated by the addition of organic matter via compost, this does not necessarily mean that decomposition rates are affected (Hadas *et al.*, 1996). Furthermore, the addition of compost adds pre-decomposed plant matter to the soil, causing microorganisms to mineralize compost in addition to tea. However, since green tea has a lower C:N ratio (~10) than compost (~19), it is still preferred.

Unexpectedly, the application of synthetic and biological pesticide cocktails significantly and slightly increased the rooibos tea decomposition in this study, respectively. Usually, with synthetic pesticide application, decomposition is inhibited because microorganisms that decompose litter are negatively affected, especially when pesticides are combined (Cornejo *et al.*, 2021). However, since the microbial biomass was not affected by biological and synthetic pesticide cocktails in this study, there must be another explanation. Besides the microbial community composition, litter quality and the physical-chemical environment are the main drivers of litter decomposition (Martínez-García *et al.*, 2021). Thereby, the climate (e.g., temperature and moisture) can influence litter decomposition (García-Palacios *et al.*, 2015) by regulating the microbial activity (Petraglia *et al.*, 2019). Warm, moist, and aerobic conditions can enhance decomposition (Gattinger *et al.*, 2008; Zimdahl, 2018). As in this study, the plant biomass was stunted in pots treated with the synthetic pesticide cocktail, which in term reduced transpiration and water uptake, resulting in higher moisture contents in these pots. In contrast, in the pots without synthetic pesticide application, the soil repeatedly dried out during hot periods between watering, resulting in slight drought stress and, therefore, decomposition was limited by moisture (Lee *et al.*, 2014; Mori *et al.*, 2021). Another reason for the increased decomposition with synthetic and biological pesticide cocktails could also be that the degradation of pesticides by bacteria and fungi can lead to the release of carbon from which microorganisms can derive energy and increase their activity and litter decomposition (Jacobsen and Hjelmsø, 2014). Especially for biological pesticides, the surplus of organic substrates and nutrients by the application may have had a slight direct stimulatory effect (Spyrou *et al.*, 2009) which may not have resulted in microbial growth but a slight increase in activity.

Thus, the effect on rooibos tea decomposition was rather due to physical differences, such as soil moisture and substance inputs, and not due to biological differences in the microbial community between the treatments.

4.5 Limitations of the experimental system and design

Pot experiments in a greenhouse allow performing an experiment under controlled conditions while having the potential to minimize biotic and abiotic stress factors (Riah *et al.*, 2014). Nevertheless, the experimental system comes with limitations. Homogenization of the soil creates artificial conditions and destroys aggregates and the natural stratification of the soil. The small soil amount and the black pot, which could heat up quickly, led to strong evaporation and thus to a high frequency of watering. Therefore, it would be advised to use larger pots for the next experiment as they are less susceptible to the heat and drying out. Another problem was that the biomass growth was faster than expected, resulting in more harvests than initially planned. Furthermore, as the biomass growth progressed, a dense root system developed throughout the whole pot and preferential flow could occur along thick roots. Therefore, the pots had to be watered very slowly so that the water could penetrate from top to bottom and not flow along the roots. Nevertheless, the experimental setup allowed good plant growth for almost all the treatments. Unfortunately, even though the synthetic pesticide cocktails was tested beforehand, the plants reacted negatively on the application. Therefore, it is recommend to test the pesticides even earlier or one could try to protect the plants from direct contact of the substances with a cover. In addition, Stomp Aqua and Pirimor should not be used for experiments with leaf lettuce, as they showed the greatest negative effect on the plant growth. This strong effect by the synthetic pesticide cocktail was likely because the substances were not applied successively at their respective application times (e.g., preemergent herbicide) but simultaneously whereby the lettuce was exposed to an enormous amount of chemicals at once. This stunting can be prevented if the substances are used one after the other at the intended time or maybe if another model plant was used.

A great difficulty was the comparability of this study with others, as no other did something comparable in a pot experiment over the short-term. In addition, the studies are generally difficult to compare because there are many different synthetic and biological pesticides on the market that can be mixed. In addition, each compost is very different in its characteristics and composition, and also inorganic fertilizers are applied in different compositions and concentrations.

Moreover, due to the low taxonomic resolution of the PLFA analysis (Frostegård *et al.*, 2011) it may be that changes could not be found because not the overall composition changed but rather the diversity within a microbial group or phyla (Lewis *et al.*, 2016; Rivera-Becerril *et al.*, 2017; Fournier *et al.*, 2020). Therefore, the diversity is more likely to provide better insight into the effect of pesticide cocktails. Furthermore, one needs to acknowledge the high variability in the PLFA data, which could have dramatically reduced the statistical power and the ability to detect statistical significance.

5 Conclusion and Outlook

This study provided new insights into the impact of pesticide cocktails and fertilizers on the soil microbial community and soil functioning. Fertilizers influenced the soil microbial community composition and structure more than pesticide cocktails. However, the interaction between pesticide cocktails and fertilizers let to an even higher impact. Neither the synthetic (H1.1) nor the biological (H1.2) pesticide cocktail, nor the organic fertilizer (H2.2), significantly affected the abundances of the soil microbial groups. Only the inorganic fertilizer caused an increase in all soil microbial abundances (Gram+ and Gram- bacteria, and saprotrophic and arbuscular mycorrhizal fungi) when applied without pesticides (H2.1). While the simultaneous application of the inorganic fertilizer in combination with either of the pesticide cocktails did diminish the positive effect of the fertilization (H3.1), organic fertilizers in combination with either of the pesticide cocktails resulted in comparable abundances as when combined with no pesticides (H3.2). Thereby, AMF abundance mainly showed the same response as the other microbial groups. A significant shift in the soil microbial community was not found by any treatment. Only the CAP analysis showed a slight separation of the inorganic fertilizer treatments from the other two fertilizer treatments, which indicates that the inorganic fertilization did influence the microbial community composition.

Unfortunately, the plant biomass of the lettuce was reduced by the synthetic pesticide cocktail to such an extent that this had a predominant indirect effect on AMF colonization and litter decomposition. In the pots treated with synthetic pesticides, the plant biomass was severely stunted, and hardly any root system formed, which could have influenced the AMF root colonization as no healthy host plant has grown. Furthermore, due to the negative effect on plant growth, water uptake decreased and increased the moisture content in the pots treated with synthetic pesticides, which increased litter decomposition.

As this study showed, organic and conventional farming can influence soil biology and functioning even in the short-term. The typical components of an organic system seemed to have fewer adverse effects on the soil microbial community, especially when applied in combination. Therefore, the substitution of synthetic products such as synthetic pesticides and inorganic fertilizers can be beneficial for the soil organisms and to make agriculture more sustainable by maintaining soil fertility.

In future studies, it might be advised to do similar experiments in the greenhouse and field. It might be interesting to also look at multiple time points during the course of the experiment to see how the composition of the soil microbial community changes over time. Furthermore, before the combined effect of pesticide cocktails in combination with different fertilizers can be studied in more detail, different pesticide cocktails and fertilizers alone must be tested more extensively. This study has also confirmed that something needs to change in the current legislation of pesticides. Today, the OECD guidelines for testing chemicals only test the effect of a single substance on the N mineralization (OECD, 2000) for the evaluation of the ecotoxicity of a pesticide. A more suitable test would also incorporate the microbial abundance, diversity, and activity (Karpouzas *et al.*, 2014). Thereby, the European food safety authority (EFSA, 2010) underlined the importance of new protection goals, including soil ecosystem services. However, to develop a more viable pesticide testing strategy, a better understanding of pesticide mixture effects should be the goal as the combination of substances can have synergistic effects (Cedergreen, 2014). Furthermore, as the interaction between pesticide cocktails and fertilizers showed the biggest effect on the soil microbial community in this study, not only pesticide mixtures but also a combination of different agrochemicals should be tested. Therefore, the larger picture of the effects of pesticides and fertilizers need to be analyzed as a part of a risk assessment before being used in agriculture.

In summary, there is a need for research in many different areas. Since pesticides and fertilizers are essential in agriculture to maintain productivity, a good balance between the two must be found in the future to allow efficient control of pests and diseases together with balanced crop nutrition while minimizing adverse effects on the soil microbial community and ultimately improving soil fertility.

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6 Appendix

6.1 Additional experiment - Application of single pesticides

Due to the potential negative effect of synthetic pesticides to leaf lettuce in the main experiment, an additional experiment was set up in which the synthetic pesticides were tested individually and as a cocktail (mixture of all the synthetic pesticides; see Table 6). There was also a control where nothing was applied (see Figure 14).

Table 6 Synthetic (Agroscope, 2021) *pesticide products, listed by their respective pesticide classification, active substances and its content, recommended dosage and product per pot according to the manufacturer.*

	Pesticide Classification	Product name	Active substance	Content of active substance	Recommended dosage	Product per pot
synthetic	Herbicide	Stomp Aqua	Pendimethalin	38.9%; 455 g/l	3.5 l/ha	0.0056 ml
	Fungicide	Filan	Boscalid	50%	0.5 kg/ha	0.8 mg
	Fungicide	Switch	Cyprodinil Fludioxonil	37.5% 25%	0.8 kg/ha	1.28 mg
	Insecticide	Pirimor	Pirimicarb	50%	5 g/m^2	0.08 g
	Insecticide	Cypermethrin	Cypermethrin	11%; 100 g/l	0.25 l/ha	0.0004 ml

Due to the lack of the same soil as the main experiment, another one with the characteristics shown in Table 7 was used. The soil's physicochemical properties were analyzed by the laboratory at Agroscope Reckenholz according to the Swiss reference methods of the Federal Agricultural Research Station (see Chapter 2.1.4; FAL, 1996). The used soil was sampled on a natural grassland (47.394567°N, 9.571833°E) with a long history of organic farming. The topsoil (5 - 20 cm) without turf was air-dried for five days, sieved < 5 mm, and thoroughly homogenized during filling.

Table 7 Physicochemical characteristics of the experimental grassland soil (n = 6). Means with ± standard errors are shown.

pH	Texture			C_{org}	Humus	N	P	K
in H_2O [-]	Sand [%]	Silt [%]	Clay [%]	[mass %]	[mass %]	[mass %]	[mg/kg]	[mg/kg LS]
7.4 ± 0.02	32.3 ± 0.57	36.4 ± 0.36	27.9 ± 0.46	2.4 ± 0.07	4.1 ± 0.11	0.2 ± 0	109.8 ± 7.21	192.3 ± 5.64

Figure 14 Plant biomass before harvest at day 20 with different treatments from left to right: Control, Stomp Aqua, Switch, Filan, Pirimor, Cypermethrin, Cocktail

The additional experiment was set up exactly as the main experiment except that it did not include pots with compost (see Chapter 2.1). After four weeks of plant establishment, the leaf lettuce was harvested, and pesticides were applied by the spraying chamber one after the other (see Chapter 2.2 and 2.3). The pots were kept at a WHC of 55 - 80 % (calculated as in Chapter 2.1.2), and the leaf lettuce was harvested

after 20 days. The above-ground biomass was dried in paper bags at 65 °C for 48 h until equilibrium weight and weighed (as the biomass in Chapter 2.4.4).
To test for the significance of the effect of the different pesticides, a Kruskal-Wallis test was conducted due to missing normality assumptions (tested with Shapiro-Wilk test). For pairwise comparison of the treatments, a Wilcox-Test was performed, and a significance level of $\alpha = 0.05$ was used for all analysis. Only the pesticides Stomp Aqua, Pirimor and the cocktail showed a clearly decreased biomass compared to the other individual pesticides tested. The control treatment suffered from a high variability and only differed from the cocktail. The cocktail showed the smallest biomass of all (Figure 15). All the statistical test results are found in Table 17 in the Appendix 6.10.7.

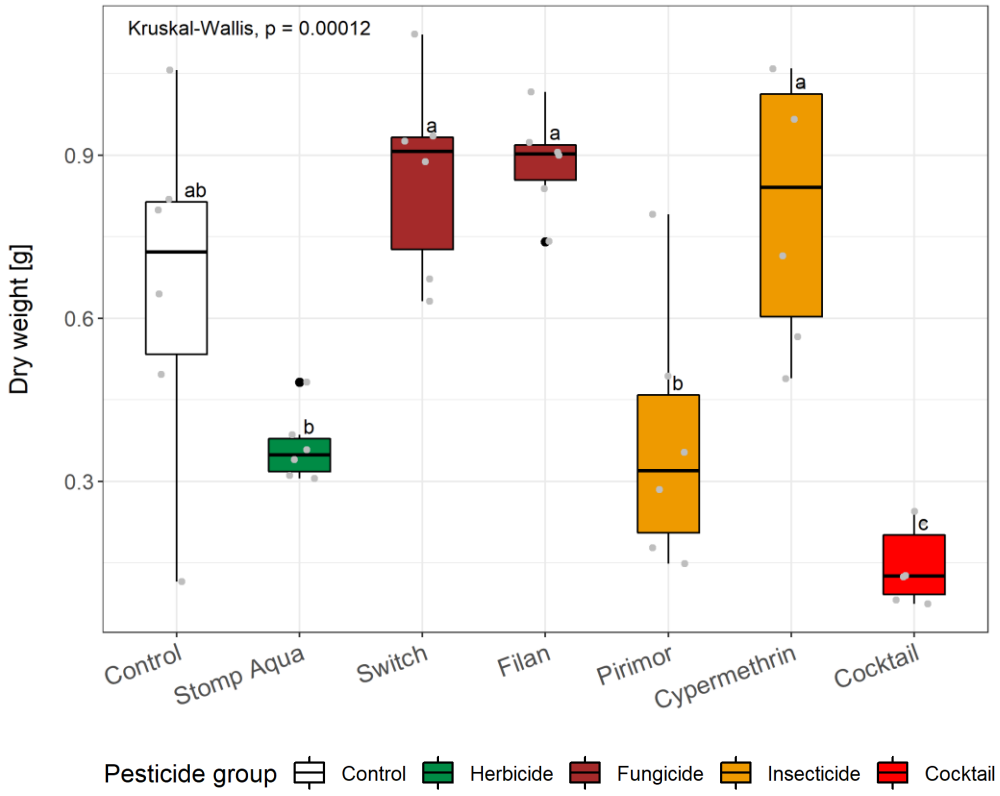


Figure 15 Leaf lettuce dry weight [g] 20 days after synthetic pesticide application (Stomp Aqua, Switch, Filan, Pirimor, Cypermethrin, Cocktail). Letters indicate Wilcox pairwise comparison with $p < 0.05$.

6.2 Physicochemical soil characteristics

As in Table 8 and according to the FAL (1997), the results of the soil textures showed that the soil with and without compost is a clay loam. With a humus content of 4.1 % and 4.6 % and organic carbon (C_{org}) of 2.4 % and 2.6 %, both soils are classified as slightly humic. The pH value of 6.4 and 6.7 indicates a slightly acidic soil.

Based on the GRUD, a nutrient assessment can be made. For the soil without compost, the general assessment of the nutrient status of soil for horticulture showed a sufficient phosphorus (P) and potassium (K) stock and enriched content in available K and a sufficient amount of available P. For the soil with compost, the general nutrient assessment showed enrichment in potassium and phosphor stocks as well as a stock in available phosphor and enriched available K content in the soil (Neuweiler and Krauss, 2017). With a C/N ratio of 8.8, the soil is within the typical range of agricultural surface soils (Weil and Bradys, 2017).

Table 8 Physicochemical characteristics of the experimental grassland soil with and without compost (each n = 3). Means with ± standard errors are shown.

Soil	**pH** in H_2O [-]	**Texture** Sand [mass %]	Silt [mass %]	Clay [mass %]	**Humus** [mass %]	**C_{org}** [mass %]	**C/N ratio** [-]
Without compost	6.4 ± 0.02	27.3 ± 0.54	41.5 ± 0.41	27.2 ± 0.23	4.1 ± 0.06	2.4 ± 0.03	8.8 ± 0.11
With compost	6.7 ± 0.02	26.6 ± 0.59	41.9 ± 0.39	27.0 ± 0.32	4.6 ± 0.1	2.6 ± 0.06	8.8 ± 0.14

N [mass %]	**P (stock)** [mg/kg]	**P (available)** [mg/kg]	**K (stock)** [mg/ kg]	**K (available)** [mg/kg]
0.3 ± 0.003	67.4 ± 0.8	2.3 ± 0.03	160.9 ± 4.9	15.7 ± 0.39
0.3 ± 0.003	92.9 ± 0.38	2.9 ± 0.06	331.3 ± 5.9	45.0 ± 2.56

Note: “Corg” = organic carbon, “C/N” = organic carbon to nitrogen ratio, “N” = nitrogen, “P” = phosphor, “K” = potassium. “Stock” indicates the nutrient reserve in the soil and “available” is the content that represents the immediately available nutrient content in the soil.

6.3 Used Pesticides - mode of action and substance characteristics

The used synthetic and biological pesticides are described in the following sections. In the subtitles, the active substances (e.g., Pendimethalin) are listed first, and the product used (e.g., Stomp Aqua) is given in parenthesis. Table 9 summarizes some characteristics of the synthetic and biological pesticides.

DT_{50} [day] is a quantitative measure of the rate of degradation of plant protection products in the environment (soil degradation in days) and represents the dissipation time (DT). The value 50 stands for a reduction of the original quantity by 50 % in soils caused by mainly microbial activity. With this value one can classify the degradability of a plant protection product in the environment and it is classified as (PPDB, 2021):

< 30	= non-persistent
30 – 100	= moderately persistent
100 – 365	= persistent
> 365	= very persistent

K_{foc} [ml/g] is the soil adsorption coefficient which measures the amount of adsorbed substance onto soil particles and is classified into (PPDB, 2021):

< 15	= very mobile
15 – 75	= mobile
75 – 500	= moderately mobile
500 – 4000	= slightly mobile
> 4000	= non-mobile

6.3.1 Synthetic pesticides

All the following synthetic pesticides are approved and commonly used in horticulture (Agroscope, 2021).

Herbicide

Herbicides are substances that help manage unwanted plants and weeds. They inhibit, interfere, disrupt, or mitigate the regular plant growth (Sherwani, *et al.*, 2015) by inhibiting cell division, photosynthesis, amino acid production, or the mimicking of natural plant hormones. Their regular application is before or during planting to minimize competition for resources during plant establishment and can be applied to the foliage or soil (EPA, 2021a). As herbicides are designed to target a specific enzyme structure in a plant, they are thought to be less harmful to soil organisms (Johnson *et al.*, 2005). Even though the microbial biomass is often not significantly influenced by herbicides, the microbial community structure can be strongly impacted (Johnson *et al.*, 2005). Herbicides can influence AMF either directly through interference in spore germination and development, where AMF colonization is reduced (Helander *et al.*, 2018), or via indirect effects by changing the physiology of the host plant (Meenakshi *et al.*, 2021).

Pendimethalin (Stomp Aqua)

Stomp Aqua is a widely applicable systemic herbicide for winter cereals, field beans, forage peas, many vegetable crops, and numerous other arable crops. It acts via the soil and the leaf against a wide range of mono- and annual dicotyledonous weeds. It remains effective for several weeks so that even weeds and grasses that germinate later are killed (BASF, 2019). The active ingredient Pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine, Figure 16) is an organic herbicide of the dinitroanilines. Its mode of action is the inhibition of microtubule assembly, which inhibits cell division and cell elongation processes (HRAC, 2021). Pendimethalin is persistent in soils (DT_{50} = 100.6), non-mobile (K_{foc} = 13'792), and has no significant adverse effect on the nitrogen or carbon mineralization of soil microorganisms (PPDB, 2021).

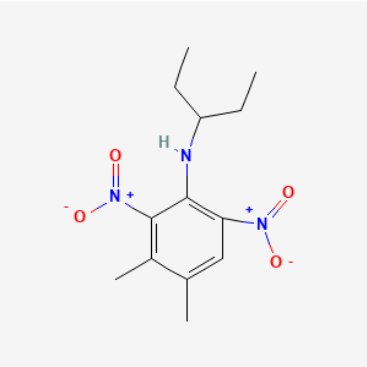


Figure 16 Chemical structure of Pendimethalin (PubchemDatabase, 2019).

Fungicide

According to the FRAC (2021), fungicides are substances that inhibit the fungal growth or spore germination by the interference of different cellular processes by inhibition of site enzymes, respiration, signal transduction, and nucleic acid, sterol, and protein synthesis at different points in the fungal lifecycle. They can act curative or protective. For the former, the active ingredient is translocated within a plant, and the latter remains on the leaf surface, forming a protective barrier against fungal infection (FRAC, 2021). Unlike herbicides, fungicides were developed to suppress soil pathogens which can also influence other beneficial soil fungi (Bünemann *et al.*, 2006). Puglisi (2017) found controversial results on the impact of fungicides on the microbial biomass, whereas the results ranged from negative to positive or no influence. The influence of fungicides on the microbial community structure was already more apparent, where in most cases, fungicides induced significant changes in the microbial structure (Puglisi, 2017). Overall, fungicides were found to have the most significant decrease in microbial biomass compared to herbicides and insecticides. Since fungicides act on fungal pests, they also affect beneficial fungi, whereby it was found that the fungicide application decreased AMF diversity (Jin *et al.*, 2013), as well as they decrease fungal spore germination and formation, germ tube elongation, and mycelium growth (Zocco *et al.*, 2008).

Cyprodinil + Fludioxonil (Switch)

Switch is a fungicide against botrytis and monilia in viti-, pomi- and horticulture. It is a water-dispensable granulate and consists of 25 % Fludioxonil (4-(2,2-Difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile, Figure 17) and 37.5 % Cyprodinil (4-Cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine, Figure 17). Fludioxonil is a non-systemic/ contact fungicide that affects the membrane-dependent transport processes of glucose phosphorylation and, therefore, reduces mycelium growth. Cyprodinil acts systemically and prevents the biosynthesis of amino acids of the fungus during the fungal penetration into the plant tissue or during mycelial growth (PubchemDatabase, 2019; FRAC, 2021; Syngenta, 2021d). While Cyprodinil

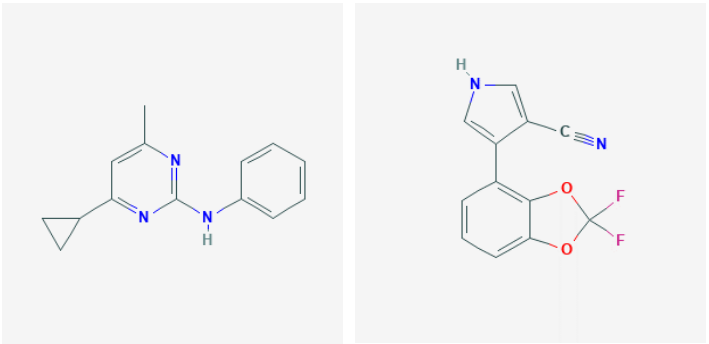


Figure 17 Chemical structures of Cyprodinil (left) and Fludioxonil (right) (PubchemDatabase, 2019).

is moderately persistent (DT_{50} = 45) and slightly mobile (K_{foc} = 2277), fludioxonil is non-persistent (DT_{50} = 16) and non-mobile in soil (K_{foc} = 132'100, PPDB, 2021).

Boscalid (Filan)

The fungicide Filan is effective against botrytis and is a water-dispersible granulate which consists of 50% Boscalid (2-chloro-N-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide, Figure 18), and is a systemic chemical substance within the family of carboxamides, which allows controlling a broad range of fungal pathogens (PubchemDatabase, 2019; Syngenta, 2021b). It interferes with mitochondrial respiration at the complex II by inhibition of the succinate dehydrogenase (FRAC, 2021) and leads to an attack on the electron transport chain of the fungal cells. This attack disturbs the energy production, prevents the production of amino acids and lipids, and thus prevents spore germination and the growth of the germ tube (Syngenta, 2021b). Boscalid is characterized by slow degradation in soil (DT_{50} = 254, persistent) due to strong sorption to soil particles and low solubility in water (Kf_{oc} = 772, slightly mobile, PPDB, 2021).

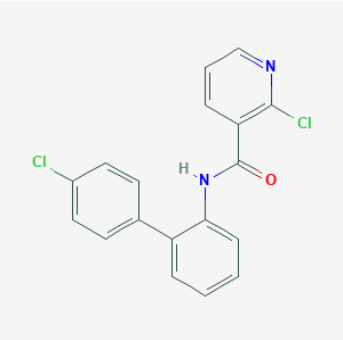


Figure 18 Chemical structure of Boscalid (PubchemDatabase, 2019).

Insecticide

Insecticides are used to prevent and inhibit insects' engagement in undesirable or destructive behavior or kill them (EPA, 2021b). They act mainly on the nervous system, while some act on growth regulators, respiration, and endotoxins (IRAC, 2021). Insecticides were found to increase microbial biomass and, in almost all cases, significantly change the microbial community structure (Puglisi, 2017). Insecticides did not have a strong impact on AMF (Schweiger and Jakobsen, 1998) or even stimulated their colonization (Spokes *et al.*, 1981).

Pirimicarb (Pirimor)

Pirimor is a water-soluble granulate that acts selective and systemic against different kinds of aphid species. Its field of application is in berries, fruits, vegetables, and field crops. 50 % is the active ingredient Pirimicarb (2-(dimethylamino)-5,6-dimethylpyrimidin-4-yl dimethylcarbamate, Figure 19) which is fast-acting and belongs to the carbamate group (Syngenta, 2021c). According to the IRAC (2021), Pirimicarb acts as an acetylcholinesterase (AChE) inhibitor whereby the hydrolysis and respiration of the neurotransmitter acetylcholine at nerve synapses are inhibited. This leads to a permanent excitation of the nerves and ultimately to paralysis and death of the insects (IRAC, 2021). It is non-persistent (DT_{50} = 9) and moderately mobile in soils (Kf_{oc} = 388, PPDB, 2021).

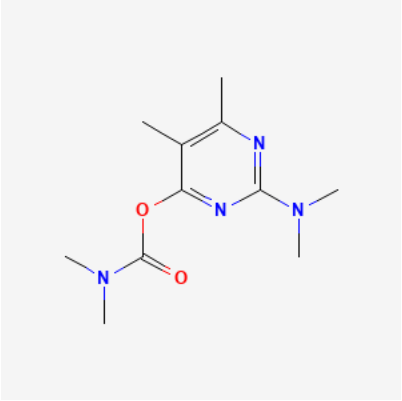


Figure 19 Chemical structure of Pirimicarb (PubchemDatabase, 2019).

Cypermethrin (Cypermethrin)

Cypermethrin is a non-systemic/ contact insecticide, is named after its active substance (Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, Figure 20) and has with a wide range of applications from arable crops, berries, vegetables, and forestry (PubchemDatabase, 2019; Sintargo, 2019). It has an effect against caterpillars, weevils, aphids, and beetles (Sintargo, 2019) and belongs to the pyrethroid group. The chemical interferes with the sodium channel modulators, preventing the sodium channels from closing, causing hyperexcitation, and leading to fatal spasms (IRAC, 2021). Due to the non-persistence (DT_{50} = -) and low solubility of Cypermethrin, it is not mobile in the soil (K_{foc} = 21.9, PPDB, 2021).

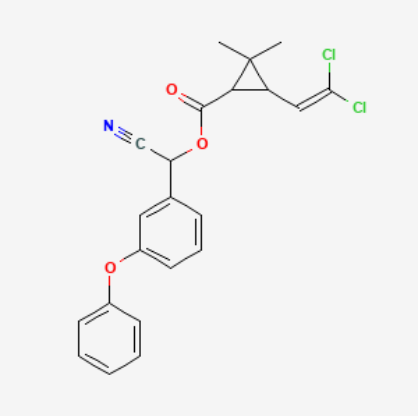


Figure 20 Chemical structure of Cypermethrin (PubchemDatabase, 2019).

6.3.2 Biological pesticides

The following biological pesticides are authorized in organic agriculture (Speiser *et al.*, 2021).

Biofungicide

Laminarin (Vacciplant)

Vacciplant is a concentrated algae filtrate used in pome fruit, strawberry, vines, cucurbits, lettuce, and tomatoes against powdery mildew, botrytis, and bacterial blotch disease (Stähler, 2021). According to Stähler, (2021), Laminarin (45 g/l), the active ingredient of Vacciplant, is extracted from the algae *Laminaria digitata* and is similar to a substance on the cell wall of plant pathogenic fungi. After applying the water-soluble concentrate, the plant reacts to the stimulation of Laminarin by activating its natural defense mechanisms. This reaction is comparable to inoculation and thus prepares the plant for later attacks by fungi, viruses, and bacteria. Thus, the effect of Laminarin has a systemic effect and is used exclusively preventive before the first symptoms appear (Stähler, 2021). Laminarin is readily biodegradable, has a moderate persistence in the soil, and a half-life of around 30 days (EFSA, 2017b).

Potassium bicarbonate (Armicarb)

Armicarb is a multi-site contact fungicide against apple scab, powdery mildew, and scotty blotch on pome fruit and against powdery mildew in horticulture. It is a water-soluble powder and containing 85 % potassium bicarbonate as an active ingredient. Potassium bicarbonate acts non-systemic and has a preventive effect against fungal diseases. By changing the pH value and the osmotic pressure, as well as by the direct ionic effect of the bicarbonate, spores and the mycelium of harmful fungi are disturbed and killed (Andermatt-Biocontrol-Suisse, 2021a). Tamm *et al.* (2006) found that Armicarb is a promising plant protection product, as it shows low risk, and no unacceptable residues were found. Potassium bicarbonate is not assumed to be very persistent or accumulative (EFSA, 2021).

Copper oxychloride (Curenox 50 WG)

The effect of copper as a protective fungicide was discovered in France in 1882. It is usually administered as oxychloride or hydroxide. Curenox 50 WG consists out of 50% copper as oxychloride ($Cu_2(OH)_3Cl$). The water-dispersing granulate is applied to the plant foliage and is effective against fungal diseases (botrytis and downy mildew) in fruit, berry, vine, vegetable, and field crops (Schneiter-Agro-AG, 2021). Copper is classified by the FRAC (2021) as a fungicide with a multi-site mode of action, which causes denaturation of proteins and inhibition of enzymes which are critical for cell functioning. As copper is an element, it cannot be degraded in soil, and only Cu^{2+} is mobile (EFSA, 2017a). It is only slightly absorbed by plants but strongly by soil particles whereby it does not leach and therefore accumulates continuously, resulting in toxic levels in soils (EFSA, 2017a). Although copper

may have some side effects, such as phytotoxicity, it is still indispensable because it can be applied in situation with high disease pressure and still does not cause resistances (BLW, 2021).

Sulfur (Netzschwefel Stulln)

Netzschwefel Stulln is an 80 % micronized sulfur with fungicidal (against scab, powdery mildew, and shot hole disease) and insecticidal (against pear leaf blister mite, blackberry mite, and rust/blister mite) effects and is applied to fruits, strawberries, blackberries, vegetables, grapes, hops, ornamental plants, and cherry laurel (Andermatt-Biocontrol-Suisse, 2021c). Sulfur is applied as a finely grounded powder (Andermatt-Biocontrol-Suisse, 2021c). It is a protective fungicide that is taken up by sensitive fungal species over the gas phase (evaporates > 18°C) which poisons the fungal cells (BLW, 2021). It is still not fully known how elemental sulfur acts on pathogens. But, it is assumed that sulfur can permeate fungal hyphae, damage the mitochondrial electron transport chain in the cytoplasm, thereby promoting the formation of toxic H_2S, which disrupts oxidative phosphorylation and stops spore germination and growth (Nwachukwu *et al.*, 2012). Possible disadvantages are phytotoxicity, toxicity to predatory mites, and the deleterious effects on beneficial organisms (BLW, 2021). Nevertheless, it is not expected to be persistent and does not accumulate in soils, whereas it is not necessary to determine a DT_{50} value (EFSA, 2008).

Bioinsecticide

Azadirachtin (Neem-Azal-T/S)

Azadirachtin is produced by the neem tree (*Azadirachta indica*) and is found in high concentrations in its seeds. The substance acts systemically and is a tetranortriterpenoid limonoid with a wide spectrum of actions and has shown high insecticidal and potential nematicidal activity (Manda *et al.*, 2020). According to Andermatt-Biocontrol-Suisse (2021b), it is used in horticulture, cucumbers, culinary herbs, fruits, tomatoes, ornamental plants and is effective against aphids, leaf miners, horse chestnut leaf miners, spider mites, thrips, whiteflies, cicadas, asparagus beetles/asparagus beetles, cherry flies . The active ingredient penetrates the leaves and is partially transported within the plant and taken up by the pests through their sucking or feeding activity. NeemAzal-T/S has an inactivating effect on the pests within a few hours, which stop feeding and thus their plant-damaging activities (Andermatt-Biocontrol-Suisse, 2021b). Azadirachtin can block receptor cells that normally stimulate feeding culminating in starvation and species death. Furthermore, it was found that Azadirachtin can act as well on the growth and molding as well as on the reproduction and protein synthesis in a variety of tissues (Mordue, 2004). The substance has a low persistence with a half-life of 7 to 21 days and a low to high mobility (Stark and Walter, 1995).

Spinosad (Audienz)

According to Omya (Schweiz) AG (2021), Audienz is a non-systematic insecticide against various pests (e.g., moths, thrips, leaf-miner flies, cutworms, etc.) in arable farming, fruits, berries, vine, and vegetables. It contains Spinosad, which consists of two active ingredients Spinosyn A and Spinosyn D. Spinosad is obtained through a fermentation process from the ray fungus *Saccharopolyspora spinosa* (actinomycetes). Insects absorb the active ingredient through feeding activities and contact with the active ingredient. The speed of action is comparable to that of synthetic insecticides. Spinosad can influence the neuronal activity of insects as it binds specifically to the nicotinic acetylcholine receptor (nAChR) and causes a permanent Na-ion influx, which leads to a discharge and thus hyperactivity of the neurons and muscle activity. The effect occurs after a few hours, and the first symptoms end with complete paralysis of the insect, which is irreversible. The degradation of Spinosad in the environment is very rapid, where the primary mechanism is through photolysis (Omya (Schweiz) AG, 2021). In soil, rapid degradation by microorganisms occurs (Omya (Schweiz) AG, 2021) with a half-life of 2 to 8 days (Thompson *et al.*, 2002).

Table 9 Active substances and mode of action for herbicides (HRAC, 2021), fungicides (FRAC, 2021), and insecticides (IRAC, 2021) with their different chemical, ecological and regulatory properties (PubchemDatabase, 2019) and some information on the manufacturer, pesticide group, active substance, and mode of action.

	Manufacturer	Pesticide group	Product	Active Substance	Mode of action (MoA)	MoA Code	Chemical family	DT_{50} in soil [days]	K_{foc} [ml/g]
synthetic	BASF Schweiz AG	Herbicide	Stomp Aqua	Pendimethalin	Inhibition of microtubule assembly	K1	Dinitroanilines	100.6 (persistent)	13792 (non-mobile)
	BASF Schweiz AG	Fungicide	Filan	Boscalid	Respiration: complex II: succinate-dehydrogenase	C2	Pyridine-carboxamides	254 (persistent)	772 (slightly mobil)
	Syngenta Agro AG	Fungicide	Switch	Cyprodinil	Amino acids and protein synthesis: methionine biosynthesis	Nr.9/D1	Anilino-pyrimidines	45 (moderately persistent)	2277 (slightly mobile)
				Fludioxonil	Signal transduction: MAP/Histidine-Kinase in osmotic signal transduction (os-2, HOG1)	Nr.12/E2	Phenylpyrroles	16 (non-persistent)	132100 (non-mobile)
	Syngenta Agro AG	Insecticide	Pirimor	Pirimicarb	Acetylcholinesterase (AChE) inhibitors	1A	Carbamates	9 (non-persistent)	388 (moderately mobile)
	Sintagro AG	Insecticide	Cypermethrin	Cypermethrin	Sodium channel modulators	3A	Pyrethroids/ Pyrethrins	21.9 (non-persistent)	- (non-mobile)
biological	Stähler Suisse AG	Fungicide	Vacciplant	Laminarin	Host plant defence induction	P04	β-(1,3)-Glucan	30 (non-persistent)	-
	Andermatt Biocontrol Suisse	Fungicide	Armicarb	Potassium bicarbonate	Suppression of the pepsin action	-	Inorganic	-	-
	Schneiter AGRO AG	Fungicide	Curenox 50 WG	Copper oxychloride	Multi-site contact activity	M01	Inorganic (electrophiles)	1000 (very persistent)	medium mobility to immobility
	Andermatt Biocontrol Suisse	Fungicide	Netzschwefel Stulln	Sulfur	Compounds of unknown or uncertain MoA	UN	Inorganic	-	low
	Andermatt Biocontrol Suisse	Insecticide	NeemAzal-T/S	Azadirachtin A	Compounds of unknown or uncertain MoA	UN	Limonoids	7-21 (non-persistent)	low to high mobility
	Omya Schweiz AG	Insecticide	Audienz	Spinosad	Nicotinic acetylcholine receptor allosteric modulator	5	Derived from microorganisms	2-8 (non-persistent)	low mobility to immobility

Note: "K_{OC}" = sorption parameter, "DT_{50}" = half-life in soil (aerobic degradation)

6.4 Calculations of fertilizer amounts and application rates

Chapter 2.2.1 of the Materials and Methods section mentioned the used inorganic and organic fertilizer amounts and application rates, for which the calculations are given in the following two chapters.

6.4.1 Inorganic fertilizer

With the fertilization recommendation for leaf lettuce in the greenhouse (50 kg N/ha, 4.4 kg P/ ha, and 41.5 kg K/ha; Neuweiler and Krauss, 2017), one can calculate the amount N, P, and K per pot:

$$\text{Pot}_{diameter} = 14.2\ cm$$

$$\text{Pot}_{area} = \pi * (0.0712\text{m})^2 = 0.016\ \frac{m^2}{pot}$$

$$N_{pot} = 50\ \frac{kg\ N}{ha} * \frac{1'000'000\ mg}{1\ kg} * \frac{1\ ha}{10'000\ m^2} * 0.016\ \frac{m^2}{pot} = 80\ \frac{mg\ N}{pot}$$

$$P_{pot} = 10\ \frac{kg\ p}{ha} * \frac{1'000'000\ mg}{1\ kg} * \frac{1\ ha}{10'000\ m^2} * 0.016\ \frac{m^2}{pot} = 7.04\ \frac{mg\ P}{pot}$$

$$K_{pot} = 41.5\ \frac{kg\ p}{ha} * \frac{1'000'000\ mg}{1\ kg} * \frac{1\ ha}{10'000\ m^2} * 0.016\ \frac{m^2}{pot} = 66.4\ \frac{mg\ K}{pot}$$

Table 10 Molar masses from different chemical elements and substances

Element	u	Substance	u
N	14.007		
P	30.974	$(NH_4)H_2PO_4$	115.02
K	39.098	NH_4NO_3	80.04
H	1.008	KOH	56.11
O	15.999		

The following three substances were used to mix the inorganic fertilizer. Based on the known nutrient amounts per pot and proportions of the element N, P, K in the various chemical components, the amount of the respective chemical compounds can be calculated:

1. Ammonium dihydrogen phosphate $(NH_4)H_2PO_4$

Proportion of N and P in the chemical compound:

$$\frac{N}{(NH_4)H_2PO_4} = 14.007u * \frac{100}{115.02u} = 12.18\ \%$$

$$\frac{P}{(NH_4)H_2PO_4} = 30.974\text{u} * \frac{100}{115.02\text{u}} = 26.93\ \%$$

Amount of chemical compound per pot:

$$7.04\ mg\ P/pot * \frac{100}{26.93\ \% \frac{P}{(NH_4)H_2PO_4}} = \mathbf{26.1\ mg}\ \frac{\boldsymbol{(NH_4)H_2PO_4}}{\boldsymbol{pot}}$$

$$\frac{26.1\ mg}{100} * 12.18\ \% = 3.18\ mg\ N$$

$$80\ mg\ N - 3.18\ mg\ N = 76.82\ mg\ N\ (Remainder)$$

2. Ammonium nitrate NH_4NO_3

Proportion of N in the chemical compound:

$$\frac{N}{NH_4NO_3} = 14.007u * \frac{100}{80.04\ u} = 35\ \%$$

Amount of chemical compound per pot:

$$76.82\ \frac{mg\ N}{pot} * \frac{100}{35\ \%\ \frac{N}{NH_4NO_3}} = \mathbf{219.5}\ \frac{\boldsymbol{mg\ NH_4NO_3}}{\boldsymbol{pot}}$$

3. Potassium hydroxide KOH

Proportion of K in the chemical compound:

$$\frac{K}{KOH} = 39.098u * \frac{100}{56.11\,u} = 69.69\ \%$$

Amount of chemical compound per pot:

$$66.4\ \frac{mg\ K}{pot} * \frac{100}{69.69\ \%\ \frac{K}{KOH}} = \mathbf{95.3}\ \frac{\boldsymbol{mg\ KOH}}{\boldsymbol{pot}}$$

Therefore 26.1 mg $(NH_4)H_2PO_4$ / pot, 219.5 mg NH_4NO_3 pot and 95.3 mg KOH/pot are needed to apply 80 mg N/pot, 7.04 mg P/pot and 66.4 mg K/pot .

6.4.2 Organic fertilizer

Compost

The compost was analyzed for nutrients and pollutants at the laboratory of lbu (Labor für Boden- und Umweltanalystik) (see Table 11).

Table 11 Results of the nutrient and pollutant analysis of the compost from the Biomassenhof AG Winterthur at the lbu laboratory (Labor für Boden- und Umweltanalytik) from the 11.09.2020.

Parameter		Result	Unit	Method
Dry matter 105°C		**71.8**	%	D-TS-lbu
Ignition residue 500°C		**72.8**	%	D-AS-lbu
Ignition loss 500°C (OS)		**27.2**	%	D-AS-lbu
Carbon (C_{org})		**158.0**	g/kg TS	D-AS-lbu
pH-value		**8.13**		RD-CC-pH-lbu
Specific weight		**0.49**	kg/l	D-VG-lbu
Total-N after Kjeldahl		**8.32**	g/kg TS	NKjeldahl-Büchi-lbu
C/N-ratio		**18.99**		Calculation
Salt content		**5.05**	gKCl/kgTS	RD-H2O10-Sal-lbu
Conductivity		**622.00**	µS/cm	H2OSU-Sal-lbu
Phosphor	P	**2.00**	kg/t TS	AD-KW-ICP-lbu
Phosphorus pentoxide	P_2O_5	**4.58**	kg/t TS	Calculation
Potassium	K	**11.2**	kg/t TS	AD-KW-ICP-lbu
Potassium oxide	K_2O	**13.44**	kg/t TS	Calculation
Calcium	Ca	**42.4**	kg/t TS	AD-KW-ICP-lbu
Magnesium	Mg	**8.84**	kg/t TS	AD-KW-ICP-lbu
Sulfur	S	**1.52**	kg/t TS	AD-KW-ICP-lbu
Cadmium	Cd	**0.3700**	g/t TS	AD-KW-ICP-lbu
Cupper	Cu	**45.8**	g/t TS	AD-KW-ICP-lbu
Quicksilver	Hg	**0.153**	g/t TS	Hg-AFS-lbu
Nickel	Ni	**24.7**	g/t TS	AD-KW-ICP-lbu
Lead	Pb	**37.4**	g/t TS	AD-KW-ICP-lbu
Zinc	Zn	**147**	g/t TS	AD-KW-ICP-lbu

The compost application of the Swiss recommendation is 25 t DM/ ha, which results in an application rate per pot of:

$$Application\ rate\ =\ 25\ \frac{t\ DM}{ha}$$

$$Pot_{area} =\ \ 0.016\ \frac{m^2}{pot}$$

$$Application\ rate\ x\ Pot_{area}\ =\ 25\ \frac{t\ DM}{ha}\ x\ \frac{1\ ha}{10'000\ m^2}\ x\ \frac{1'000'000\ g}{1\ t}\ x\ 0.016 \frac{m^2}{pot} = \mathbf{40}\ \frac{\boldsymbol{g\ DM}}{\boldsymbol{pot}}$$

Table 12 Chemical composition of the mature compost from the Biomassenhof AG in Winterthur

pH	C/N	Total-N [g/kg DM]	P (P_2O_5) [kg/t DM]	K (K_2O) [kg/t DM]
8.13	18.99	8.32	2 (4.58)	11.2 (13.44)

As we know the chemical composition of the used compost (as in Table 12) and only 10% of the N is available from compost, one can calculate the available N amount per pot with the application of 40 g compost/ pot:

$$Application\ rate\ =\ 25 \frac{t\ DM}{ha}$$

$$N_{tot} =\ 8.32\ \frac{g}{kg\ DM}$$

$$P_{tot} =\ 2\ \frac{g}{kg\ DM}$$

$$K_{tot} =\ 11.2\ \frac{g}{kg\ DM}$$

$$N_{availability}\ =\ 10\ \%$$

$$N_{pot} =\ N_{tot} x\ application\ rate\ x\ N_{availability}\ x\ Pot_{area}$$

$$= 8.32\ \frac{g\ N}{kg\ DM}\ x\ 25\ \frac{t\ DM}{ha} x\ \frac{1'000\ kg}{1\ t}\ x\ \frac{1\ ha}{10'000\ m^2}\ x\ 0.1$$

$$= \mathbf{2.08}\ \frac{\boldsymbol{g\ N}}{\boldsymbol{m^2}}\ x\ \frac{1'000\ mg}{1\ g}\ x\ 0.016\ \frac{m^2}{pot} = \mathbf{33.28}\ \frac{\boldsymbol{mg\ N}}{\boldsymbol{pot}}$$

The same applies for P and K, when assumed that 100% is available and 40 g compost/ pot is applied:

$$P_{pot} =\ P_{tot} x\ application\ rate\ x\ Pot_{area}$$

$$=\ 2\ \frac{g\ P}{kg\ DM}\ x\ \frac{1'000\ mg}{1\ g} x\ 25\ \frac{t\ DM}{ha}\ x\ \frac{1'000\ kg}{1\ t}\ x \frac{1\ ha}{10'000\ m^2} x\ 0.016\ \frac{m^2}{pot} = \mathbf{80}\ \frac{\boldsymbol{mg\ P}}{\boldsymbol{pot}}$$

$$K_{pot} =\ K_{tot} x\ application\ rate\ x\ Pot_{area}$$

$$= 11.2\ \frac{g\ K}{kg\ DM}\ x\ \frac{1'000\ mg}{1\ g} x\ 25\ \frac{t\ DM}{ha}\ x\ \frac{1'000\ kg}{1\ t}\ x \frac{1\ ha}{10'000\ m^2} x\ 0.016\ \frac{m^2}{pot}$$

$$= \mathbf{448}\ \frac{\boldsymbol{mg\ K}}{\boldsymbol{pot}}$$

Biorga Quick

Only 10 % of the N is available in compost, and therefore, only 2.08 g of the 5 g N/m² (= 50 kg N/ ha) were applied. The difference in N is applied through Biorga Quick. It should be noted that in Biorga Quick only 70% of the N is available to the plants. N is as well available as NO in Biorga Quick (see Table 13). Therefore only 5.6 % of Biorga Quick is N:

$N_{difference} = 5 \frac{g\ N}{m^2} - 2.08 \frac{g\ N}{m^2} = 2.92 \frac{g\ N}{m^2}$

$N_{availability}$ = 70%

NO_{amount} = 12%

Molar mass of NO = M_{NO} = 14.0067 u + 15.999 u = 30.0057 u

Molar mass of N = M_N = 14.0067 u

Molar mass of O = M_O = 15.999 u

N_{Biorga} = 5.6 % N

Table 13 Chemical composition of Biorga Quick from Hauert

NO	OS	Cl
12 %	80 %	0 %

$$N_{Biorga} = \frac{M_N\ x\ 100}{M_{NO}}\ x\ NO_{amount} = \frac{14.0067\ \text{u}\ x\ 100}{30.0057\ \text{u}}\ x\ 12\% = 5.6\ \%\ N$$

Thus, one can calculate the application rate of Biorga Quick and the amount of Biorga Quick per pot, as well as the applied amount of N per pot:

$$Application\ rate\ of\ Biorga\ Quick = \frac{N_{difference}}{N_{availability}\ x\ N_{Biorga}} = \frac{2.92\ \frac{g\ N}{m^2}}{70\ \%\ x\ 5.6\ \%\ N} = 74.41 \frac{g}{m^2}$$

$$Biorga\ Quick\ per\ pot = Application\ rate\ x\ Pot_{area} = 74.41 \frac{g}{m^2}\ x\ 0.016 \frac{m^2}{pot} = \mathbf{1.2} \frac{\boldsymbol{g}}{\boldsymbol{pot}}$$

$$N_{pot} = N_{difference} x\ Pot_{area} = 2.92\ \frac{g\ N}{m^2}\ x\ \frac{1'000\ mg}{1\ g}\ x\ 0.016\ \frac{m^2}{pot} = \mathbf{46.72}\ \frac{\boldsymbol{mg\ N}}{\boldsymbol{pot}}$$

6.4.2.1 Fertilizer application amounts per pot

With all the calculations above, one can determine the applied amounts of N, P, and K per pot through organic and inorganic fertilization. The recommended application rates are 50 kg N/ha, 4.4 kg P/ ha, and 41.5 kg K/ha (Neuweiler and Krauss, 2017), results in 80 mg N/ pot, 7.04 mg P/ pot, and 66.4 mg K/ pot. With an application rate of 40 g DM/ pot of compost and 1.2 g Biorga Quick/ pot this results in N, P, and K amounts per pot for the organic fertilization of:

Table 14 Comparison of applied amounts of N, P and K by inorganic and organic fertilization

		Application amount	N [mg/pot]	P [mg/pot]	K [mg/pot]
Inorganic			80	7.04	66.4
Organic	Compost	40 g DM/ pot	332.8	80	448
	Biorga Quick	1.2 g/ pot	46.7	0	0
	Sum		380	80	448
	Portion Inorganic / Organic		**21**	**8.8**	**14.8**

Even though 21 times more N was applied by organic fertilization, the same amounts of N should be plant available. There are not yet many studies on the availability of P and K, so it can be assumed that 8.8 times more P and 14.8 times more K was applied by the organic fertilizer than with the inorganic fertilizer.

6.5 Lab protocol for phospholipid fatty acid analysis (PLFA)

@PHYSLABS (GIUZ, UZH), Guido Wiesenberg & Cyrill Zosso, v1.5 (07/2019)

Preparation

Freeze dry soil or work with field moist soil. Note that for field moist soil moisture needs to be determined separately and later corrections need to be made.

For every sample, amount of solvent needs to be adjusted:

- peat and other organic soil samples: 2-4 g dry soil (up to ca. 8 g moist) — 12 mL/g dry soil
- mineral soil: 5-10 g dry soil (up to ca. 15 g moist) — 4 mL/g dry soil.
- Amount of solvent might be required to be adjusted after measuring test samples of every sample set.

Notes:

- When working with an Eppendorff type pipette, the bodies and tips are not solvent resistance against $CHCl_3$ and partially also MeOH (otherwise one might get contamination by plasticizers). Note that the pipette needs to be equipped with a filter to avoid organic solvents (vapor) pass into the body of the pipette. To avoid contamination, one should replace tips regularly.

Extraction solution (storage max. app. 4 weeks):

- Citric buffer (0.15 M)
  - Weigh 6.30 g citric acid monohydrate into 200 mL volumetric flask using spatula, weighing paper and scale in K92.
  - Fill volumetric flask with de-ionized water (ELGA) until ca. 1 cm below mark. (NOTE: Keep in mind that it slightly gains more volume in the next step, which is why you might take a few drops less rather than a few drops more water).
  - Transfer solution into 250 mL Erlenmeyer beaker and add a magnet.
  - Place Erlenmeyer beaker on magnetic stirrer and turn on stirrer.
  - Check pH using pH-meter (pre-calibrate with the calibrants of pH 4 and 7).
  - Adjust solution to pH 4.0 by adding KOH pellets (ca. 20-40 pellets; Be careful not to add too much pellets; After adding a few pellets always wait a moment until KOH dissolved and check pH; If you see pH approaching 4, add subsequently less pellets).
  - To properly adjust the volume, return the adjusted solution to the volumetric flask and fill until mark.
- Extraction solution
  - Use 100 mL volumetric cylinder and stock bottle for extraction solution. (If stock is not too old, i.e. 1-2 weeks, one might add fresh solvent to the existing stock; Otherwise discard stock; Note that volume of stock bottle is 500 mL.)
  - Add 80 mL citric acid buffer
  - Add 200 mL MeOH
  - Add 100 mL chloroform ($CHCl_3$)
  - The fixed relationship of $CHCL_3$:MeOH:citric acid buffer (v:v:v) should be always 1:2:0.8!

Transaction/extraction (day 1)

- Weigh amount of soil (see **Preparation**) into glass centrifuge tubes with lid. Note the exact weight.
- Add the required amount of extraction solution (4-12 mL/g; see **Preparation**) with a 5 or 10 mL Eppendorf type pipette. Use a fresh tip every 5 times of soaking up solution.
- Add 50 µg PC 19:0 PLFA (c=1.0 mg/mL), which is dissolved in $CHCl_3$.
- Close centrifuge tubes well and vortex them for 15 sec.
- Shake centrifuge tubes 2h on the horizontal shaker (K92) at 200 rpm using a white metal/plastic rack that you need to cover by paper tissues to avoid damage to centrifuge vials.
- Shake manually before centrifuging for 10 min at 2500 rpm. Note that centrifuge always needs to be equilibrated with identical weights on opposing positions to avoid damages on the centrifuge.
- Carefully transfer supernatant to separation funnels using a Pasteur pipette.
- Add 5 mL extraction solution to centrifuge vial, vortex, shake for 30 min and centrifuge, followed by transfer to separation funnel.
- Repeat the last steps - the supernatant should get a more pale color.

- Add 0.34 times total volume of added extraction solution each $CHCl_3$ and citric buffer. Only use glass syringes or glass pipettes with solvent resistant pipetting-ball whenever handling $CHCl_3$. Note that using Eppendorff pipette tips releases plastizisers which happens for CHCl3 already the first time you soak up solvent. For other solvents it is a bit less critical: tests tell that plastizisers can be released for $CHCL_3$ and CH_2Cl_2 during first use, methanol after ca. 5 uses and acetone and hexane after ca. 10 uses, respectively.
- Close separation funnel with stopper, shake and open carefully to release overpressure.
- Repeat the last step.
- Close separation funnel and fix stopper by red plastic clamp and shake for 15 min on horizontal shaker (about 70 rpm to avoid sample loss via the stopper when shaking stronger, which is not entirely tight). Note that separation funnels are tightly closed and fix the separation funnel carefully on the shaker.
- Place separation funnels on holders in the fume hood and leave them standing for separation over night.
- Activate 1 g silica 60 per sample in oven over night (at 110-120°C).

Transaction/extraction (day 2)

- Extraction
    - After separation overnight, carefully release the lower (organic phase) into 100 mL round bottom flasks. Make sure that you do not release part of the upper phase into the round bottom flask!
    - Add another aliquot (ca. 10 mL $CHCl_3$) to the separation funnel, shake for 15 min on horizontal shaker and add lower phase to round bottom flask.
    - Repeat the last step at least two more times.
    - Clean outlet of separation funnel as well as the ground neck of the round bottom flasks with a few drops of $CHCl_3$ using a Pasteur pipette.
    - Reduce volume of $CHCl_3$ to ca. 100 µL in Büchi Multivapor by slowly adjusting underpressure and temperature of water bath in order not to spoil pump with solvent. Note that evaporation conditions provided in Multivapor controller are too strong to start with, i.e. start with 45°C of the water bath and ca. 500 mbar.
- Separation of fatty acid fractions
    - Prepare separation cabinet in the way that you add per sample (from bottom to top): long stainless steel connector, stop cock, 6 mL glass column with glass fiber filter.
    - Add 0.5 g activated silica (column height 1 cm) to the column and add a little plug of pre-extracted glass wool on top.
    - Sequentially wash the whole setup with two-three syringe fillings of $CHCl_3$ including stop cock by turning this two times during washing and the tip of the connector by a few extra drops of solvent. If required, carefully remove air bubbles from silica by stirring with one Pasteur pipette in the beginning. Make sure that the silica does not fall dry again. Cover with aluminum foil, if you leave it standing for a while.
    - Release $CHCl_3$ until the top of the silica and close stop cock.
    - Add sample to the column by using a Pasteur pipette.
    - Add 1 mL of $CHCl_3$ to round bottom flask by use of a glass syringe, gently shake and transfer sample to column.
    - **Neutral lipids:** Open stop cock and release this fraction into a pre-weighed 8 mL vial, if one wants to keep this fraction for later analyses. As this is not relevant for the analysis of phospholipid fatty acids, this fraction might be directly disposed and NOT collected in pre-weighed 8 mL vials.
    - Repeat the last two steps 4 times. This yields 5 mL of **neutral lipids**.
    - Close stop cock shortly before the column falls dry.
    - Rinse steel connector with $CHCl_3$
    - **Glycolipids:** Add 5 mL acetone to round bottom flask using a glass syringe, wash it and transfer it to the column. Release the solvent into a 50 mL round bottom flask, if one wants to keep this fraction for later analyses. As this is not relevant for the analysis of phospholipid fatty acids, this fraction might be directly disposed and NOT collected in round bottom flasks.
    - Repeat the last step 3 times, which yields 20 mL **glycolipids**.

- Close stop cock shortly before the column falls dry.
- Rinse steel connector and ground neck of round bottom flask with acetone.
- **Phospholipids:** Add 5 mL methanol to round bottom flask using a glass syringe, wash it and transfer it to the column. Release the solvent into a 50 mL round bottom flask.
- Repeat the last step 3 times, which yields 20 mL **phospholipids**.
- Rinse steel connector and ground neck of round bottom flask with methanol.
- Reduce volume of methanol of the phospholipid fraction to ca. 100 µL in Büchi Multivapor by slowly adjusting underpressure and temperature of water bath in order not to spoil pump with solvent. Note that evaporation conditions provided in Multivapor controller are too strong to start with, i.e. start with 50°C of the water bath and ca. 400 mbar. It might be necessary to remove solvent from the reservoir before adjusting the pressure and temperature of the water bath.
- Prepare separation cabinet in the way that you add per sample (from bottom to top): long stainless-steel connector, 3 mL glass column with glass fibre filter and ca. 1 g Na_2SO_4 (column height ca. 2 cm). Wash with ca. 5 mL methanol and place a pre-weighed 4 mL vial underneath.
- Transfer restricted phospholipid fraction to glass column with a Pasteur pipette.
- Wash round bottom flask with a small amount of methanol and transfer to glass column.
- Repeat the last step at least 3 additional times or until the 4 mL vial is filled.
- Reduce volume of methanol of the phospholipid fraction to ca. 100 µL in concentrator under gentle stream of N_2 at 40°C in K91.

6.6 Methylation of phospholipid fatty acids

Section 3.7 in Wiesenberg and Gocke (2017**):**

Reproducible derivatization techniques are required for quantitative investigations of polar lipid fractions such as fatty acids as well as lowest interferences for compound-specific isotope analysis. For fatty acids, methylation has been proven to fulfill both criteria, which is why we recommend this method for fatty acid analysis.

1. Dissolve the total fatty acid fraction or an aliquot of total fatty acids (< 2 mg fatty acids can be methylated with the described method) in 300 µL dichloromethane (GC grade) in fraction vial.
2. Optional: If required, add an internal standard to the fraction vial using a glass syringe.
3. Add 500 µL boron trifluoride/methanol to the fraction vial.
4. Close the vial and make sure that it is properly tightened. Place the vial in a heating block or drying cabinet at 60°C for 15 min. If required, leave 1–2 min more for equilibration of the temperature.
5. Remove the vial afterward from the heating block, and let it cool down to room temperature before opening the vial.
6. Add 500 µL water of millipore quality to the fraction vial, close the vial again, and use the vortex mixer to properly mix the liquids.
7. Centrifuge the vial for 1 min at 300 g.
8. Insert a small plug of glass wool into a glass pasteur pipette, and push it downward, e.g., by using the tip of another pasteur pipette.
9. Add 0.5–1 g sodium sulfate to the pasteur pipette, and rinse it with ca. 1 mL dichloromethane (GC grade). Afterward, place an autosampler vial underneath the pasteur pipette to collect the methylated fatty acids.
10. Transfer the lower (organic) phase from derivatization vial to the filled glass pasteur pipette.
11. Add another 100 µL dichloromethane (GC grade) to the derivatization vial, use the vortex mixer, and repeat steps 7 and 10–11 at least five times or until the organic phase remains colorless at least three times to enable quantitative transfer of sample.
12. Add another 200–400 µL dichloromethane (GC grade) to the pasteur pipette for complete elution of fatty acids from sodium sulfate.
13. Afterward, the solvent volume in the autosampler vial can be reduced under a gentle N_2 stream, and the methylated fatty acids can be transferred to a micro insert, if required.

6.7 GC oven and MMI temperature program

Table 15 GC oven and MMI temperature program for PLFA measurements, same as in Zosso and Wiesenberg (2021).

GC oven temperature program			Multimode inlet temperature program		
Rate [°C/ min]	Temperature [°C]	Hold time [min]	Rate [°C/ min]	Temperature [°C]	Hold time [min]
-	50	4	-	60	0.5
10	150	0	850	400	5
2	160	0	50	250	-
0.5	170	10			
0.2	175	10			
0.2	180	10			
0.2	185	5			
0.2	190	5			
2	210	5			
5	320	15			

6.8 Derivation of the PLFA quantification formula

For PLFA quantification, the formulas from the paper by Quideau *et al.* (2016) were used and transformed as the following,

$$\text{PLFA content}\left(\frac{\text{nmol}}{\text{g}}\right) = \frac{F \times \frac{\text{area PLFA}}{\text{area } D_{39}C_{20:0}} \times D_{39}C_{20:0} \times \frac{\text{C19:0 std added}}{\text{C19:0 sample}}}{\text{sample weight}}$$

$$\text{C19:0 sample} = F \times \frac{\text{area C19:0}}{\text{area } D_{39}C_{20:0}} \times D_{39}C_{20:0} \text{ std added}$$

$$F = \frac{M_{C19:0}}{M_{PLFA}}$$

$$\text{PLFA content}\left(\frac{\text{nmol}}{\text{g}}\right) = \frac{\frac{F \times \text{area PLFA} \times \text{C19:0 std added}}{F \times \text{area C19:0}}}{\text{sample weight}}$$

$$\text{PLFA content}\left(\frac{\text{nmol}}{\text{g}}\right) = \frac{\frac{\text{area PLFA} \times \text{C19:0 std added}}{\text{area C19:0}}}{\text{sample weight}} \times \frac{M_{C19:0}}{M_{PLFA}}$$

where F is an adjustment factor that takes molarity differences between PLFAs into account (Christie and Han, 2010), area PLFA is the peak area for each identified PLFA, $C_{19:0}$ std added is the amount of added $C_{19:0}$ [nmol] to each sample, area C19:0 is the peak area of the C19:0, sample weight is the weight of the oven-dried soil [g] added to the centrifuge tube before extraction, $M_{C19:0}$ is the molar weight of the $C_{19:0}$ and M_{PLFA} is the molar weight of the identified PLFA (Quideau *et al.*, 2016).
The C19:0 std added was converted by the following formula,

$$\text{C19:0 std added} = \frac{[19{:}0]_{std} \; x \; V_{(19:0 \; std \; added)} \; C19{:}0}{M_{19:0}} \; x \; 2$$

where $[19:0]_{std}$ is the concentration of C19:0 nonadecanoate surrogate standard dissolved in chloroform and added to the soil prior to extraction [mg/L], $V_{(19:0\text{std added})}$ is the added volume of C19:0 nonadecanoate surrogate standard [ml] and $M_{19:0}$ is the molar mass of C19:0. The factor of two was included because PC19:0 is methylated into two C19:0 compounds.

6.9 PLFA data variability of replicates

As described in the materials and method section (Chapter 2), some samples were repeatedly extracted and measured. The mean value of all replicates of a sample, as well as the absolute and relative standard error, were calculated. As seen in Table 16, in some cases, the relative standard error was above 10 % (red filling). The variability between the replicates and within a sample was very high, complicating the interpretation of the results. Due to the small sample amount (5 g dry soil), the results can end in a high variability due to the heterogeneity of a sample (Zosso and Wiesenberg, 2021). In general, Zosso and Wiesenberg (2021) showed that the preparation and amount of the sample, age of the extraction solution, and methylation method could lead to differences in the abundances of functional microbial groups. Even if everything was kept constant throughout the measurements, differences and variabilities could still occur.

Table 16 Mean values per sample of total abundances, absolute abundances of bacteria, and absolute abundances of fungi in mol/g DM (dry matter) with the respective standard errors, relative standard errors, and the number of samples considered (n). A green filling indicates a relative standard error above 5 %, a yellow filling above 10 %, a red filling above 20 %, and no filling below 5 %.

		Total abundance [nmol/ g DM]			**Absolute abundance bacteria [nmol/ g DM]**			**Absolute abundance fungi [nmol/ g DM]**			
Sample	Treatment	Mean	Standard error	Relative standard error [%]	Mean	Standard error	Relative standard error [%]	Mean	Standard error	Relative standard error [%]	**n**
25821GW2	C+C	420.1	1.7	0.4	271.1	3.2	1.2	46.9	2.7	5.8	2
25821GW3		483.2	5.8	1.2	320.3	10.0	3.1	42.8	1.9	4.4	2
25821GW5		572.0	45.1	7.9	373.3	29.3	7.9	64.9	3.7	5.7	2
25821GW7	C+Org	643.8	88.9	13.8	407.5	59.0	14.5	71.8	13.9	19.4	4
25821GW8		708.1	153.5	21.7	449.7	98.3	21.8	90.5	22.0	24.3	2
25821GW10		740.4	9.4	1.3	477.9	5.6	1.2	89.8	4.1	4.5	2
25821GW11		567.5	2.9	0.5	368.8	5.4	1.5	71.1	1.6	2.3	3
25821GW16	C+Inorg	782.0	134.1	17.1	513.0	90.0	17.6	91.8	15.8	17.2	3
25821GW17		775.3	85.2	11.0	527.7	68.3	12.9	84.9	10.2	12.0	3
25821GW21	Bio+C	578.4	36.4	6.3	381.5	25.3	6.6	59.9	5.8	9.6	2
25821GW26	Syn+C	465.4	37.3	8.0	304.2	26.4	8.7	50.8	0.3	0.6	2
25821GW27		648.0	39.3	6.1	426.2	22.8	5.3	68.4	9.2	13.5	2
25821GW29		619.0	16.4	2.7	410.9	5.3	1.3	65.1	5.4	8.3	2
25821GW33	Bio+Org	890.3	71.9	8.1	571.0	37.2	6.5	97.2	17.3	17.8	2
25821GW37		558.0	33.9	6.1	363.2	14.2	3.9	59.4	3.0	5.1	2
25821GW38	Bio+Inorg	625.8	118.7	19.0	414.9	78.6	18.9	62.8	15.7	25.0	2
25821GW45	Syn+Org	515.3	64.0	12.4	325.8	39.5	12.1	59.2	6.2	10.4	2
25821GW48		662.2	67.9	10.3	435.5	46.9	10.8	67.7	4.6	6.8	3
25821GW49	Syn+Inorg	734.4	107.3	14.6	493.9	80.2	16.2	61.1	4.5	7.3	2
25821GW54		539.8	0.7	0.1	359.3	2.6	0.7	55.6	2.3	4.2	2

6.10 Additional figures and results of the statistical tests

6.10.1 Actinobacteria

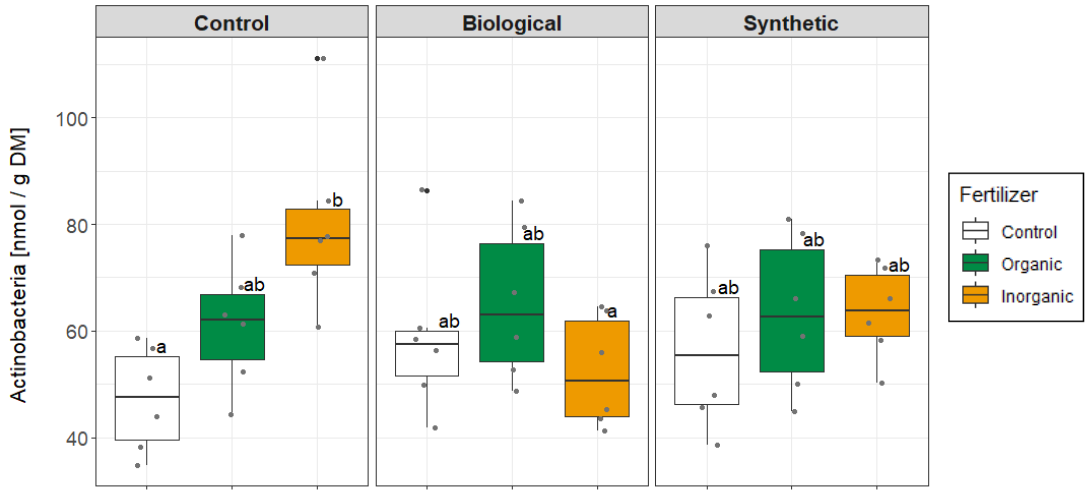


Figure 21 Absolute abundance of actinobacteria in response to the different pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

For the absolute abundance of **actinobacteria**, the fertilizer application significantly influenced the absolute abundance (η^2 = 11%) and a significant interaction was found (η^2 = 23 %). The inorganic fertilizer application significantly increased, and the organic fertilizer application almost showed a marginal effect (p = 0.11) on the abundance of actinobacteria compared to the fertilizer control. While there was a distinct increase with fertilization together with no pesticides, no clear trend was seen for the other two pesticide applications (see Figure 21). All statistical results are in Table 17 in the Appendix 6.10.7.

6.10.2 Multidimensional scaling (MDS) of PLFA profiles

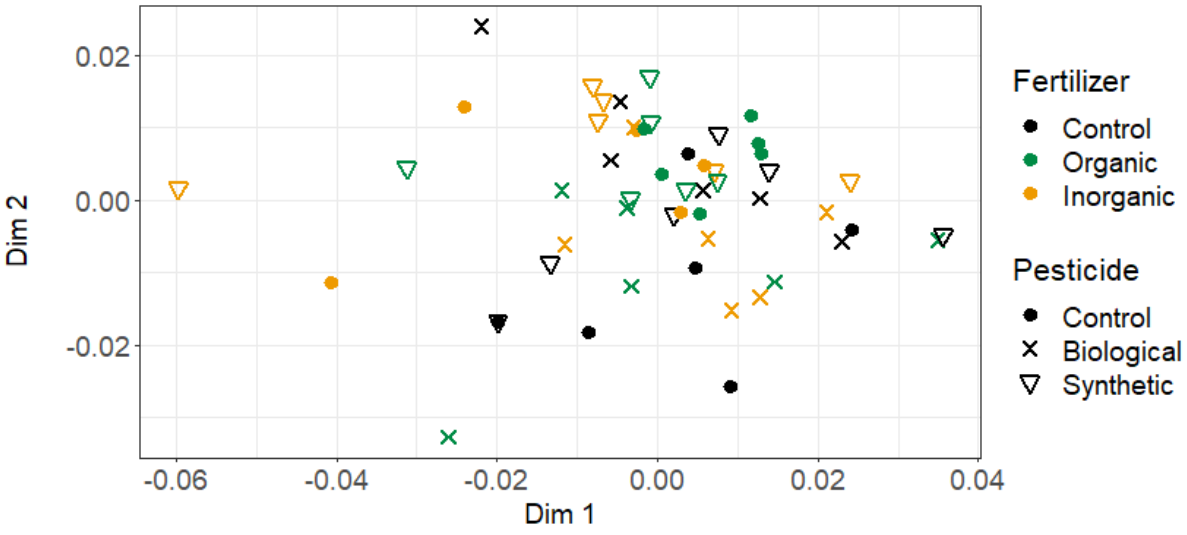


Figure 22 Multivariate statistical analysis (MDS) plot of PLFA profiles of the soil microbial community. The figure shows the unconstrained multidimensional scaling of the square-root transformed Bray-Curtis similarity matrices of the PLFA profiles (mol %). The distances between the datapoints does display the relative differences in similarity.

6.10.3 Relative abundances of PLFA

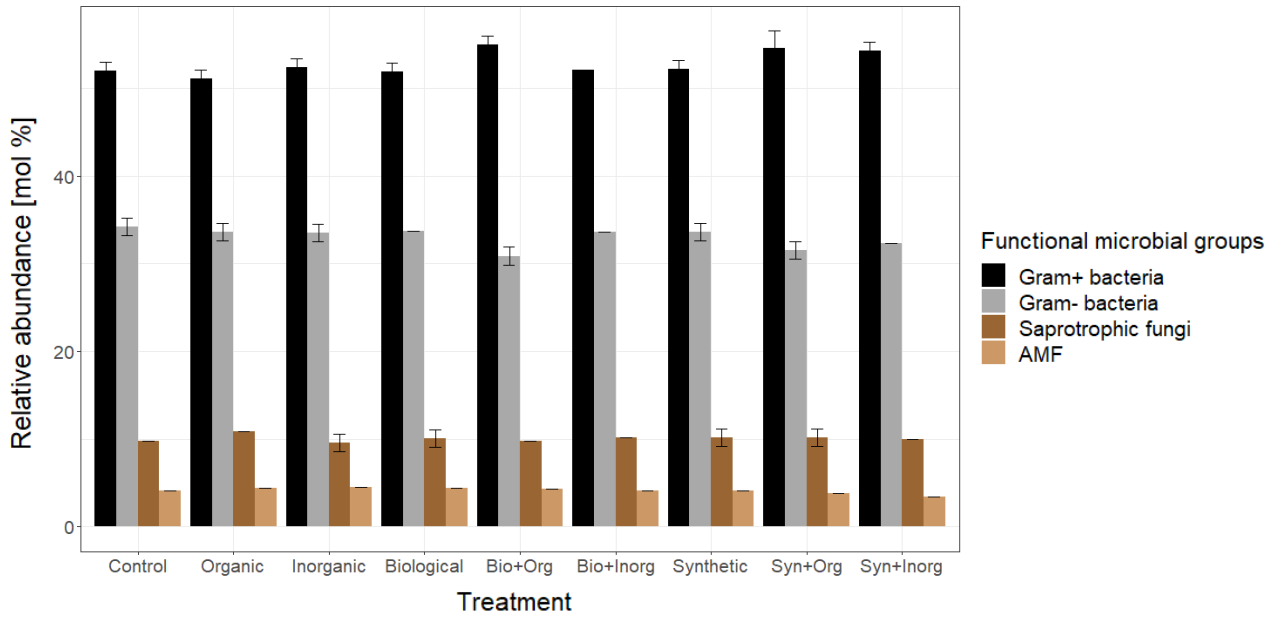


Figure 23 Average relative abundances [mol %] of functional microbial groups by treatment. Bars represent the standard error of the mean.

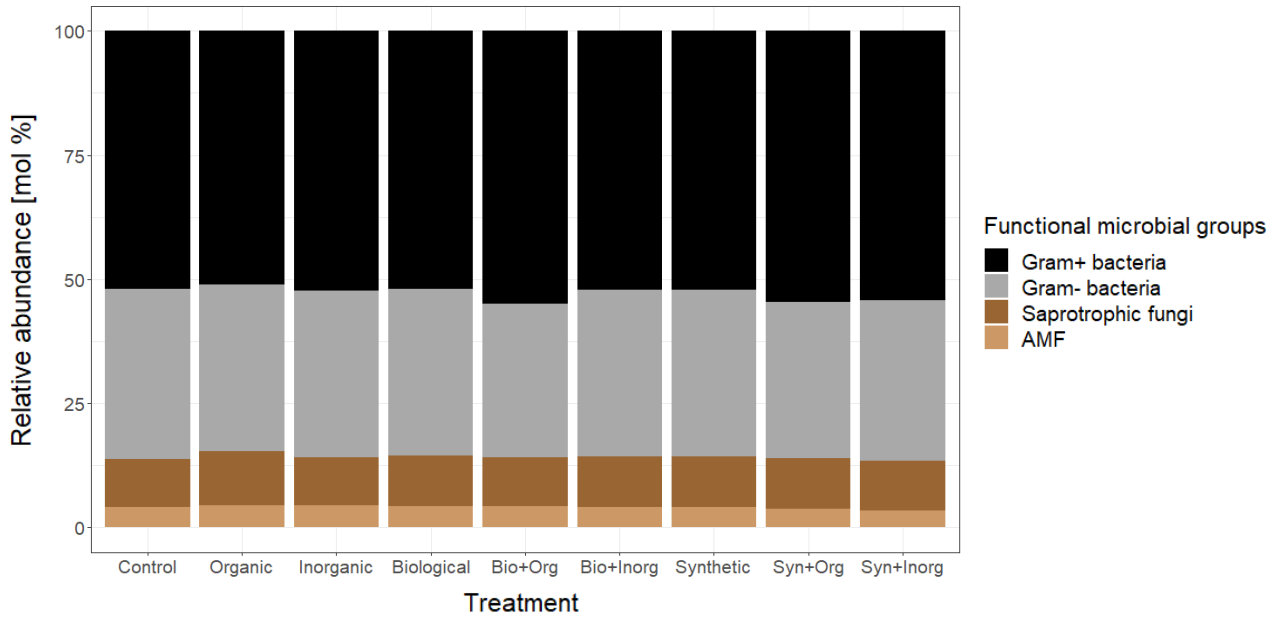


Figure 24 Average relative abundances [mol %] of different functional microbial groups by treatment.

6.10.4 AMF root colonization

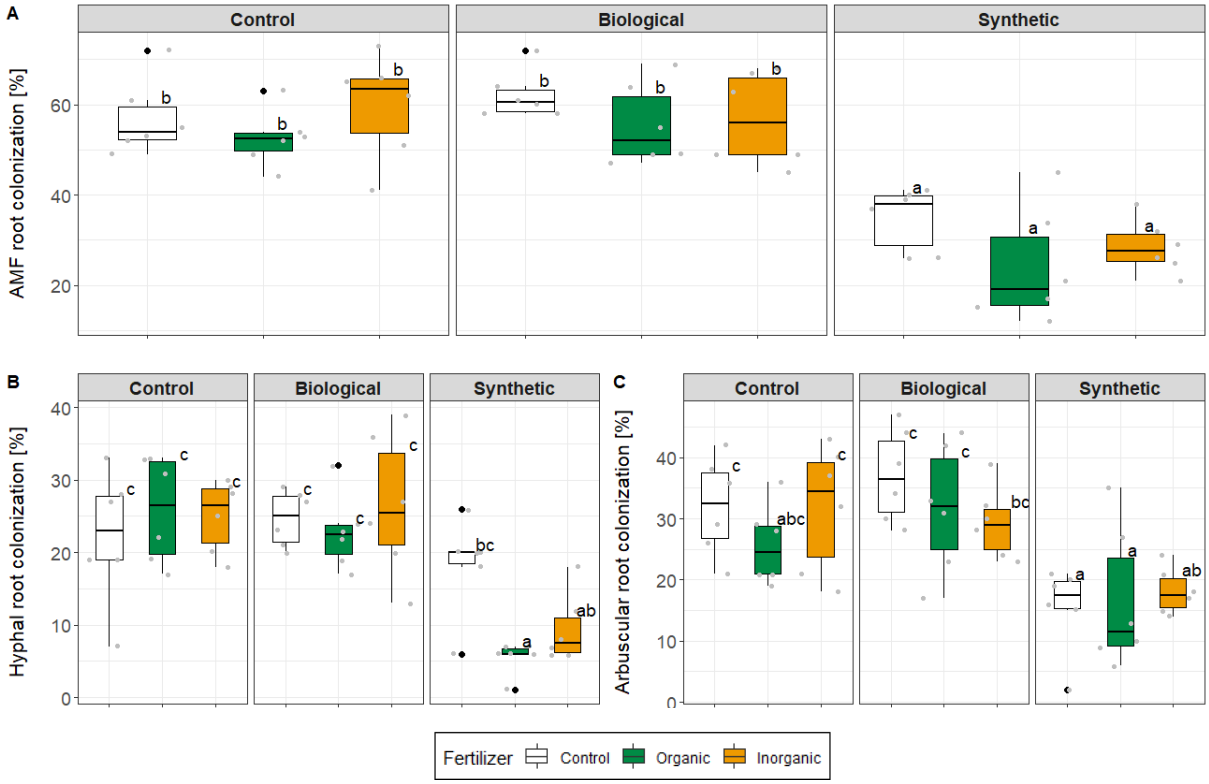


Figure 25 Total AMF (A), hyphal (B), and arbuscular (C) root colonization with pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

6.10.5 Litter decomposition

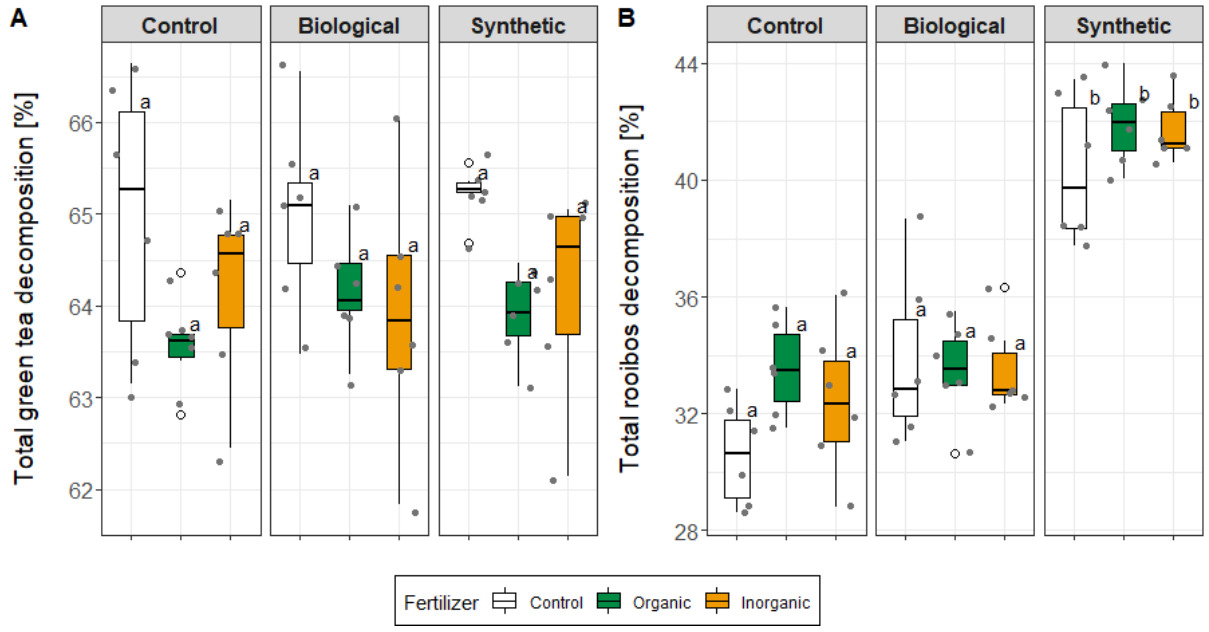


Figure 26 Total green (A) and rooibos (B) tea decomposition with pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications after 20 and 60 days, respectively. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

6.10.6 Nutrient content

This is additional information to the nutrient contents already mentioned in Chapter 3.2.2 of the result section. The interaction between both factors was almost negligible but was still significant at day 20 (N: 9 %; P: 3 %; K: 3 %). The positive effect of organic fertilization can be seen within the pesticide control (C+Org) and biological pesticide (Bio+Org) treatments for all nutrients whereby this was also seen in combination with synthetic pesticides (Syn+Org) only for the K content. Furthermore, inorganic fertilizer application increased the nutrient content for the N and K content with exception when used in combination with synthetic pesticides (see Figure 27 A-C).

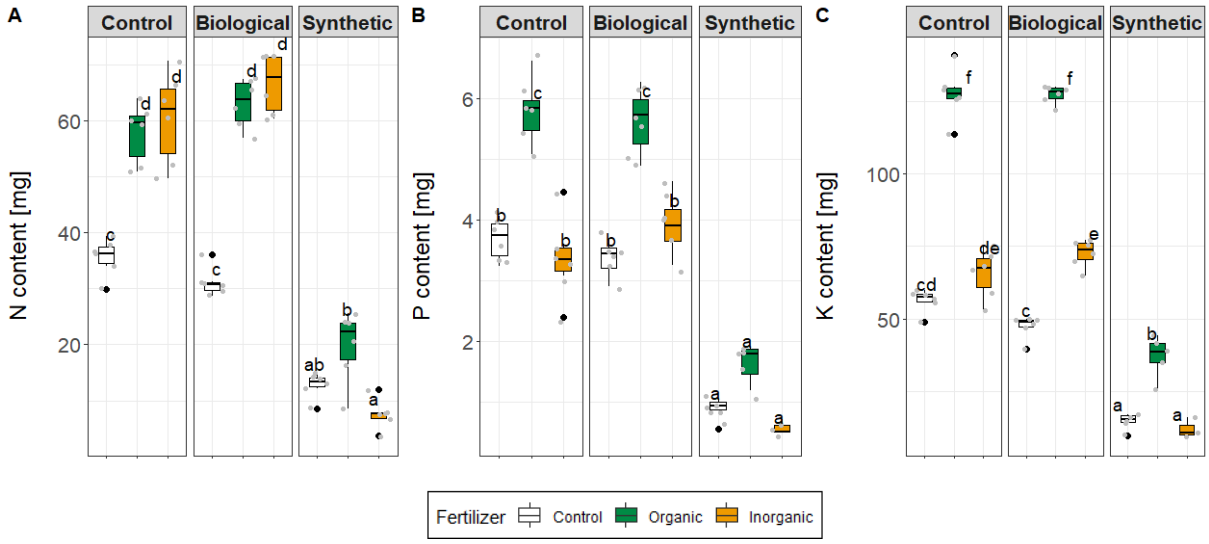


Figure 27 Nutrient content per pot [mg] for nitrogen (A), phosphorus (B), and potassium (C) 20 days after pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

Furthermore, at day 60, an interaction was found (P: 7 %; K: 9 %) where the positive effect of both fertilizer applications was not found together with synthetic pesticides for all nutrients (Figure 28 A-C).

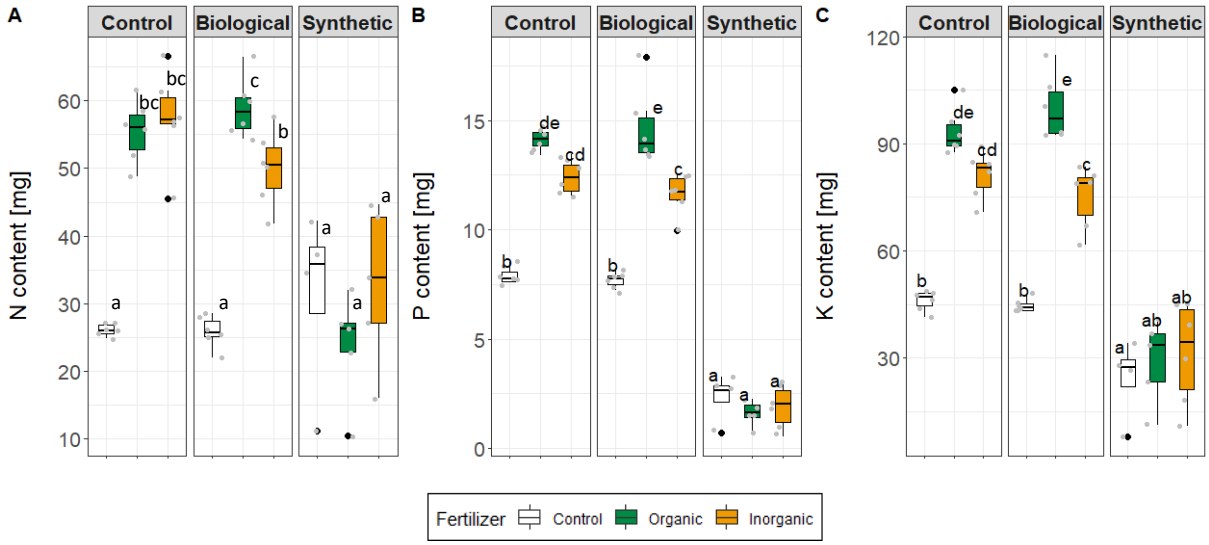


Figure 28 Nutrient content per pot [mg] for nitrogen (A), phosphorus (B), and potassium (C) 60 days after pesticide (Control, Biological, Synthetic) and fertilizer (Control, Organic, Inorganic) applications. Letters indicate differences based on Tukey pairwise comparison with $p < 0.05$.

6.10.7 Tables of statistical results

The following tables show all statistical results of the functional and biological parameters for the result Chapter 3.

*Table 17 Summary tables of the two-way ANOVA for the different response variables (e.g., green tea decomposition) by the factors block, pesticide (P), and fertilizer (F) with their interaction (FxP). Df = degrees of freedom, Sum Sq = sum of squares, Mean Sq = mean squares, Pr(>F) = p-value. Data transformations prior to the analysis are marked as sqrt (square root) and log (logarithm). For the N content at Day 60 and the additional experiment a Kruskal Wallis test was conducted with the factors pesticide (P), fertilizer (P) and treatment. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

Soil functioning												
Day 20							**Day 60**					
Green tea decomposition	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**Rooibos tea decomposition**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	4.29	0.858	0.894	0.49422		Block	5	31.4	6.3	1.723	0.1516	
P	2	0.27	0.133	0.139	0.87066		P	2	870.9	435.5	119.634	<2e-16	***
F	2	14.73	7.364	7.673	0.00151	**	F	2	18.4	9.2	2.521	0.0931	.
F x P	4	1.23	0.307	0.32	0.86271		F x P	4	17.9	4.5	1.231	0.3131	
Residuals	40	38.39	0.96				Residuals	40	145.6	3.6			
Sqrt(Dry weight)	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**Dry weight**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.036	0.007	0.876	0.50608		Block	5	0.19	0.038	0.927	0.474	
P	2	6.781	3.391	414.24	< 2e-16	***	P	2	16.98	8.49	206.799	< 2e-16	***
F	2	1.354	0.677	82.698	6.16E-15	***	F	2	4.91	2.455	59.795	9.57E-13	***
F x P	4	0.149	0.037	4.561	0.00396	**	F x P	4	2.659	0.665	16.193	5.78E-08	***
Residuals	40	0.327	0.008				Residuals	40	1.642	0.041			
Sqrt(N content)	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**N content (Kruskal-Wallis)**	Df	Chi-Sq	p-value			
Block	5	0.64	0.13	0.641	0.67		P	2	8.7688	0.01247			*
P	2	156.31	78.16	393.272	< 2e-16	***	F	2	17.825	1.35E-04			***
F	2	24.97	12.48	62.821	4.55E-13	***	Treatment	8	39.167	4.576E-06			***
F x P	4	19.87	4.97	24.994	2.02E-10	***							
Residuals	40	7.95	0.2										

P content	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**P content**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	3.2	0.64	2.978	0.02378	*	Block	5	12.8	2.6	3	0.0226	*
P	2	100.6	50.3	234.011	<2.00E-16	***	P	2	944.6	472.3	553.69	<2.00E-16	***
F	2	34.88	17.44	81.132	4.60E-14	***	F	2	182.9	91.5	107.23	4.01E-16	***
F x P	4	5.23	1.31	6.084	0.000759	***	F x P	4	86.6	21.6	25.38	3.60E-10	***
Residuals	36	7.74	0.21				Residuals	37	31.6	0.9			
Sqrt(K content)	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**K content**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	5.87	1.17	7.44	7.03E-05	***	Block	5	771	154	1.906	0.117	
P	2	187.35	93.68	594.18	<2.00E-16	***	P	2	20650	10325	127.613	<2.00E-16	***
F	2	117.88	58.94	373.87	<2.00E-16	***	F	2	12927	6464	79.887	3.74E-14	***
F x P	4	8.79	2.2	13.94	5.78E-07	***	F x P	4	3624	906	11.197	4.72E-06	***
Residuals	36	5.68	0.16				Residuals	37	2994	81			

Soil biology

Log(Total abundance)	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.2805	0.0561	1.501	0.2114	
P	2	0.0875	0.04374	1.17	0.3207	
F	2	0.2537	0.12686	3.394	0.0435	*
F x P	4	0.3915	0.09788	2.618	0.0492	*
Residuals	40	1.4953	0.03738			

Absolute abundance							**Relative abundance**						
Bacteria	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	45849	9170	1.314	0.2777		Block	5	40.82	8.165	6.772	0.000117	***
P	2	15604	7802	1.118	0.3369		P	2	3.05	1.525	1.265	0.293196	
F	2	36972	18486	2.649	0.0831	.	F	2	2.34	1.168	0.969	0.388185	
F x P	4	88953	22238	3.187	0.0231	*	F x P	4	7.43	1.857	1.54	0.208999	
Residuals	40	279140	6978				Residuals	40	48.22	1.206			
Gram+	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	26007	5201	1.753	0.1449		Block	5	200.35	40.07	8.676	2.30E-04	***
P	2	2912	1456	0.491	0.6158		P	2	32.03	16.01	3.467	0.0409	*
F	2	20158	10079	3.397	0.0434	*	F	2	21.55	10.78	2.333	0.1101	
F x P	4	38051	9513	3.206	0.0225	*	F x P	4	39.86	9.97	2.158	0.0914	.
Residuals	40	118692	2967				Residuals	40	184.74	4.62			

Actinobacteria	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	1138	227.7	1.352	0.263		Block	5	6.091	1.218	2.29	0.06382	.
P	2	148	74.2	0.441	0.6468		P	2	6.039	3.02	5.677	0.00676	**
F	2	1279	639.3	3.795	0.031	*	F	2	7.953	3.977	7.476	0.00174	**
F x P	4	2724	680.9	4.042	0.0076	**	F x P	4	1.996	0.499	0.938	0.45183	
Residuals	40	6738	168.4				Residuals	40	21.276	0.532			
Gram-	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.3121	0.06243	1.623	0.1761		Block	5	61.23	12.246	7.561	4.47E-05	***
P	2	0.1942	0.0971	2.524	0.0928	.	P	2	17	8.501	5.249	0.009459	**
F	2	0.1212	0.06062	1.576	0.2194		F	2	30.66	15.329	9.464	0.000431	***
F x P	4	0.4097	0.10242	2.663	0.0463	*	F x P	4	14.79	3.698	2.283	0.077217	.
Residuals	40	1.5385	0.03846				Residuals	40	64.79	1.62			
Fungi	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.4186	0.08373	2.498	0.04645	*	Block	5	40.82	8.165	6.772	0.000117	***
P	2	0.1495	0.07473	2.229	0.1208		P	2	3.05	1.525	1.265	0.293196	
F	2	0.2643	0.13216	3.942	0.02737	*	F	2	2.34	1.168	0.969	0.388185	
F x P	4	0.5422	0.13555	4.043	0.00759	**	F x P	4	7.43	1.857	1.54	0.208999	
Residuals	40	1.3409	0.03352				Residuals	40	48.22	1.206			
Log(Saprotrophic fungi)	Df	Sum Sq	Mean Sq	F value	Pr(>F)			Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.4638	0.09276	2.607	0.0393	*	Block	5	32.22	6.443	6.797	0.000113	***
P	2	0.0944	0.04721	1.327	0.2767		P	2	0.07	0.036	0.038	0.962453	
F	2	0.291	0.14551	4.09	0.0242	*	F	2	1.29	0.644	0.68	0.512565	
F x P	4	0.4073	0.10182	2.862	0.0355	*	F x P	4	5.18	1.294	1.365	0.263128	
Residuals	40	1.4232	0.03558				Residuals	40	37.92	0.948			
Log(AMF)	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**AMF**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	0.4427	0.08854	2.391	0.05472	.	Block	5	0.82	0.1641	1.563	0.19275	
P	2	0.4354	0.21769	5.878	0.005783	**	P	2	3.395	1.6973	16.168	7.15E-06	***
F	2	0.2111	0.10557	2.85	0.069616	.	F	2	0.277	0.1385	1.32	0.2786	
F x P	4	0.9568	0.23921	6.459	0.000416	***	F x P	4	1.951	0.4877	4.645	0.00357	**
Residuals	40	1.4815	0.03704				Residuals	40	4.199	0.105			

Root colonization by												
Total AMF	Df	Sum Sq	Mean Sq	F value	Pr(>F)		**Arbuscules**	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Block	5	1196	239	4.065	0.00446	**	Block	5	1298.5	259.7	5.896	0.000358	***
P	2	9549	4774	81.17	8.31E-15	***	P	2	2585.6	1292.8	29.349	1.43E-08	***
F	2	489	245	4.16	0.02285	*	F	2	110.3	55.1	1.252	0.297021	
F x P	4	173	43	0.735	0.57368		F x P	4	252.9	63.2	1.435	0.240177	
Residuals	40	2353	59				Residuals	40	1762	44			
Additional experiment													
Biomass Day 20 (Kruskal – Wallis)	Df	Sum Sq	Mean Sq	F value	Pr(>F)								
Block	5	0.25869	0.05174	7.889	3.04E-05	***							
P	2	0.05942	0.02971	4.53	0.0168	*							
F	2	0.06654	0.03327	5.073	0.0109	*							
F x P	4	0.06313	0.01578	2.406	0.0654	.							
Residuals	40	0.26234	0.00656										

*Table 18 Results of PERMANOVA of the effect of pesticide (P) and fertilizer (F) application and their interaction (FxP) on the relative abundance (mol %) of PLFAs specific to bacteria with Gram+ and Gram- bacteria, and fungi with saprotrophic fungi and AMF. Df = degrees of freedom, Sum Sq = sum of squares, Mean Sq = mean squares, R^2 = R-square ,Pr(>F) = p-value Signifance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1*

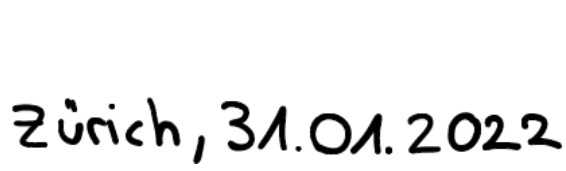
	Df	Sum Sq	Mean Sq	F value	R^2	Pr(>F)	
P	2	0.001930	0.00096505	1.8393	0.05861	0.009	**
F	2	0.002964	0.00148221	2.825	0.09002	0.001	***
P x F	4	0.004424	0.00110600	2.108	0.13435	0.001	***
Residuals	45	0.023610	0.00052468		0.71701		
Total	53	0.032929			1		

Table 19 Root colonization by hyphae, arbuscules, vesicles, arbuscules and vesicles, and no colonization (nothing). The sum of all (without nothing counts) resulted in the total AMF root colonization. The numbers represent the counts of positive intersections (see Chapter 2.4.2) divided into treatments. Values are mean ± standard error.

Treatment	Intersections					
	Hyphae	Arbuscules	Vesicles	Arbuscules and Vesicles	Nothing	Total
C + C	22.2 ± 4	32 ± 3	1.3 ±1	1.5 ± 1	43.2 ± 3	57 ± 3
C + Org	25.8 ± 3	25.7 ± 3	1 ± 0	0	47.5 ± 3	52.5 ± 3
C + Inorg	25 ± 2	31.8 ± 4	2.3 ± 1	0.5 ± 0	40.3 ± 5	59.7 ± 5
Bio + C	24.7 ± 2	37 ± 3	0.3 ± 0	0.2 ± 0	37.8 ± 2	62.2 ± 2
Bio + Org	22.8 ± 2	31.7 ± 4	0.8 ± 1	0.2 ± 0	44.5 ± 4	55.5 ± 4
Bio + Inorg	26.5 ± 4	29.3 ± 3	0.7 ± 0	0.3 ± 0	43.2 ± 4	56.8 ± 4
Syn + C	18.3 ± 3	15.5 ± 3	0.8 ± 1	0.2 ± 0	65.3 ± 3	34.8 ± 3
Syn + Org	5.5 ± 1	16.7 ± 5	1.7 ± 1	0.2 ± 0	76 ± 5	24 ± 5
Syn + Inorg	9.5 ± 2	18.2 ± 2	0.8 ± 0	0	71.5 ± 2	28.5 ± 2

Declaration of independence

I hereby declare that the submitted Thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the Thesis.



Zürich, 31 January 2022 — Celina Steffani