# Plastic mulching in agriculture – Impacts on soil properties and processes and the consequences for soil quality

Insights gained from a three-year field study in strawberry cultivation

#### **Angenommene Dissertation**

Zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat) Fachbereich 7: Natur- und Umweltwissenschaften

Universität Koblenz-Landau, Campus Landau

von

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Tag der Disputation: 06.05.2022

#### **Declaration**

I hereby declare that this PhD thesis, entitled "Plastic mulching in agriculture – Impacts on soil properties and processes and the consequences for soil quality: Insights gained from a three-year field study in strawberry cultivation", was conducted and created by my own. All assistances, contributors and authors are declared and clearly indicated in this thesis. This PhD thesis has never been submitted elsewhere for an exam; neither to any other university nor to any other scientific institution.

Landau in der Pfalz, 11.08.2021

Place, date

**Signature (Maximilian Meyer)** 

#### **Contributions to this PhD thesis**

This PhD thesis includes four chapters, which have been published as research papers. The described field experiments were conducted on a commercial strawberry field, located near 'Offenbach an der Queich' in Southwestern Germany (49°11'N, 8°10'E), enabled by a cooperation with a professional strawberry farmer, who cultivated the field according to the common agricultural practices in strawberry cultivation. All soil analyses were conducted in the laboratories of the Institute of Environmental Sciences at University of Koblenz-Landau, Campus Landau. Funding for this research project was received by the Ministry for Education, Sciences, Further Education and Culture of the State of Rhineland-Palatinate (MBWWK) in the frame of the Interdisciplinary Research Group for Environmental Studies (IFG-Umwelt) of University Koblenz-Landau, the Prof. B. Gedek and W. Gedek foundation and the research fund of the University of Koblenz-Landau. The concept and the research questions of this work were developed by myself in cooperation with Prof. Dr. Gabriele Ellen Schaumann and Dr. Katherine Andrea Muñoz Sepúlveda. Soil samplings and laboratory analyses were planned and conducted by my own, with practical support from technical and student assistants. Calculations, statistics, data interpretation and paper writing were done by myself in cooperation with Prof. Dr. Gabriele Ellen Schaumann, Dr. Katherine Andrea Muñoz Sepúlveda and Dr. Dörte Diehl. The contributions to the individual papers are subsequently stated:

#### Chapter 3 (Meyer et al. 2020) was published in SN Applied Sciences:

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2020): Analysis of biogeochemical processes in plastic-covered soil during establishment period in strawberry cultivation. SN Applied Sciences. 2(10): 1-16

The concept and the experimental design of this paper was developed by myself in cooperation with Prof. Dr. Gabriele Ellen Schaumann and Dr. Katherine Andrea Muñoz Sepúlveda. I planned and conducted all soil samplings, laboratory analyses, data evaluations and statistics. I had practical support from colleagues and technical staff during soil samplings and from technical and student staff during laboratory analyses. Data interpretation and paper writing was done in collaboration with all three co-authors.

Chapter 4 (Meyer et al. 2021a) was published in Environmental Science and Pollution Research:

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Agricultural mulching and fungicides – Impacts on fungal biomass, mycotoxin occurrence and soil organic matter decomposition. Environmental Science and Pollution Research. 28(27):36535-36550

The concept and the experimental design of this paper was developed by myself in cooperation with Dr. Katherine Andrea Muñoz Sepúlveda. All soil samplings, data evaluations and statistics were planned and conducted by myself. Colleagues and technical staff supported me during soil samplings. Fungicide measurements in soil samples were performed by Collins Ogbeide in the frame of his master thesis. Mycotoxin and ergosterol measurements in soil samples were respectively performed by Johanna Giradi and Maria Olivares in the frame of their research projects. All students were supervised by Dr. Katherine Andrea Muñoz Sepúlveda and myself. The remaining laboratory analyses were conducted by myself with assistance of technical and student staff. Data interpretation and paper writing was done in collaboration with all three coauthors.

#### Chapter 5 (Meyer et al. 2021b) was published in the Journal of Soils and Sediments:

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Multiannual soil mulching in agriculture – Analysis of biogeochemical soil processes under plastic and straw mulches in a three-year field study in strawberry cultivation. Journal of Soils and Sediments. 21:3733-3752

The concept of this paper was developed by myself together with Prof. Dr. Gabriele Ellen Schaumann and Dr. Katherine Andrea Muñoz Sepúlveda. Experimental design, all soil samplings, data evaluations and statistics were planned and conducted by myself. Colleagues and technical staff supported me during soil samplings. The laboratory analyses were conducted by myself with assistance of technical and student staff. Data interpretation and paper writing was done by myself with support of all three co-authors.

#### Chapter 6 (Meyer et al. 2022) was published in Mycotoxin Research:

Meyer, Maximilian; Schaumann, Gabriele Ellen; Muñoz, Katherine (2022): How does multiannual plastic mulching in strawberry cultivation influence soil fungi and mycotoxin occurrence in soil? Mycotoxin Research. 38(2):93-105

The paper concept was developed by myself in collaboration with Dr. Katherine Andrea Muñoz Sepúlveda. Experimental design, all soil samplings, data evaluations and statistics were planned and conducted by myself. Colleagues and technical staff supported me during soil samplings. Mycotoxin and ergosterol measurements in soil samples were partly performed by Johanna

Giradi and Maria Olivares, respectively, in the frame of their research projects. Both students were supervised by Dr. Katherine Andrea Muñoz Sepúlveda and myself. All other laboratory analyses were conducted by myself with assistance of technical and student staff. Data interpretation and paper writing was done by myself with support of Dr. Katherine Andrea Muñoz Sepúlveda and Prof. Dr. Gabriele Ellen Schaumann.

#### Acknowledgements

First of all, I want to thank Prof. Dr. Gabriele Ellen Schaumann for offering me the opportunity to work on this interesting and challenging research project. I am grateful for her competent and trusting supervision during the past years and appreciated her support of my work with all her ideas, experience and knowledge. Especially, I want to thank her for improving my abilities in critical thinking, scientific working and writing and focusing on process understanding.

Furthermore, I want to thank Dr. Katherine Andrea Muñoz Sepúlveda for her creative and inspiring ideas, her tireless commitment and support and the always positive working atmosphere. I am particular grateful for her expertise on fungi and mycotoxins and our discussions about these topics, which were always helpful and widened my knowledge enormously.

I also want to thank Dr. Dörte Diehl for her practical ideas, her experience in soil scientific methods and analyses and her always constructive and targeted criticism, which helped me a lot to design my experiments and write my papers.

Additionally, I want to thank Angelika Holderle for taking care of all the administrative and organizational stuff and for her open, gentle and helpful nature.

My special thanks go to my office colleagues and friends, Christian, Zacharias and Kilian, for all the support, the discussions, the always good mood, the laughter and all the beers we shared together.

To my colleagues Mathilde, Julius, Allan, Aaron and all other members of our working group 'Environmental and Soil Chemistry', I thank you for your warm welcome, cooperativeness and support during the last years.

Furthermore, I want to thank our technical assistants, Karin, Doris, Silvia and Anna, for their support, skills and professional commitment in the laboratory and during the numerous soil samplings in the strawberry fields.

I thank all my student assistants, especially Katharina, Camilla, Johanna and Sirma, for their support and good work.

I am very grateful to all my friends for all the conversations, the moments and the adventures we shared. Our get-togethers were always a welcome change from daily routine and an encouraging and recreational time.

Finally, I want to express my deepest gratitude to my family, especially my parents Elisabeth & Ernst, for all the love, care and support through all those years.

Last but not least, I want to thank all funders and our cooperation partner Christof Steegmüller for making this research project possible.

All of this, has made my doctoral phase a cheerful, pleasant and memorable time, which has led me grown on a professional level as well as on a personal level.

#### **Abstract**

Agriculture requires a sustainable intensification to feed the growing world population without exacerbating soil degradation and threatening soil quality. Globally, plastic mulching (PM) is increasingly used to improve crop growth and yields and consequently agronomic productivity. However, recent literature reported also critical aspects of PM for soil quality and showed contradictory outcomes. This might result from the numerous applications of PM in different climates across various crops, soils and agricultural techniques. Thus, a closer look is necessary on how PM influences soil processes under certain climate and cultivation conditions to obtain a comprehensive understanding of its effects, which is important to evaluate PM in terms of a sustainable agriculture.

The aim of this PhD thesis was to understand how multiannual PM influences soil properties and processes under the temperate, humid Central European cultivation conditions and to evaluate the resulting consequences for soil quality. I designed a three-year field study to investigate the influence of PM (black polyethylene,  $50 \,\mu m$ ) on microclimate, structural stability, soil organic matter (SOM) and the concentrations of selected fungicides and mycotoxins in three soil layers (0–10, 10–30 and 30–60 cm) compared to straw mulching (SM). Both mulching types were applied in a drip-irrigated ridge-furrow system in strawberry cultivation.

PM shifted the soil microclimate to higher soil temperatures and lower soil moistures. The higher soil temperature seems thus to be the key factor for the increased crop growth and yields under the present humid climate. The reduced soil moisture under PM indicated that under PM the impeded rainfall infiltration had a stronger effect on the water balance than the reduced evaporation. This indicate an inefficient rainwater use in contrast to arid climates. PM changed the water cycling in the ridges from downward directed water flows to lateral water flows from furrows to ridges. This reduced nitrogen leaching in the topsoil (0-10 cm) in the strawberry establishment period. The plastic mulches avoided aggregate breakdown due to rapid soil wetting and excess water during rainfalls and thus maintained a loose and stable soil structure in the surface soil, which prevents soil compaction and made soil less prone to erosion. PM changed carbon fluxes and transformation so that a larger total and more stable SOM was observed. Thus, the higher belowground biomass productivity under PM compensated the impeded aboveground biomass input and the temperature-induced SOM decomposition. However, SM increased the labile and total SOM in the topsoil after the first experiment year and promoted microbial growth due to the aboveground biomass incorporation. PM reduced fungicide entry into soil compared to SM and reduced consequently the fungal biomass reduction and the biosynthesis of the mycotoxin deoxynivalenol. The modified microclimate under PM did not increase mycotoxin occurrence. In this context, PM poses no risk for an increased soil contamination, impairing soil quality. This PhD thesis demonstrated that the PM effects on soil can vary depending on time, season and soil depth, which emphasizes the importance to include soil depth and time in future studies.

Compared to semiarid and arid regions, the PM effects found in this PhD thesis were small, absent or in another way. I attributed this to the fact that PM under humid climate reduced instead of increased soil moisture and that SM had due to straw und strawberry canopy a similar 'covering effect' as PM. Thus, generalizing the PM effects on soil across different climates seems hardly possible as they differ in type and extent depending on climate. A differentiated consideration is hence necessary to evaluate the PM effects on soil quality. I conclude that PM under temperate, humid climate might contribute to reduce soil degradation (e.g., SOM depletion, erosion, nutrient leaching, soil compaction and soil contamination), which sustains soil quality and helps to enable a sustainable agricultural intensification. However, further research is necessary (1) to support my findings on a larger scale, longer time periods and across various soil and crop types, (2) to address remaining open questions and (3) to develop optimization to overcome the critical aspects of PM (e.g. macro- and microplastic waste in soil, mulch disposal).

#### Zusammenfassung

Eine nachhaltige Intensivierung der Landwirtschaft ist notwendig, um die wachsende Weltbevölkerung zu ernähren ohne die Bodenqualität durch verstärkte Bodendegradation zu verschlechtern. Plastikmulche (PM) werden weltweit zunehmend eingesetzt, um Wachstum und Ertrag von Feldfrüchten zu verbessern und somit die landwirtschaftliche Produktivität zu steigern. Zunehmend finden sich aber auch kritische Aspekte der PM-Anwendung auf die Bodenqualität sowie widersprüchliche Ergebnisse in der wissenschaftlichen Literatur. Grund könnte die Anwendung in verschiedenen Klimaten und bei unterschiedlichen Feldkulturen, Böden und landwirtschaftlichen Techniken sein. Ein genauerer Blick ist somit notwendig, um den PM-Einfluss auf die Bodenprozesse unter verschiedenen Klima- und Anbaubedingungen umfassend zu verstehen und hinsichtlich einer nachhaltigen Landwirtschaft zu bewerten.

Ziel dieser Doktorarbeit war es, zu verstehen, inwieweit eine mehrjährige PM-Anwendung verschiedene Bodeneigenschaften und -prozesse unter gemäßigt, humidem Klima in Mitteleuropa beeinflusst und die Folgen für die Bodenqualität zu bewerten. Hierfür untersuchte ich in einer dreijährigen Feldstudie, wie PM (schwarzes Polyethylen, 50 µm) das Mikroklima, die Strukturstabilität, die organische Bodensubstanz (OBS) und die Konzentrationen bestimmter Fungizide und Mykotoxine in drei Bodenschichten (0–10, 10–30 and 30–60 cm) im Vergleich zu Strohmulch (SM) beeinflusst. Beide Bodenabdeckungen wurden in einer Dammkultur mit Tröpfchenberegnung im Erdbeeranbau eingesetzt.

Die PM veränderten das Mikroklima des Bodens hin zu höheren Temperaturen und niedrigeren Wassergehalten. Hauptfaktor für das gesteigerte Pflanzenwachstum unter gegebenem Klima dürfte somit die höhere Bodentemperatur sein. Die niedrigere Bodenfeuchte unter PM zeigte, dass die verhinderte Niederschlagsversickerung stärker den Wasserhaushalt beeinflusste als die reduzierte Evaporation, was auf eine ineffiziente Niederschlagsnutzung hinweist. Die PM veränderten den Wasserkreislauf hin zu vermehrt seitlichen Wasserflüssen von der Furche zum Damm und weniger vertikalen Sickerwasserflüssen im Damm. Letzteres verringerte die Stickstoffauswaschung im Oberboden (0-10 cm) in der Anwachsphase der Erdbeeren. PM verhinderte eine abrupte Bodendurchnässung und Überschusswasser bei Regenfällen und somit Aggregatzerstörung. So wurde eine lockere und stabile Bodenstruktur erhalten, die Bodenverdichtung und Bodenerosion vorbeugt. PM veränderte Kohlenstoffaustausch und -umwandlung hin zu einer größeren und stabileren OBS. Somit kompensierte die unterirdische Biomassenproduktion unter PM den temperaturbedingt beschleunigten OBS Abbau sowie den fehlenden Eintrag oberirdischer Biomasse. Das SM erhöhte jedoch die labile und totale OBS im Oberboden nach dem ersten Versuchsjahr und steigerte das mikrobielle Wachstum durch den oberirdischen Biomasseeintrag. PM verringerte den Fungizideintrag in den Boden und verursachte kein erhöhtes Mykotoxinvorkommen. Somit stellt PM kein erhöhtes Risiko für Bodenkontaminationen und die Bodenqualität dar. Diese Doktorarbeit zeigte, dass sich die PM-Effekte zeitlich, saisonal und zwischen den Bodenschichten unterschieden, womit die Bedeutung der Faktoren Bodentiefen und Zeit für zukünftige Studien belegt wurde.

Verglichen mit ariden Gebieten, waren die beobachteten PM-Einflüsse klein, ausgeblieben oder anders. Als Grund hierfür vermute ich, dass PM in humidem Klima die Bodenfeuchte verringerte anstatt zu erhöhen und dass unter SM das Stroh und Blätterwerk einen PM-ähnlichen "Abdeckungseffekt" verursachte. Eine Generalisierung der PM-Effekte über verschiedenen Klimazonen ist somit kaum möglich, da sich die Effekte in Art und Ausmaß in Abhängigkeit vom Klima unterscheiden. Die PM-Effekte auf die Bodenqualität müssen somit differenziert beurteilt werden. Ich schlussfolgere, dass PM in humiden Klimaten Bodendegradationen vermindern könnte (z.B., OBS Abbau, Erosion, Nährstoffauswaschung, Verdichtung und Kontamination) und somit hilft, Bodenqualität zu erhalten und eine nachhaltige, landwirtschaftliche Intensivierung zu ermöglichen. Allerdings ist weitere Forschung nötig um meine Ergebnisse auf größeren Skalen, über längere Zeitperioden und bei verschiedenen Böden und Feldfrüchte zu überprüfen, verbleibende offene Fragen zu beantworten und Verbesserungen zu entwickeln, um die Nachteile der PM zu überwinden (z.B. Bodenverunreinigung mit Plastik, Entsorgung der Mulche).

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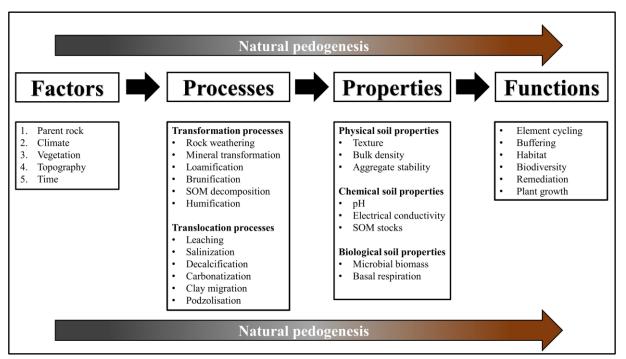
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### **CHAPTER 1**

Introduction

#### 1.1 Soil – Functions and services

The pedosphere is the top layer of the earth's surface and embraces the entirety of the earth's soils. Soils consist of a mixture of mineral particles, organic materials and the interspaces between them, the pores, which are filled with water and air and are inhabited by a multitude of soil organisms (Blume et al., 2016). Soils developed over the millennials and were shaped by the five soil-forming factors: parent rock, topography, vegetation, climate and time (Kibblewhite et al., 2008; Dominati et al., 2010). Soil formation is driven by precipitation and temperature, which define the velocity of chemical and biological reactions and enable physical weathering (e.g., temperature bursting), chemical weathering (e.g., hydrolysis and hydration), relocation of weathering products and vegetation growth (Dominati et al., 2010; Blume et al., 2016). The mineralogy of parent rock defines the mineral composition of a soil and also influences weathering velocity (Dominati et al., 2010). Furthermore, vegetation promotes biological weathering (e.g., root exudation) and physical weathering (e.g., root bursting), whereas topography indirectly influences soil formation due to effects of gravity, local microclimate and water availability (Dominati et al., 2010; Blume et al., 2016). The aforementioned pedogenic processes drive soil formation and determine, as function of time and intensity, the characteristic properties and functions of a soil (Figure 1).



**Figure 1** Soil formation: The soil-forming factors trigger pedogenic processes, which define as function of time and intensity the characteristic properties and functions of a soil (based on Kuzyakov & Zamanian, 2019; Blume *et al.*, 2016)

Soil properties are usually divided in physical (e.g., texture, bulk density and aggregate stability), chemical (e.g., pH, electrical conductivity and soil organic matter (SOM) stocks) and biological (e.g., microbial biomass and basal respiration) properties (Dominati et al., 2010; Kuzyakov & Zamanian, 2019). Soil functions refer to one or more soil processes or properties, which contribute to one or more ecosystem services (Costanza et al., 1997; Bünemann et al., 2018). For example, the SOM pool is the most central key soil property, which influences a multitude of soil processes and other soil properties (Figure 2) and thus contributes to several ecosystem services such as climate and water regulation, nutrient cycling, erosion control and food production (Costanza et al., 1997; Kibblewhite et al., 2008; Powlson et al., 2011). Ecosystem services are generally defined as the benefits which humanity derives from ecosystem functions and have an estimated annual value of US\$ 16–54 trillion (Costanza et al., 1997). Soils and its functions have a vital role in sustaining various ecosystem services, which are increasingly used to incorporate ecological sustainability in political decision-making (Greiner et al., 2017). The ecosystem services supplied by soils can be divided in three categories (Table 1): Providing services include all products that humans obtain from ecosystems, regulating services control soil processes which enable humans to live in a stable, healthy and resilient environment and cultural services comprise all nonmaterial human benefits such as aesthetic experiences, cultural heritage, spiritual enrichment and recreation (Dominati et al., 2010; Powlson et al., 2011). In the public opinion, soils lack the attractiveness and charisma of other natural elements (Mendes et al., 2016). Because of that, they are often neglected in conservation and protection planning, although they are fundamental to maintain critical ecosystem services such as food and biomass production, water and climate regulation, raw material source and biodiversity (Dominati et al., 2010; Mendes et al., 2016; Greiner et al., 2017).

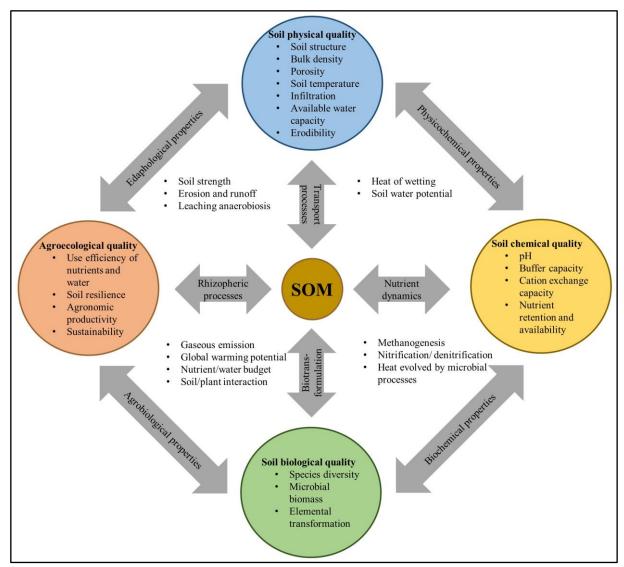


Figure 2 The influence of SOM on various soil properties and processes (redrawn from Lal, 2013)

Although all ecosystem services of soils provide large benefits for humanity, the primary interest in soil was traditionally on its potential for agricultural production and hence the soil function plant growth was of paramount importance (Bünemann *et al.*, 2018; Kuzyakov & Zamanian, 2019). In terms of maximizing agronomic productivity to feed the growing world population, many natural soils were modified by humans to such an extent, that humanity was recently suggested as the sixth soil-forming factor (Kuzyakov & Zamanian, 2019). Increasing all the functions in an agricultural soil which are necessary to improve crop growth will inevitably change soil properties and processes and decrease other soil functions or services, as maximizing all ecosystem services simultaneously is not possible (Kibblewhite *et al.*, 2008; Powlson *et al.*, 2011; Kuzyakov & Zamanian, 2019). Thus, a trade-off for agricultural soil management has to be made between conserving all ecosystem services and optimizing agricultural yields (Powlson *et al.*, 2011). However, intensifying agricultural production should avoid irreversible soil damage and include a sustainable development (Kibblewhite *et al.*, 2008;

Powlson *et al.*, 2011), which was defined in the Brundtland report as: "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland *et al.*, 1987).

**Table 1** Ecosystem services and functions provided by soils (summarized from Costanza *et al.*, 1997; Dominati *et al.*, 2010; Powlson *et al.*, 2011; Pereira *et al.*, 2018)

<b>Ecosystem service</b>	<b>Ecosystem functions</b>	Examples		
Providing services				
Food production	Enabling plant growth	Production of crops, nuts and fruits by gathering and farming.		
Raw materials	Source of raw materials.	Resource of peat for fuel and clay for potting		
Physical support	Strength, intactness and resilience of soil structure	Physical base for human infrastructures		
Biodiversity	Providing genetic resources and habitat for species	Nurseries for beneficial insects, genetic materials for medical purposes		
Regulating services				
Climate regulation	Carbon storage and regulation of CO <sub>2</sub> , N <sub>2</sub> O and CH <sub>4</sub> emissions	SOM decomposition and denitrification		
Disturbance regulation	Responding to environmental fluctuations due to the capacity to retain water and soil within an ecosystem	Mitigating floods and droughts, prevention of soil loss by wind and runoff		
Water regulation	Storage and retention of water, regulation of hydrological flows.	Provisioning of water for agricultural or industrial processes or transportation.		
Nutrient cycling	Storage, cycling, processing and acquisition of nutrients.	Nitrogen fixation, N, P and other elemental or nutrient cycles.		
Filtering and	Filtering, immobilization or	Waste treatment, groundwater filtration,		
detoxification	transformation of nutrients or xenic compounds.	detoxification.		
Disease regulation	Controlling the proliferation of pests and harmful disease vectors	Competition between microorganisms		
<b>Cultural services</b>				
Recreation	Providing structures for recreational activities.	Eco-tourism, outdoor recreational activities.		
Cultural heritage	Storing geological and archeological heritage	Educational, spiritual and scientific values of ecosystems.		

In the context of a sustainable agriculture, soil quality (or the similar concept of soil health) is discussed as an integrative property that describes the soil's capacity to respond to agricultural intervention (Kibblewhite *et al.*, 2008; Bünemann *et al.*, 2018). Soil quality is commonly defined as "the capacity of a soil to function within ecosystem and land-use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal

health" (Doran & Parkin, 1994, 1996). In terms of a sustainable agricultural development, agricultural practices have to maintain soil quality on a sufficient level to support the agricultural production as well as the provision of other ecosystem services and have therefore to be estimated for their impacts on soil quality (Kibblewhite *et al.*, 2008; Bünemann *et al.*, 2018).

# 1.2 The importance of sustainable land use for a productive future agriculture

In the coming decades, agriculture will face a big challenge to (1) provide food security for an ever-increasing world population and (2) achieve this in an environmentally and socially sustainable way without driving further the progressing climate change and the ongoing soil degradation of the already scarce fertile agricultural areas (Godfray *et al.*, 2010; Tilman *et al.*, 2011). It is forecasted that the world population will reach 9 billion people in 2050 (Godfray *et al.*, 2010). This will result in a doubled global crop demand from 2005 to 2050, not only due to population growth but also according to changing eating habits from a more plant-based to a more animal-based diet caused by higher per capita incomes (Lal, 2008; Tilman *et al.*, 2011; Mueller *et al.*, 2012). The increasing demands for food, feed, (bio-) fuel and fiber require either larger areas for agriculture and/or an intensification of the already cultivated agricultural lands (Foley *et al.*, 2005; Kuzyakov & Zamanian, 2019).

However, the agricultural area increased by only ~9 % in the past five decades, although grain production has almost doubled (Godfray *et al.*, 2010). The increase was only minor because agricultural lands cover already large parts of the global land area (Kuzyakov & Zamanian, 2019) and compete with the remaining areas (e.g., commercial timberlands and natural ecosystems), which are needed for other important human activities and purposes (Godfray *et al.*, 2010). Nowadays, 34 % of the global land area are covered by agricultural lands (crop- and grasslands), which made up almost 50 % of the area suitable for agriculture as many areas, such as deserts, mountainous, ice-covered and settled regions, are unsuitable for agriculture (Kuzyakov & Zamanian, 2019). Because of that, a further increase in agricultural lands would be a very costly solution, especially if protecting biodiversity, climate and ecosystems services of natural ecosystems (Godfray *et al.*, 2010; Kuzyakov & Zamanian, 2019). It can thus be expected, that the future increase in agricultural production will mainly rely on intensification (Kuzyakov & Zamanian, 2019).

In the last decades, agriculture has already undergone a strong intensification by introducing and increasing the use of heavy machinery, mineral fertilizers, pesticides, irrigation and high-yielding plants (Foley et al., 2005; Kuzyakov & Zamanian, 2019). However, overuse and inappropriate land management have led to several types of soil degradation such as soil erosion, soil compaction, SOM depletion, biodiversity losses, salinization, acidification and greenhouse gas emissions (Lal, 2008, 2015; Tilman et al., 2011; Reay et al., 2012). For example, large machinery inputs increased soil compaction and erosion and thus, reduce soil structural stability and SOM (Pimentel & Burgess, 2013; Borrelli et al., 2017; Shah et al., 2017), pesticides and natural habitat destruction decreased beneficial organisms (e.g., plant pollinators) (Foley et al., 2005; Ndakidemi et al., 2016), irrigation enhanced soil salinization, affecting already > 25 % of all agricultural areas (Qadir et al., 2000) and the high fertilizer use has decreased water quality in many regions (Foley et al., 2005). Additionally, agriculture causes ~60 % of anthropogenic nitrous oxide emission (Reay et al., 2012) and ~25 % of the global anthropogenic greenhouse gas emission (Tilman et al., 2011). Today, 33 % of the global land area are affected by at least one type of soil degradation, which is detrimental to soil quality and fertility and threatens agronomic productivity (Lal, 2015).

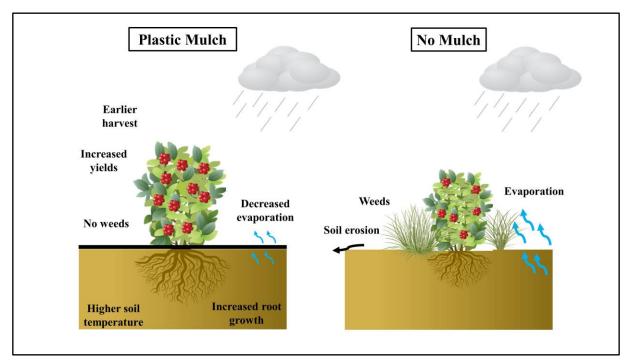
However, maintaining soil quality and fertility are pivotal to meet the future challenges in agriculture (Pimentel & Burgess, 2013; Borrelli et al., 2017; Shah et al., 2017). To this end, a 'sustainable intensification' of agricultural lands has been proposed (Mueller et al., 2012; Lindblom et al., 2017). This requires, first of all, a change in the perception of soil from solely being a medium for plant growth and raw materials (Lal, 2008) to being a provider of various ecosystem services, which are not only important for the health of humanity and adjacent ecosystems but also for the long-term sustainability of the agricultural system itself (Robertson & Swinton, 2005). Furthermore, a sustainable intensification of agriculture needs to implement new techniques, which mitigate the negative environmental impacts of an intensive agriculture and maintain ecosystem services (Oenema & Pietrzak, 2002; Morris et al., 2010; Mueller et al., 2012; Lindblom et al., 2017). Such techniques include precision agriculture, conservation tillage, high-yielding hybrids, mulching, improved nutrient management and multifunctional landscape management (Mueller et al., 2012; Lal, 2018). Additionally, a science is needed that provide a comprehensive understanding of agricultural soils and ecosystems to identify the impact of agricultural practices on soil properties and processes and hence reveal the consequences for soil quality and ecosystem services (Robertson & Swinton, 2005).

# 1.3 Plastic mulching – An expanding agricultural practice with global importance

Mulching is usually defined as covering the soil surface with different materials to improve growth conditions of crops (Prosdocimi et al., 2016; Iqbal et al., 2020). Mulching techniques such as lithic and organic mulching have a long history in agriculture for improving yields and agronomic productivity (Lightfoot, 1996; Haapala et al., 2014; Ray & Biswasi, 2016). Lithic mulching includes materials such as stones, gravel, volcanic ash, cinder and other lithic materials, which adsorb and store solar energy and reduce evaporation, surface crusting, wind velocity and water runoff, and thus improving crop growth by increasing soil temperature and moisture and reducing soil and nutrient losses by wind and water erosion (Lightfoot, 1996). However, lithic mulching is a locally confined mulching technique, which is primarily applied in arid and semiarid regions with large deposits of lithic materials (Lightfoot, 1996; Gan et al., 2013). The more common organic mulching uses cover materials such as straw, grass, bark and crop stubbles and improves crop growth and yields by reducing weed growth, evaporation, soil and nutrient losses by mitigating erosion and leaching and by increasing SOM, microbial activity and water infiltration (Haapala et al., 2014; Ray & Biswasi, 2016; Li et al., 2020a). Beside the beneficial impacts on crop growth and yields, lithic and in particular organic mulching materials have the advantage to be cheap, abundant and the application often requires only a low labor input (Lightfoot, 1996; Li et al., 2020a).

In recent decades, plastic mulching (soil covering with plastic films) has become the most important mulching technique (Ray & Biswasi, 2016; Sintim & Flury, 2017). Similar to lithic and organic mulching materials, plastic mulches influence heat transfer, gas and mass exchange between soil surface and surrounding by its optical properties and impermeability (physical barrier) for many substances (Ham *et al.*, 1993; Ham & Kluitenberg, 1994; Khan *et al.*, 2000), which increase soil temperature, reduce evaporation, prevent weed growth and mitigate soil erosion by wind and water (Figure 3) (Gan *et al.*, 2013; Steinmetz *et al.*, 2016). However, plastic mulching performs better on the aforementioned attributes than the traditional mulching techniques and thus achieve higher yields with lower water, fertilizer and pesticide inputs due to an improved water and fertilizer use efficiency and an almost complete weed suppression (e.g., Bu *et al.*, 2013; Qin *et al.*, 2015; Li *et al.*, 2018; Yang *et al.*, 2018; Gao *et al.*, 2019a). Plastic mulching is often combined with drip irrigation, which is an even more efficient practice to save irrigation water (Vázquez *et al.*, 2006), retain soil water (Berger *et al.*, 2013) and increase crop yields (Ospanbayev *et al.*, 2017; He *et al.*, 2018). Different types of plastic

mulches, such as black, white, transparent, red, blue or yellow mulches, provide a wide array of application scenarios (Tarara, 2000). For example, black mulches warm soil and hence extend growing season in temperate climates, transparent mulches are used in hot climates for soil solarization due to an extreme soil warming, white mulches are used to reduce soil temperature in the warm season by light reflection and colored mulches are used in vegetable production to repel harmful insects or attract beneficial insects (Tarara, 2000; Steinmetz et al., 2016). Polyethylene is the most frequently used material to manufacture plastic mulches (Kasirajan & Ngouajio, 2012) because of its easy processability, high durability, optimal mechanical properties and cheapness (Espí et al., 2006; Kasirajan & Ngouajio, 2012; Haapala et al., 2014). Because of the aforementioned advantages and the versatile application possibilities, plastic mulching has become a globally applied agricultural practice in recent decades (Sintim & Flury, 2017), which has largely replaced the traditional mulching techniques (Haapala et al., 2014; Ray & Biswasi, 2016). In future, the agricultural area covered with plastic mulches is expected to increase globally in the coming decade, for example, from 20 to 30 million ha in China (the largest user of plastic mulches worldwide) but also in other regions like Europe and the US (Liu et al., 2014; Sintim & Flury, 2017; Mordor Intelligence, 2020).



**Figure 3** Beneficial effects of plastic mulching on the conditions of crop growth compared to an uncovered plot (redrawn from Sintim & Flury, 2017)

# 1.4 Plastic mulching – Chance or risk for a sustainable agricultural development?

The agricultural intensification faces severe problems due to overuse and inefficiency of management practices, which have to be optimized in order to obtain a more efficient utilization of resources in terms of a 'sustainable intensification'. For example, an improved water management is necessary as global agricultural production is seriously affected by water shortage (Piao et al., 2010; Sternberg, 2011) due to limited water resources, increasing regional water shortages enhanced by climate change and an unproductive water use (30-60 % water loss) in croplands (Piao et al., 2010; Raes et al., 2012; Valipour et al., 2015). Similarly, agriculture requires an improved fertilizer use efficiency because the strongly increased fertilizer application (especially nitrogen fertilizers) during the last century resulted in large, unproductive losses into the environment (e.g., 60–70 % of applied N gets lost), impairing ecosystems and human health due to eutrophication and ozone, N<sub>2</sub>O and NO<sub>x</sub> emission (Mueller et al., 2012; Van Grinsven et al., 2013; Mohanty et al., 2020). Furthermore, soil is the largest reservoir of organic carbon in the terrestrial biosphere (Batjes, 1996; Lal, 2013) and the continuing SOM depletion of agricultural soils by tillage and erosion increases atmospheric CO<sub>2</sub> concentrations (Lal, 2004; Kirkels et al., 2014). This drives climate change and leads to warmer and more extreme climate condition, which can, for example, modify microbial ecology and host susceptibilities and thus complicate agricultural production due to increased incidence and intensity of crop infections (Tirado et al., 2010). In this context, plastic mulching can be discussed as a promising agricultural practice to achieve sustainable intensification, which improves water and fertilizer use efficiency and mitigates soil loss and thus SOM depletion by reducing wind and water erosion (Gan et al., 2013; Steinmetz et al., 2016).

However, also critical aspects of plastic mulching were increasingly reported in recent years and are briefly discussed in the following: Removing plastic mulches from the fields is labor-intensive and time-consuming and often results in an incomplete removal from fields (Kasirajan & Ngouajio, 2012; Huang *et al.*, 2020), which leads to an accumulation of plastic residues in soil because plastics are hardly degradable (Shah *et al.*, 2008). An improper and extensive use of plastic mulching was reported to contaminate soil with plastic residues of up to 325 kg ha<sup>-1</sup> of macroplastic and 1076 pieces kg<sup>-1</sup> of microplastic and let assume that plastic mulching might be a major source for microplastics in terrestrial environments (Astner *et al.*, 2019; Huang *et al.*, 2020). Plastic residues were reported to decrease yields if the plastic residues exceed 240 kg ha<sup>-1</sup> because of a reduced nutrient availability, decreased microbial

diversity, damaged soil structure and hampered root development (Gao et al., 2019a). Additionally, plastic mulches and their residues can release carcinogenic and mutagenic phthalates into soil (Wang et al., 2016; Shi et al., 2019), which might increase the risk of human exposure when taken up by food crops (Steinmetz et al., 2016) or leached into groundwater, serving as drinking waters. Further critical aspects resulted from the modified microclimate under plastic mulching, which was shown to increase microbial biomass and activity (Li et al., 2004; Yang et al., 2018), enhance labile and dissolved organic carbon (DOC) fractions (Zhou et al., 2012; Tian et al., 2013; Luo et al., 2015), shift microbial community (Buyer et al., 2010; Muñoz et al., 2015; Farmer et al., 2017) and reduce SOM (Li et al., 2004; Zhou et al., 2012; Zhang et al., 2015). This was interpreted as an enhanced microbial SOM decomposition, which point to an accelerated C-cycling, resulting in continuous SOM depletion, reduced SOM quality and increased greenhouse gas emissions (Steinmetz et al., 2016). However, it was suggested that the reported SOM losses might only occur in the short-term and could be compensated by higher (root) biomass inputs after several seasons due to an enhanced plant growth under plastic mulching (Gan et al., 2013). Furthermore, first studies showed that plastic mulching can shift the microbial community composition towards mycotoxigenic fungi and increases the concentration of deoxynivalenol in soil (Muñoz et al., 2015, 2017). Deoxynivalenol is a mycotoxin, which is biosynthesized by several fungal species of the genus Fusarium under unfavorable growth condition and are harmful to animals and humans (Murphy et al., 2006; Vanhoutte et al., 2016). Thus, plastic mulching might pose a risk for an increasing mycotoxin contamination of soils and, depending on mycotoxin fate, possibly also for crops, ground- and running waters. These reports have led to increasing concerns about the sustainability of plastic mulching, especially regarding its long-term effects on soil quality (Steinmetz et al., 2016).

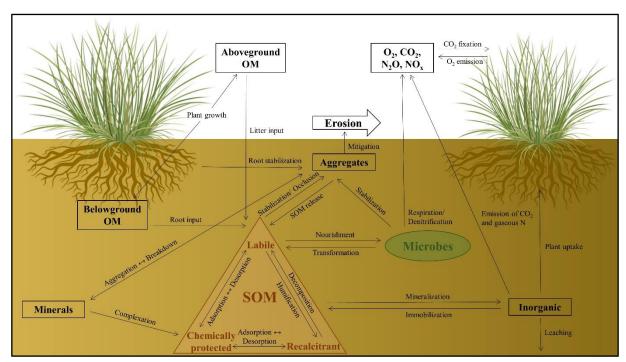
#### 1.5 Open questions

The scientific literature about the influence of plastic mulching on physicochemical and biological soil properties remains inconsistent and contradictory (Ma *et al.*, 2018), for example, with regard to microbial biomass (Moreno & Moreno, 2008; Wang *et al.*, 2014), SOM (Li *et al.*, 2007; Qin *et al.*, 2016) and aggregate stability (Tindall *et al.*, 1991; Wang *et al.*, 2017). I assumed that the different effects might result from the fact that plastic mulches are applied in various:

- mulching arrangements (e.g., ridge-furrow vs. flat, ridge or furrow coverage vs. full coverage),
- film thicknesses (e.g., 8 μm vs. 20 μm),
- periods (before maturity vs. full growing phase or annual vs. multiannual),
- agricultural practices (different types of irrigation, fertilization and pesticide treatment)
- as well as to different crops, soils and climates (Kasirajan & Ngouajio, 2012; Liu *et al.*, 2014; Ma *et al.*, 2018; Zhao *et al.*, 2018).

Furthermore, the plastic mulching application varies regionally: In China, for example, plastic mulching is mainly used to improve water (precipitation) use efficiency under water scarce conditions in arid and semiarid farmland regions (Deng et al., 2006; Gan et al., 2013; Han et al., 2014; Gao et al., 2019a). Conversely in Europe, plastic mulching is often used for premium and seasonal products such as strawberries, asparagus and other vegetables (Scarascia-Mugnozza et al., 2011; Steinmetz et al., 2016) to improve product quality and to promote earliness or extend harvest periods (Scarascia-Mugnozza et al., 2011; Neri et al., 2012). The numerous applications of plastic mulching can thus be assumed to influence soil processes in different magnitudes or directions, such as the soil processes regulating SOM, which is the most central soil property and closely interlinked with many soil properties and processes (Figure 4). In a review about plastic mulching, Steinmetz et al. (2016) concluded, that most studies about plastic mulching focused on its individual effects (mainly short-term agronomic benefits), whereas a substantial process understanding of its impact on various soil properties and processes is still missing but necessary to evaluate the impacts of plastic mulching on soil quality in the long-term. Thus, to evaluate the plastic mulching influence on soil quality and to discuss it in terms of a sustainable agricultural development it was necessary for me to contribute to the following overarching questions to improve our process understanding of the multifaceted effects of the complex cultivation system:

- Which soil processes and properties are influenced by plastic mulching under which cultivation condition?
- How strong (and in which direction) are soil processes influenced by plastic mulching under certain cultivation condition?
- Which effects can be attributed to the mulching type alone and which are possibly intensified, mitigated or caused by other cultivation factors (e.g. fertilization, irrigation, pesticides, tillage)?



**Figure 4** The main soil processes influencing the most central soil property SOM (based on information given in McLauchlan, 2006; Dignac *et al.*, 2017)

Furthermore, the scientific literature about plastic mulching (e.g., reviewed by Steinmetz et al. (2016) and Gan et al. (2013)) reveals several open questions or rather only marginally discussed topics, which have to be addressed for a comprehensive and reliable estimation of the plastic mulching impacts on soil quality:

(1) Almost no information is available about plastic mulching under the typical European cultivation systems and conditions, despite its expanding application in Europe (Mordor Intelligence, 2020). It needs to be examined whether plastic mulching under humid conditions with lower evaporation and higher precipitation rates than arid regions also strongly increases the soil moisture or whether the soil moisture is rather decreased because the water-impermeable plastic mulch impedes rainfall infiltration. This is important as changes in soil moisture are a main driver of plastic mulching effects (Gan *et al.*, 2013) and can affect SOM decomposition and aggregation and thus shift SOM composition and soil structural stability (Bronick & Lal, 2005; Dignac *et al.*, 2017). The SOM and the soil structural stability influence, for example, water and nutrient cycling, aeration, erosion and soil microorganisms and are thus essential for soil fertility and quality (Loveland & Webb, 2003; Bronick & Lal, 2005; Grego & Lagomarsino, 2008; Lal, 2015). With regard to this, I aimed to answer the following questions:

- How does plastic mulching influence the soil moisture with regard to the higher precipitation and lower evaporation rates under temperate, humid climate conditions in Central Europe compared to arid and semiarid areas?
- How does plastic mulching influence SOM decomposition and aggregation and thus the SOM quantity and composition and soil structural stability under European cultivation conditions?
- (2) Plastic mulching is often combined with fungicide application. But how the fate and effects of fungicides are affected by the plastic mulch was not investigated yet. This is important because fungicides are soil contaminants which can impair soil quality (Mendes *et al.*, 2016; Bünemann *et al.*, 2018). In a first step, I wanted to investigate how the plastic mulch influences the soil entry of selected fungicides and how the residual soil concentrations and effects of the fungicides on microbial biomass and SOM decomposition change within a time period long enough to ensure complete fungicide degradation in soil:
  - How does plastic mulching influence the fungicide fate in soil (soil entry and degradation time)?
  - Which effects have the fungicide residues in soil on microbial biomass and SOM decomposition and occurs a recovery within a certain time period?
- (3) Similar to fungicides, mycotoxins have received very little attention in plastic mulching research until yet. However, they may be relevant and possibly overlooked soil pollutants, which can influence microbial biomass and activity and hence inevitably impact on soil quality (Muñoz *et al.*, 2015; Venkatesh & Keller, 2019). Furthermore, mycotoxins are a stress indicator, indicating unfavorable growth conditions for the fungal community (Magan *et al.*, 2002; Schmidt-Heydt *et al.*, 2008; Reverberi *et al.*, 2010). Thus, I wanted to assess in a first step whether the modified soil microclimate under plastic mulching can influence mycotoxin occurrence:
  - Does plastic mulching increase the mycotoxin occurrence in soil?
  - Do the mycotoxins occur at certain stages during multiannual plastic mulching?
- (4) Most studies about plastic mulching focused only on the effects in the topsoil (0–10 cm) and after a full one-year or two-year application period. However, whether the influence of plastic mulching can also reach into deeper soil layers or can change within the temporal course of its application in dependence of season or time is still unknown. Thus, I wanted to investigate

which influence soil depth and time (continuous plastic mulching) have on the plastic mulching effects with regard to the aforementioned soil processes and properties:

- Can the effects of plastic mulching also reach subsoil layers (below 10 cm)?
- How do the potential effects change with increasing soil depth?
- Are there effects which appear only below the topsoil layer?
- Do the plastic mulching effects increase or decrease with time?
- Do some effects only occur at certain stages of the cultivation or during the seasonal cycle (e.g. establishment period of plants or summer season)?

Furthermore, agricultural practices are usually evaluated on basis of their short-term economic benefits, whereas the important ecosystem services provided by soils (beside plant growth) are often neglected (Foley et al., 2005; Dominati et al., 2010; Piñeiro et al., 2020). However, preventing soil degradation and sustaining soil quality and ecosystem services is also imperative for agricultural productivity itself and thus for the long-term sustainability of the agricultural system (Robertson & Swinton, 2005; Mueller et al., 2012; Piñeiro et al., 2020). Especially when reaching for a sustainable intensification of agriculture in the future, we need agricultural techniques that mitigate the negative environmental impacts of an intensive agriculture and maintain ecosystem services and soil quality (Oenema & Pietrzak, 2002; Morris et al., 2010; Mueller et al., 2012; Lindblom et al., 2017). Plastic mulching is known to increase yields and helps thus to feed a growing world population (Godfray et al., 2010; Qin et al., 2015; Li et al., 2018; Ma et al., 2018; Gao et al., 2019b). However, how it influences soil degradation processes (e.g., SOM depletion, erosion, nutrient leaching, soil compaction and soil contamination), which complicate a sustainable agricultural development because they reduce soil quality and ecosystem services (Powlson et al., 2011; Lal, 2015; Mendes et al., 2016; Kuzyakov & Zamanian, 2019), has not yet been evaluated. Because of this, I wanted to discuss, on basis of my findings, how plastic mulching can impact on soil degradation processes. This contributes to a more comprehensive understanding of the consequences of plastic mulching for soil quality and ecosystem services, which finally helps to enable a sustainable agricultural development.

### **CHAPTER 2**

**Objectives and Hypotheses** 

#### 2.1 Research aims and methodological approach

The main objectives of this PhD thesis were (1) to understand how multiannual plastic mulching influence soil properties and soil processes under Central European cultivation conditions (2) and to evaluate the resulting consequences for soil quality with respect to major soil functions such as carbon transformation, nutrient cycles and soil structure (Kibblewhite *et al.*, 2008). According to the aforementioned open questions, I focused on how plastic mulching influences (1) the soil microclimate, (2) the soil structural stability, (3) the quantity and quality of SOM, (4) the soil concentrations of selected fungicides and (5) the mycotoxin occurrence in soil compared to organic mulching (more traditional but still frequently used). Furthermore, I aimed to identify whether the influence of plastic mulching on the aforementioned soil attributes (6) can also reach the subsoil layers (> 10 cm) and (7) how the effects of plastic mulching vary during the temporal course of a multiannual application.

My central hypotheses were that, compared to organic mulching, (1) plastic mulching increases soil temperature and moisture by modifying water, gas and heat exchange at the soil surface, (2) the water-impermeable plastic film impedes rainfall infiltration and seepage water flows and thus mitigates nutrient depletion in soil due to reduced leaching, (3) the plastic mulch prevents aggregate breakdown in the topsoil during rainfall events and thus soil crusting and compaction, maintaining a stable soil structure, (4) plastic mulching reduces SOM in the topsoil due to an impeded entry of aboveground biomass into soil and an accelerated microbial SOM decomposition due to the higher soil temperature and moisture, (5) the reduced entry of aboveground biomass and the increased microbial SOM decomposition under plastic mulching shift the SOM composition to a more hardly degradable SOM, (6) the impermeable plastic mulch mitigates fungicide entry into soil during fungicide application and lead to smaller soil concentrations of fungicides after fungicide application, (7) the adaption of the fungal community to the modified microclimate under plastic mulching increases mycotoxin occurrence.

In order to evaluate the hypotheses, I designed a field experiment that enabled to compare the two different coverage types (plastic vs. organic mulch) but had otherwise the same soil and crop type, agricultural treatment (irrigation, pesticides and fertilizers) and management history and furthermore reflected the current agricultural practice in Central Europe under temperate, humid climate. This experiment design enabled me to study exclusively the effects of the plastic mulch on soil properties and processes (compared to organic mulch) in a homogeneous soil without masking of treatment effects by landscape variation, edge effects and management

factors. The field experiment compared plastic mulch (black polyethylene, 50 µm) in a typical application scenario (ridge-furrow system combined with subsurface drip irrigation) to the same system covered with wheat straw as organic mulch in a multiannual strawberry cultivation system for three years (July 2016 until July 2019). In the organic mulched area, the ridges were covered with straw before the first harvest in April 2017 (as usual in strawberry cultivation) and have been left uncovered until then. The three-year sampling period included the complete growth period of the perennial strawberry plant from planting (establishment of the system) until exchange (re-planting). Beside the topsoil layer (0–10 cm), where usually the largest mulching effects can be expected, I also investigated the root layer (10–30 cm), referring to the main root zone of strawberry plant, and the subsoil layer below root and plough zone (30–60 cm). In order to emphasize that I focused exclusively on the effects of the two different coverage types, henceforth the terms plastic coverage (PC) and straw coverage (SC) were used.

I chose a commercial farmland area with strawberry cultivation as experiment site because (1) strawberry cultivation is a frequent and economically relevant example for the application of plastic mulching in Central Europe, (2) it commonly uses year-round, multiannual plastic mulching for the perennial strawberry plants and (3) the current agricultural practices can be conducted by a professional strawberry farmer. Before the experiment period, a raster sampling was conducted on the field to identify potential gradients and inhomogeneities of soil properties that may interfere with the experiment design. The raster sampling was conducted in May 2016 after tillage and fertilization (April 2016) but before setting up the ridge-furrow system with subsurface drip irrigation and soil coverage at the ridges (late-June 2016) and strawberry planting (mid-July 2016) and indicated no gradients or inhomogeneities of soil properties. I expected that the strawberry establishment period with a fast strawberry growth from bare-root plants to full-grown plants and the lingering consequences of the field set-up (tillage, fertilization and establishing the ridge-furrow system) might induce initial, transient effects on soil properties and processes. Because of this, the first three soil samplings were conducted in short time intervals (two months) on 25 July 2016 (T0), 26 September 2016 (T1) and 29 November (T2). Afterwards, five further samplings were conducted in extended time intervals (≥ six months) on 25 April 2017 (T3), 9 October 2017 (T6), 3 May 2018 (T7), 11 October 2018 (T8) and 23 July 2019 (T9) to assess the mulching effects on soil during the three-year sampling period. To estimate the residual concentrations and effects of fungicides in soil, two additional soil samplings were conducted on 19 June (T4) and 18 July (T5) in 2017 after the fungicide treatments with cyprodinil, fludioxonil and fenhexamid (usually applied in strawberry cultivation) in May/June 2017. Thus, soil concentrations of the fungicides could be measured one month prior to fungicide treatments (T3) and respectively one week, five weeks and four months afterwards (T4, T5 and T6).

To assess the soil properties and processes that influence the major soil functions and thus soil quality, I used soil parameters which were appropriate as fast and sensitive indicators for the usually slow changes in soils due to land use and management and also met the practical requirements such as costs, reliability and ease of determination (Kibblewhite *et al.*, 2008; Powlson *et al.*, 2011; Bünemann *et al.*, 2018). Soil temperature and moisture were hourly tracked with a field station to investigate the microclimate in soil. I used pH, electrical conductivity and total nitrogen to assess nutrient status and bulk density, macroaggregate fraction and stability and pore size distribution to determine soil structural stability. The SOM pool was estimated with soil organic carbon, DOC and active, intermediate and passive SOM pools, while soil microorganisms were assessed with microbial biomass carbon and nitrogen, ergosterol (proxy for fungal biomass) and elemental and eco-physiological ratios. Furthermore, I determined the soil concentrations of the *Fusarium* mycotoxins deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN) and the fungicides fludioxonil, cyprodinil and fenhexamid as potential soil contaminants.

#### 2.2 Structure of the PhD thesis

The results of the three-year field experiment were subdivided and presented in four subtopics:

In Chapter 3, the effects of PC on soil properties and processes in the strawberry establishment period were presented and discussed, compared to the uncovered plots (NC). My main assumption was that the PC increases soil temperature and moisture compared to NC, leading to an enhanced microbial growth and hence to changes the amount and composition of SOM. Furthermore, I assumed that the impeded rainfall infiltration under PC reduces aggregate breakdown (in the surface soil) and nutrient leaching.

In Chapter 4, the effects of PC and SC on fungicide entry and the soil concentrations of the fungicides were shown. Furthermore, I described and discussed how long and large the consequences of the fungicide concentrations in soil were for soil microorganisms and microbial-mediated processes like SOM decomposition. My main assumption was that the impermeable PC reduces fungicide entry into soil and had thus a smaller and more transient effect on soil fungi and hence on SOM decomposition and mycotoxin occurrence.

The Chapter 5 represents the main part of the study and discusses the influence of the continuous, three-year plastic mulching application compared to SC on aggregation, nutrient leaching, SOM transformation and microbial growth in dependence of soil depth and time. I hypothesized that the water-impermeable PC impedes rainfall infiltration and raindrop impact at the soil surface and thus reduces nutrient leaching and soil compaction due to mitigated seepage water flows and aggregate slaking and dispersion, respectively. The increased soil temperature and moisture under PC was expected to promote growth of roots and microorganisms and thus macroaggregate formation and stability. Furthermore, the impeded aboveground SOM entry under PC together with a temperature- and moisture-induced larger microbial SOM decomposition was expected to reduce SOM and shift SOM composition to a more hardly degradable SOM.

In Chapter 6, I discussed how PC and SC influenced microbial community, soil fungi and mycotoxin occurrence during the three-year sampling period. I expected that the modified microclimate under PC changes microbial community by favoring fungal growth and leads to a higher mycotoxin occurrence due to adaption processes of the soil fungi to competition and the changed growth condition.

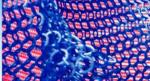
### **CHAPTER 3**

Analysis of biogeochemical processes in plastic-covered soil during establishment period in strawberry cultivation

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2020): SN Applied Sciences. 2(10): 1-16







Research Article

# Analysis of biogeochemical processes in plastic-covered soil during establishment period in strawberry cultivation



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Received: 2 April 2020 / Accepted: 16 September 2020 © The Author(s) 2020 OPEN

#### **Abstract**

Plastic mulching (PM) has become a widely applied agricultural practice to optimize plant growth. However, it is still under debate how PM influences biogeochemical soil processes and thus important factors of soil quality, such as soil organic matter (SOM) composition, aggregate stability and microbial biomass. Our objective was to identify the impact of PM on biogeochemical soil processes. Therefore, we compared a plastic-covered strawberry cultivation system (PC) with an uncovered system (NC) in three soil layers (0–10, 10–30 and 30–60 cm) at three dates during a 4-month period of strawberry establishment from their transplanting in summer to the beginning of winter. The PC shifted the microclimate of the soil towards higher temperatures but lower moistures in the 0–35 cm soil layer compared to uncovered soil. Predominantly in the surface layer, the PC reduces leaching processes, which can improve nutrient (fertilizer) use efficiency. PC increased SOM and shifted SOM to a more stable SOM. The higher SOM under PC despite larger microbial biomass and elevated temperatures, indicate that belowground biomass inputs compensate the potential SOM losses by an enhanced SOM decomposition under PC. We demonstrated that PC influenced soil processes already within the 4-month period of strawberry establishment, partially down to the 30–60 soil layer. Further, long-term studies are required to estimate the influence of multi-annual PM application on biogeochemical soil processes and on soil quality.

Keywords Plastic mulching · Strawberry cultivation · Soil aggregates · Soil organic matter · Soil microbial biomass

<b>Abbrevia</b>	tions	BD	Bulk density
PM	Plastic mulching	WSA	Water-stable aggregates
SOM	Soil organic matter	MBC	Microbial biomass carbon
SOC	Soil organic carbon	MBN	Microbial biomass nitrogen
PC	Plastic-covered ridge-furrow system with	DOC	Dissolved organic carbon
	subsurface drip irrigation	C:N ratio	Carbon-to-nitrogen ratio
NC	Uncovered ridge-furrow system with subsur-	frSOM	Free soil organic matter
	face drip irrigation	oSOM	Aggregate occluded soil organic matter
CEC	Cation exchange capacity	aSOM	Mineral associated soil organic matter
<b>ICP-OES</b>	Inductively coupled plasma-optical emission	LFOM	Light fraction organic matter
	spectrometry	<b>HFOM</b>	Heavy fraction organic matter
EC	Electrical conductivity		
TN	Total nitrogen		

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s42452-020-03528-x) contains supplementary material, which is available to authorized users.

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SN Applied Sciences (2020) 2:1749 | https://c

https://doi.org/10.1007/s42452-020-03528-x

Published online: 30 September 2020

#### 1 Introduction

Plastic mulching (PM) has become a widely applied practice in agriculture [30]. By increasing soil temperature and reducing soil water evaporation PM can lead to higher yields, earlier harvests and improved product quality, which offers important economic benefits for farmers. Furthermore, PM can suppress weed growth and reduce fertilizer leaching, which in turn allows reducing herbicide and fertilizer application. In recent years, PM has also been associated with reduced soil organic matter (SOM) contents, entry of plastic waste and plasticizers into soil and shifts in microbial composition towards mycotoxigenic fungi (reviewed in Steinmetz et al. [58]). This has raised concerns regarding the sustainability of PM application, which is, particularly in Central Europe, increasingly used for out of season products, such as strawberries [42].

In strawberry cultivation in natural soil, strawberry plants are usually transplanted as seedlings into readily fertilized and drip-irrigated plastic-covered raised beds (ridge-furrow systems) during summer [42]. Plant development and establishment of the appropriate field structure, including tillage, fertilization, drip irrigation and building the ridge-furrow system can impact on various soil processes and parameters, such as aggregate formation, SOM decomposition and microbial biomass [36, 57]. Additionally, the PM modify the soil microclimate and can influence hence various soil processes in the establishment phase of strawberries: For example, the soil temperature is elevated compared to uncovered areas [19], which can increase plant growth [34, 72] as well as microbial growth and activity [5, 29]. This can increase root growth and root and microbial exudation, which might increase SOM stocks and promote microand macroaggregate formation [36]. On the other hand, an enhanced microbial activity and growth can promote SOM decomposition and thus nutrient release [7, 14]. The impermeable plastic cover can alter soil moisture by impeding evaporation and rainfall infiltration [26, 38] and thus influence plant growth, microbial activity and growth [7, 57]. Additionally, the impeded rainfall infiltration avoids excess water [38], which can reduce nutrient leaching [59] and aggregate breakdown caused by slaking and the mechanical impact of raindrops [36]. The PM impedes entry of aboveground biomass into soil and thus might reduce SOM stocks.

However, the partially inconsistent observations of PM influence on soil, reported e.g. for microbial biomass [47, 67], SOM [41, 51] and aggregate stability [60, 65], suggest that underlying processes are still not fully understood. Probably because the PM impact on

biogeochemical processes also depend on season and soil depth, as the plastic-cover-induced changes in soil temperature and moisture decrease with soil depth [15, 22] and with season change from summer to late-autumn [34, 70]. Additionally, PM is applied in various arrangements and periods and is often compared to other management systems, which differ not only in coverage but also in tillage, irrigation, fertilization and pesticide treatment [39, 41, 48, 74]. Thus, to identify the effects of plastic coverage alone in various soil depths and during a seasonal course will help to understand which soil processes predominate under certain conditions and allow a better estimation of PM impact on soil quality.

Because of that, our objective was to investigate the soil processes under plastic coverage, influencing SOM quality, aggregate stability and microbial biomass during plant establishment period in strawberry cultivation. We compared a plastic-covered ridge-furrow system with subsurface drip irrigation (PC) to the same system without plastic coverage (NC) in three soil layers (0-10, 10-30 and 30-60 cm) at three dates within a 4-month period of strawberry establishment after field set-up and transplantation of seedlings. Our hypotheses were: (1) The PC increase the soil temperature and moisture in the establishment phase of strawberries but with declining degree with soil depth and season change from summer to late-autumn. (2) These shifts in the soils microclimate enhance plant and microbial growth and hence changes amount and composition of SOM towards larger contents of microbial biomass and SOM as well as larger carbon-to-nitrogen ratios (C:N ratios) due to SOM input. However, we also expect lower contents of dissolved organic carbon (DOC) and free SOM (frSOM) due to enhanced microbial activity. (3) Additionally, the impeded rainfall infiltration by PC reduces waterstable aggregate (WSA) breakdown and nutrient leaching, leading to higher WSA, aggregate occluded SOM (oSOM), nitrogen content and electrical conductivity (EC) under PC. In order to investigate these hypotheses, we designed a semi-controlled field experiment that reflected current agricultural practice while enabling us to study soil processes in a homogeneous soil type and avoiding masking of treatments effects by landscape variation and edge effects.

#### 2 Material and methods

#### 2.1 Site description

The sampling site, a commercial strawberry field, is located near 'Offenbach an der Queich' in Southwestern Germany (49° 11′ N, 8° 10′ E, 130 m a.s.l.) in a temperate, humid

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climate with an annual average rainfall of 643 mm  $a^{-1}$ (Weather station of Landau-Wollmesheim, Agrarmeteorologie Rheinland-Pfalz). Soil texture consisted of  $7 \pm 2\%$ sand,  $83 \pm 5\%$  silt and  $10 \pm 3\%$  clay in the 0–60 cm soil layer and was analyzed via hydrometer method [4]. The soil type was a silty loam (Anthrosol) according to FAO soil classification [24]. In April 2016, the field, cultivated with winter wheat in the previous season, was tilled and fertilized with 800 kg ha<sup>-1</sup> of an organic fertilizer, consisting mainly of barley malt culms and sugar beet components (MALTaflor®, Maltaflor Europa GmbH, Andernach, Germany) and a mineral fertilizer (15 kg N, 5 kg P, 30 kg K, 2 kg Mg). In late-June 2016, a ridge-furrow system was established with subsurface drip irrigation and black plastic film covered ridges (Polyethylene, 50 µm) and bare furrows. In mid-July 2016, the strawberries (*Fragaria x ananassa*, 'Malwina') were transplanted in double rows in the ridges with 40 cm distance between plants (8 plants per m<sup>2</sup>).

#### 2.2 Experimental design and soil sampling

For the semi-controlled field experiment with the same soil type, crop type and agricultural treatment (apart from coverage type) a raster sampling was conducted in May 2016 to identify potential gradients and inhomogeneities of soil properties that may interfere with our experiment design (Table S1-S40, Online Resource).

For both treatments (PC and NC), we selected a treatment area (21×10 m), consisting of five ridges (and furrows) aligned in north-south direction. In both treatment areas, five plots (10×1.5 m) were randomly chosen for soil sampling: PC (n=5) and NC (n=5). Three soil samplings were conducted during the establishment phase of strawberries in 2016: 1 week after transplanting the strawberries on 25 July (T0) and two (T1) and four (T2) months later, respectively (on 26 September and 29 November).

During the sampling period, no manual weed control was necessary and no pesticides or fertilizers were applied. Subsurface drip irrigation (three emitters per meter) was applied identically in both treatment areas and took place from strawberry transplantation in July until mid-October. The field was irrigated depending on weather conditions for 3–4 h, resulting in 7–11 L water per meter.

At each sampling date, a composite sample was taken in the ridges from three selected soil layers (0-10, 10-30 and 30-60 cm) in each of the randomly-selected plots in both treatment areas. Each composite soil sample consists of soil from five single cores. Beside the surface soil layer (0–10 cm), which was investigated in most former studies as the largest impact of PM was expected there (e.g. [47, 73]), we additionally included the root layer, which refers to the main root zone of the strawberries (10-30 cm), and the subsoil layer below the root zone (30-60 cm)

to differentiate between potential depth-dependent PC impacts. The surface soil samples were collected with stainless steel soil sampling rings (d=5 cm, h=5 cm), whereas the sub-surface soil samples (> 10 cm) were collected with a boring rod. Composite soil samples were homogenized in a bucket, filled in labelled plastic bags and stored at 4 °C for further analyses.

All analyses with field-fresh soil were conducted directly after sampling. For all other analyses, the soil was either air-dried for 14 days at room temperature, sieved (< 2 mm) and stored in plastic bags at room temperature or ovendried at 105 °C for 24 h, sieved (< 2 mm) and milled (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany) and stored in closed centrifuge tubes. All analyses with air- and oven-dried soil were performed within 2–4 months after sampling.

#### 2.3 Soil temperature and moisture

https://doi.org/10.1007/s42452-020-03528-x

Soil temperature and moisture were recorded hourly in both treatments by a measuring station (ecoTech®, Bonn, Germany) at the soil depths 5, 15 and 35 cm, according to the three soil layers selected for soil analysis. Therefore, one sensor was installed at each soil depth in both treatments (in total: six sensors). Air temperature and precipitation data were obtained from the weather station Landau-Wollmesheim (Agrarmeteorologie RLP).

#### 2.4 Soil characterization

The cation exchange capacity (CEC) was determined according to DIN ISO 11260:1997-05. In brief, 3 g of fieldfresh soil were extracted three times with 0.1 M barium chloride solution. After a washing step with 0.0025 M barium chloride solution, the samples were extracted with 0.02 M magnesia sulfate solution. Finally, the magnesia concentration was measured in the extract with inductively coupled plasma-optical emission spectrometry (ICP-OES) (Agilent 720 Series, Thermo Fisher Scientific, Karlsruhe, Germany) and used for CEC calculation. Soil pH was determined in 0.01 M CaCl<sub>2</sub>, according to DIN EN 15933:2012-11. The EC was measured in deionized water, based on DIN CEN/TS 15937:2013-08. For pH and EC analyses, air-dried and sieved soil samples were used. Total nitrogen content (TN) was analyzed in milled, oven-dried soil with a CHNS Analyzer (vario MicroCUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany).

#### 2.5 Soil structure indicators

The bulk density (BD) and the WSA fraction are indicators to assess the influence of agricultural practices on soil structural stability and soil aeration [36, 45, 61]. Larger WSA fractions are indicative for a higher soil structural stability [36]. The WSA fraction (soil aggregates > 0.2 mm) of 1-2 mm aggregate fraction was measured with the wetsieving procedure, described in Buchmann et al. [11]. In brief, 10 g of 1–2 mm aggregates (M<sub>total</sub>) were submerged in deionized water on a 0.2 mm sieve for 10 min. Then, 20 manual 5 cm oscillations in deionized water were conducted within 1 min and the remnants on the sieve were dried at 105 °C for 24 h and then weighed ( $M_{aggregates + sand}$ ). Sand fraction of soil was determined by dispersing 5 g of 1–2 mm aggregates in 20 mL sodium hexametaphosphate (0.01 M) in 50 mL centrifuge tubes within 24 h on a horizontal shaker. Subsequently, the dispersion was poured over a 63 µm sieve and the remnants on the sieve were dried at 105 °C for 24 h and then weighed (M<sub>sand</sub>). The WSA fraction was calculated with Eq. 1:

$$WSA(\%) = \frac{\left(M_{aggregates + sand} - M_{sand}\right)}{\left(M_{total} - M_{sand}\right)} \bullet 100 \tag{1}$$

Dry BD was determined according to DIN ISO 11272:2014-06. Briefly, soil cores were sampled with stainless steel soil sampling rings (d=5 cm, h=5 cm) in the 0–5 cm soil layer. The weight of each soil core was corrected by subtracting the water content, gravimetrically determined after drying at 105 °C, and finally divided by the volume of the sampling ring.

#### 2.6 Characterization of SOM

The SOC was used to quantify SOM, whereas its ratio to TN, the C:N ratio, indicates degradability and transformation velocity of SOM [32]. The soil microbial biomass was quantified by soil microbial carbon (MBC), whereas its ratio to microbial biomass nitrogen (MBN), MBC:MBN, and to SOC, MBC:SOC, were used to estimate the microbial community composition and the quantity of nutrients in microbial biomass [46]. The MBC and DOC have both rapid turnover rates and can thus serve as indicators for changes in SOM e.g. due to management practices [21]. To identify potential changes in different SOM pools, the SOM was separated depending on density into free (frSOM), aggregate occluded (oSOM) and mineral associated SOM (aSOM). According to their turnover rates, these SOM fractions are associated to the active, intermediate and passive SOM pool, respectively [64].

#### 2.6.1 Analysis of MBC and MBN

The chloroform-fumigation method was used to determine MBC and MBN, represented as the difference in C and N between fumigated and non-fumigated soil samples [6, 62]: For fumigation, 20 g of field-fresh soil were filled in

glass jars and fumigated with chloroform in an evacuated desiccator for 24 h. Afterwards, soil samples were filled in 100 mL PE bottles, 80 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution were added and shaken for 30 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany). Finally, the extraction solutions were filtered over paper filters (MN 615 1/4, Macherey-Nagel, Düren, Germany). The extraction solution of the non-fumigated soil samples was obtained by following the same procedure without the fumigation step. Carbon content in extraction solutions was determined with TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany), whereas the nitrogen content was determined with the ninhydrin method described in Joergensen and Brookes [27]: Ninhydrin reactive nitrogen was quantified photometrical at 570 nm (Specord50, Analytik Jena GmbH, Jena, Germany).

#### 2.6.2 Analysis of SOC and DOC

The SOC was measured in accordance with Harris et al. [20]. Briefly, carbonates were dissolved by fumigation of the soil samples with concentrated HCl (12 M) for 6 h. After soil drying for 4 h at 60 °C, the carbon content of the soil samples was measured with CHNS Analyzer (vario Micro-CUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany). The DOC was measured in filtrated soil extracts (0.45  $\mu m$ , 1:5 soil-to-water ratio, w/v) of field-fresh soil samples via TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany) according to DIN EN 1484:1997-05. The C:N ratios correspond to the SOC divided by the TN of the same sample.

### 2.6.3 Separation of SOM into frSOM, oSOM and aSOM fractions by density fractionation

The density fractionation was conducted in an adapted method, principally based on the methods described by Cerli et al. [13] and Ontl et al. [49]: 10 g of air-dried, sieved soil (< 2 mm) were filled in 50 mL centrifuge tubes and 30 mL of sodium polytung state solution (1.6 g cm $^{-3}$ ) were added. When the soil was completely wetted, the centrifuge tubes were turned upside-down five times. After 60 min of resting, the suspensions were centrifuged for 20 min at 5600 g (Heraeus Multifuge 4KR, Thermo Fisher Scientific, Waltham, USA). The supernatants were filtered through a 20 µm nylon filter (Carl Roth GmbH + Co.KG, Karlsruhe, Germany). The SOM remaining on the filter was cleaned with deionized water, dried at 60 °C and quantified gravimetrically (frSOM). Once again, 30 mL of sodium polytungstate solution were added to the centrifuge tubes with the soil, followed by a re-suspendation step on a vortexer  $(2 \times 2 \text{ s})$  before disrupting the soil aggregates with an ultrasonic probe (350 J mL<sup>-1</sup>) (Sonoplus HD 2070, Bandelin

https://doi.org/10.1007/s42452-020-03528-x

electronics GmbH & Co.KG, Berlin, Germany). After resting for 60 min, the suspension was centrifuged for 20 min at 5600 g and the supernatants were filtered over a 20 µm nylon filter. The SOM remaining on the filter was cleaned with deionized water, dried at 60 °C and quantified gravimetrically (oSOM). The aSOM fraction was obtained by subtracting frSOM and oSOM from the total SOM. Total SOM was obtained by multiplying the SOC values by a factor of 2 [7]. A density fractionation was conducted only at T0 and T2, because of its labor- and cost-intensity.

### 2.7 Statistical analyses

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In both treatments (PC and NC), five composite samples were taken (from five randomly- selected plots) in each soil layer at each sampling, respectively, resulting in respectively five replicates per soil layer per sampling per treatment. Mixed factorial ANOVAs with coverage time (sampling) and soil layer as repeated factors and treatment as fixed factor were performed to determine significant differences between means. If significant interaction effects were occurring, additional ANOVAs, with least significance distance (LSD) testing as post-hoc test, were applied to identify significant differences. Differences were reported as statistically significant if the probability of error was < 0.05. Variance homogeneity was confirmed with Levene's test. Normality distribution was examined graphically, using histograms and quantile-quantile plots. Correlation of two variables was estimated with Pearson's correlation coefficient or Spearman's p if the data were not normally distributed. All statistical analyses were conducted with IBM SPSS Statistics 23 and Microsoft Excel 2010.

### 3 Results

### 3.1 Soil temperature and moisture

The daily mean soil temperature under PC and NC, corresponding to each soil depth (5, 15 and 35 cm), and the mean ambient temperature (2 m above ground) are shown in Fig. 1. In both treatments, the soil temperature profile followed that of the ambient temperature and was mostly higher than the ambient temperature. The soil temperature and moisture data exhibits a data gap from July to mid-August, due to technical malfunction of the measuring station. During the observed time period, 12 August–November, the soil temperature was always higher under PC than under NC (Table S41, Online Resource). This effect was observed at all soil depths. In August, the monthly mean soil temperature at 5, 15 and 35 cm soil depth was by  $2.0 \pm 1.4$ ,  $1.4 \pm 0.5$  and  $0.7 \pm 0.4$  °C higher under PC than under NC. In both treatments, soil temperature was highest in the surface soil and decreased with soil depth in August and the first half of September. From October on, the opposite effect, an increasing temperature with soil depth, was observed. Differences in soil temperature between PC and NC decreased with soil depth and from August until November. Largest soil temperature differences between PC and NC occurred at 5 cm soil depth and were up to 6 °C at midday on sunny days in August. The maximum temperature under PC reached 34.2 °C at 5 cm soil depth and it coincided with the highest ambient temperature.

Soil moisture (Fig. 2) was lower under PC compared with NC at all soil depths over the observed timed period (exception: 15 cm soil depth at the end of October/ November), with differences of up to 11%. In general, soil moisture increased with soil depth in both treatments (exception; 15 cm soil depth under NC at the end of October/November). Precipitation events were followed by a direct increase in soil moisture at all soil depths of both treatments. These increases in soil moisture were considerably larger under NC than under PC, especially at both upper soil depths.

### 3.2 Physicochemical soil properties

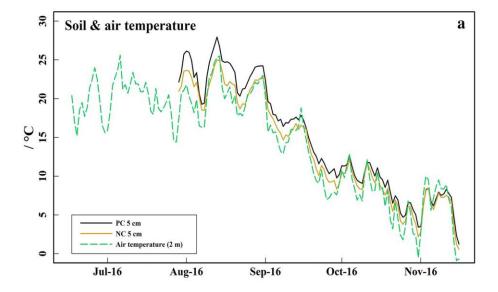
The CEC at T0 was  $1064 \pm 52$ ,  $1059 \pm 33$  and  $1071 \pm 36 \,\mathrm{mmol\,kg^{-1}}$  under PC and  $973 \pm 19$ ,  $1019 \pm 62$  and  $1026 \pm 20 \text{ mmol kg}^{-1}$  under NC, respectively in the 0–10, 10-30 and 30-60 cm soil layers.

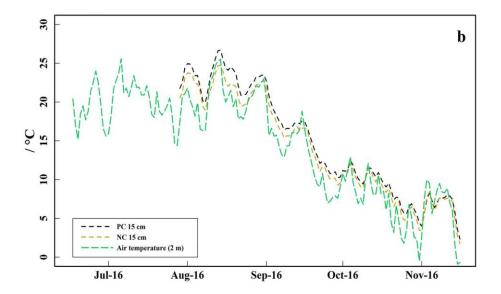
At T0 (late-July, 1 week after strawberry transplanting), pH (Fig. 3a) varied between 7.7 and 7.9, decreased to 7.5–7.6 at T1 (late-September) and remained constant until T2 (late-November). During the sampling period, the pH showed no clear dependence on soil depth or cover type.

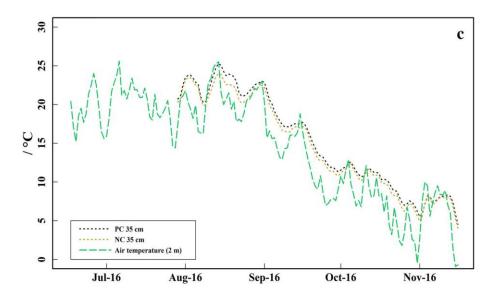
Compared to PC, the EC (Fig. 3b) under NC was higher at T0 and T1 and significantly lower at T2 (p = 0.016) in the 0-10 cm soil layer. In both treatments, the EC in the 0-10 cm soil layer was higher at T0 and T1 (significantly in NC: p < 0.016) and lower at T2 (significantly in NC; p < 0.002) compared to both sub-surface soil layers. During the sampling period, the EC dropped in the 0-10 cm soil layer of both treatments (significantly in NC: p = 0.001). In both sub-surface soil layers, the EC ranged between 152–174 μS cm<sup>-1</sup> and showed no differences between treatment, soil depth and sampling time.

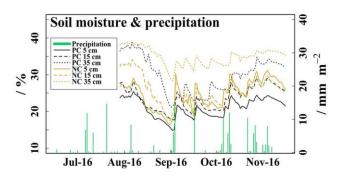
The TN (Fig. 3c) ranged between 0.07-0.14% and showed a trend towards slightly higher TN under PC compared to NC in all soil layers at T1 and T2 (exception: 30-60 cm soil layer at T2). In both treatments, the TN decreased significantly during the sampling period (p < 0.001). This decrease was stronger from T1 to T2 than from T0 to T1. In both treatments, the TN in the 30-60 cm **SN Applied Sciences** 

Fig. 1 Soil and air temperature a-c Daily mean soil temperature in strawberry cultivation, measured at 5, 15 and 35 cm soil depth under plastic coverage (PC) and no coverage (NC), respectively, and daily mean air temperature measured 2 m above ground. The data gap in soil temperature from July to mid-August was due to a technical malfunction of the measuring station









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Fig. 2 Daily mean soil moisture in strawberry cultivation, measured at 5, 15 and 35 cm soil depth under plastic coverage (PC) and no coverage (NC), respectively, and daily precipitation. The data gap in soil moisture from July to mid-August was due to a technical malfunction of the measuring station

soil layer was significantly lower than in both soil layers above (p < 0.005).

### 3.3 Soil structure indicators

The BD in the 0–5 cm soil layer was  $1.23 \pm 0.10$ ,  $1.21 \pm 0.02$ and  $1.25 \pm 0.05$  g cm<sup>-3</sup> under PC and  $1.18 \pm 0.06$ ,  $1.23 \pm 0.06$  and  $1.12 \pm 0.05$  g cm<sup>-3</sup> under NC at T0, T1 and

T2, respectively. The BD results showed only small variation between treatment and sampling time. Only at T2, the BD was significantly higher under PC compared to NC (p=0.049).

Higher WSA fractions (Fig. 3d) were measured under NC at T0 and T1 than under PC, whereas at T2 no differences were observed. In both treatments, the WSA fractions showed a similar pattern: they increased from 30-41% at T0 to 40-51% at T1 and decreased afterwards to 28-32% at T2. Overall, no effects of the soil layers were observable in the WSA fractions.

### 3.4 Soil organic matter characteristics

#### 3.4.1 Soil microbial biomass characteristics

The MBC (Fig. 4a) ranged between 63.9–510.4 mg kg<sup>-1</sup>. At T0 and T1, MBC was lower under PC than under NC (significantly at 10–30 and 30–60 cm, p < 0.028), whereas at T2, the opposite was observed. In both treatments, MBC increased significantly from T0 to T1 (p < 0.001). In PC, the MBC increased further from T1 to T2, whereas the MBC decreased in NC. The strongest increase in MBC under PC was found in the 10-30 cm soil layer. In both treatments,

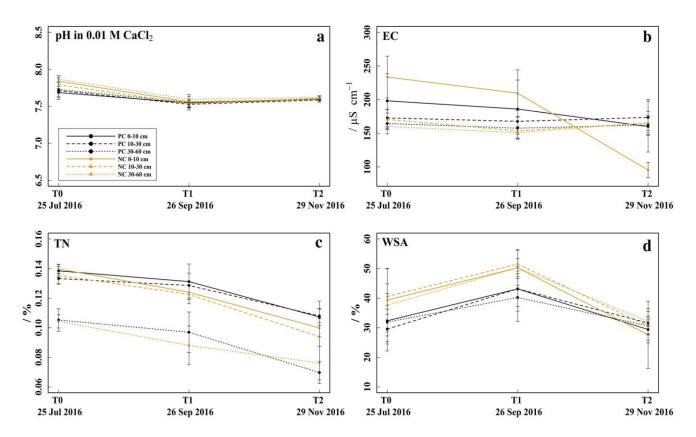
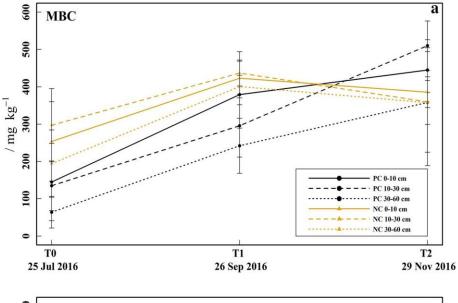
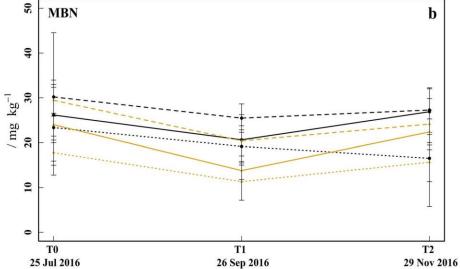


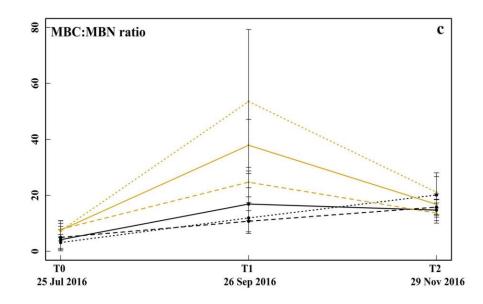
Fig. 3 Physicochemical soil properties a pH (in 0.01 M CaCl<sub>2</sub>) determined immediately after strawberry plantation (T0), 2 months (T1) and 4 months (T2) in the 0-10, 10-30 and 30-60 cm soil layer

under plastic coverage (PC) and no coverage (NC), respectively, shown as mean with standard deviation (n = 5). b Electrical conductivity (EC), c total nitrogen (TN), d water-stable aggregates (WSA)

Fig. 4 Soil microbial biomass a Soil microbial biomass carbon (MBC) determined immediately after strawberry plantation (T0), 2 months (T1) and 4 months (T2) in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and no coverage (NC), respectively, shown as mean with standard deviation (n=5). b Soil microbial biomass nitrogen (MBN), c MBC:MBN ratio







the lowest values for MBC were measured in the 30–60 cm soil layer.

The MBN (Fig. 4b) ranged between  $11.3-30.2 \text{ mg kg}^{-1}$  and was significantly higher under PC compared to NC (p=0.002). Generally, MBN decreased from T0 to T1 and increased again from T1 to T2 in both treatments. With respect to all other soil layers, MBN was highest in the root layer of both treatments.

The MBC:MBN ratios (Fig. 4c) ranged between 3.1–53.5. At T1, the MBC:MBN ratios were lower under PC compared with NC, whereas no clear differences were observed between treatments at T0 and T2. Under PC, MBC:MBN ratios became significantly wider from T0 to T2 (p=0.013). Under NC, MBC:MBN ratios showed a significant increase (p=0.003) from T0 to T1, whereas from T1 to T2, the ratios dropped again significantly (p=0.014) but were still larger than at T0. The C:N ratios correlated positively with the MBC:MBN ratios (r=0.291, p=0.005).

### 3.4.2 SOC, DOC, C:N and MBC:SOC ratio

The SOC (Fig. 5a) ranged between 0.84–1.16% and showed slightly higher values under PC compared to NC. From T0 to T1, the SOC increased significantly in all soil layers in

both treatments (p < 0.001), whereas from T1 to T2, the SOC remained nearly constant. In both treatments, SOC was significantly lower in the 30–60 cm soil layer compared with both soil layers above (p < 0.005).

At T2, the highest DOC value (26.9 mg kg $^{-1}$ ) was found in the 0–10 cm soil layer under NC (Fig. 5b), which differed significantly from the PC treatment (p < 0.001). Under NC, the DOC increased in both upper soil layers during the sampling period, significantly in the 0–10 cm soil layer from T1 to T2 (p < 0.001), whereas in the 30–60 cm soil layer, there was a strong decrease in DOC from T0 to T1, followed by an increase back to the initial level from T1 to T2. Under PC, only small fluctuations on DOC levels were observed.

No differences were observed in the C:N ratios between both treatments (Fig. 5c). At T0, the values ranged between 7.1–8.7 and increased significantly during the sampling period to 10.6–15.1 at T2 (p = 0.001). In both treatments, the C:N ratios increased with soil depth, however, not significant.

The MBC:SOC ratios (Fig. 5d) ranged between 0.7–4.4%. At T0 and T1, the MBC:SOC ratios were lower under PC compared with NC, whereas the MBC:SOC ratios were slightly higher under PC compared with NC at T2 (exception: 30–60 cm soil layer). In both treatments, the MBC:SOC

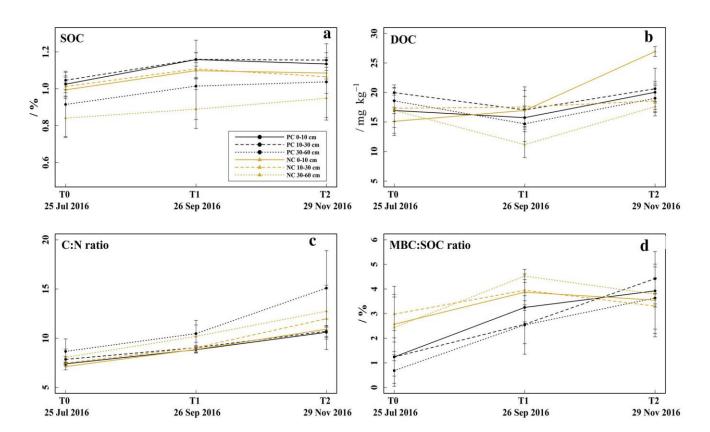


Fig. 5 Soil organic matter **a** Soil organic carbon (SOC) determined immediately after strawberry plantation (T0), 2 months (T1) and 4 months (T2) in the 0–10, 10–30 and 30–60 cm soil layer under

plastic coverage (PC) and no coverage (NC), respectively, shown as mean with standard deviation (n=5). **b** Dissolved organic carbon (DOC), **c** C:N ratio, **d** MBC:SOC ratio

ratios increased from T0 to T2 (significantly under PC:

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### 3.4.3 SOM fractions

The values for frSOM, oSOM and aSOM fractions, separated by density fractionation, ranged between 1.0-3.7, 1.1–7.2 and 10.4–19.5 g  $kg^{-1}$ , respectively (Fig. 6a–c). The aSOM fraction showed a slight impact of treatment with the highest values found under PC at T2 (significant in the 10–30 cm soil layer: p = 0.017). In both treatments, the aSOM fractions increased significantly from T0 to T2 (p < 0.001), whereas the frSOM and oSOM fractions decreased significantly from T0 to T2 (p < 0.004).

### 4 Discussion

### 4.1 Soil temperature and moisture

Our results corroborate the well described increase of soil temperature under PC compared to NC (e.g. [70]) as well as the decreasing influence of PC on soil temperature with increasing soil depth [37, 52]. Former studies mostly focused on the impact of PM on the surface soil layer (e.g. [47, 73]) but we found temperature increases even at 35 cm soil depth under PC compared to NC. This confirmed our assumption that also deeper soil layers should be included, when investigating the influence of PC on soil processes, especially as soil temperature is seen as a main driver of PC-effects on soil (e.g. [19]). Differences in soil temperature decreased during the sampling period from July to November and revealed hence a strong seasonal effect.

Because PC reduces evaporation, it is often used to increase the water content in soil, especially in arid and semiarid climates (e.g. [40, 70]). In contrast to this, we found lower soil moisture under PC than in the uncovered soil. Similarly, Schirmel et al. [54] reported lower soil moisture under PC compared to organic mulching in strawberry cultivation in southwestern Germany. We attributed this mainly to additional water inputs from rainfall events. In NC treatment, precipitation water infiltrates the soil directly as observed by increasing soil moisture after rainfalls (Fig. 2). Under PC, only a fraction of the rainfalls reaches the soil, presumably through lateral water flow from the furrows induced by lateral pressure heads [53], resulting in a lower increase of soil moisture after rainfalls. Furthermore, higher transpiration rates of the plants under PC due to higher soil temperatures [67] might also affect differences in soil moisture between treatments. We conclude from our results that the reduced water infiltration after rainfalls (and the higher transpiration losses of the plants) under PC overbalances the water saving effect of PC due to mitigated evaporation in our study. Our soil temperature and moisture results confirm our basic assumption that PC shifts soil temperature and moisture and thus, the microclimate in the establishment phase of the strawberries, although the reduced soil moisture was the opposite of the expected direction.

### 4.2 Effects on leaching processes

The lower EC decrease under PC compared to NC during the sampling period in the surface soil layer (0–10 cm) indicates that PC can reduce leaching processes by impeding rainfall infiltration as displayed by the lower soil moisture under PC. The slight EC drop under PC between late-July and late-November (T0-T2) in the surface soil layer can be attributed to the nutrient uptake of the growing strawberry plants [34]. The larger EC drop under NC between late-July and late-November occurred likely

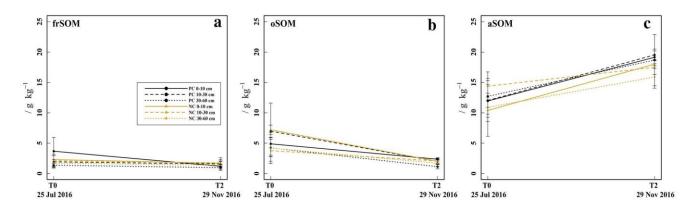


Fig. 6 Soil organic matter fractions separated by density fractionation. a Free soil organic matter (frSOM) determined immediately after strawberry plantation (T0) and 4 months (T2) in the 0-10, 10-30 and 30-60 cm soil layer under plastic coverage (PC) and no

coverage (NC), respectively, shown as mean with standard deviation (n=5). **b** Aggregate occluded soil organic matter (oSOM). **c** Mineral associated soil organic matter (aSOM)

due to an increased leaching caused by larger rainfall amounts and an increasing water saturation of the soil. Reduced leaching under PC is additionally supported by the smaller TN reduction under PC compared to NC during the sampling period in both upper soil layers. We attribute the TN decrease in both treatments, during the establishment phase of the strawberries to plant uptake of nitrogen and microbial mediated denitrification processes [7, 67], whereas the difference in TN between treatments in late-September (T1) and late-November (T2) presumably occurred due to reduced nitrogen leaching and impeded ammonia volatilization under PC [67]. The higher TN value under NC in the 30-60 cm soil layer in late-November might be interpreted as an accumulation of nitrogen leachate in the subsoil. The higher TN in both upper soil layers under PC indicate that the reduced nitrogen leaching under PC can overbalance the expected higher nitrogen loss under PC by increased plant uptake and larger microbial denitrification processes (higher MBC) [34, 67]. The differences in leaching between both treatments did not influence the pH. The slight drop in soil pH from July to September (T0–T1) can be attributed to the H<sup>+</sup> entry of the growing strawberry plants, induced by their root exudation and respiration [7]. Generally, these reduced leaching processes can point to a better nutrient (fertilizer) use efficiency under PC, which can reduce the applied amounts of mineral fertilizers, and potentially mitigate groundwater contamination with nitrate.

### 4.3 Effects on soil aggregation

Plastic coverage can impede the mechanical impact of raindrops at soil surface as well as aggregate slaking after rainfalls [31]. Therefore, we hypothesized that this will increase the WSA fraction in the surface layer, however, this was not confirmed at all by our results. Possibly the fast-growing strawberry leave canopy strongly mitigated the mechanical impact of rainfall events on soil surface. Unexpectedly, we found larger WSA fractions under NC compared to PC in late-July (T0). As microbial exudates are known to promote aggregate formation [36], the also higher MBC under NC at this time might have caused the larger WSA fractions (reasons for the differences in MBC discussed in microbiology section). These differences between PC and NC treatment in WSA fractions were still observable in late-September. We attributed the general increase of the WSA fractions in both treatments from late-July to late-September to the mechanical impact and the root exudation of growing strawberry roots, the increased microbial exudation induced by higher microbiological activity, and the decreased soil moisture [7, 36]. We assume that in the colder and wetter season (T1 to T2), the reduced microbial and root exudation, the dying-off of the strawberry roots and the increasing soil moisture decreased the WSA fractions again. This decrease was smaller under PC and balanced out the initial differences in WSA fractions between PC and NC. We suggest that the higher temperatures under PC decelerate the root dyingoff and mitigated the reduction of root and microbial exudation which in turn might be responsible for the smaller decrease in WSA fractions. The changes in the WSA fraction seemed to have no influence on BD. We found a significantly lower BD under NC compared to PC in late-November, which we attributed to higher bioturbation induced by an increased earthworm activity under NC [54, 56] and to extended soil pores induced by moderate frost in the night before the respective sampling.

### 4.4 Soil organic matter characteristics

### 4.4.1 Effects on microbial biomass growth and composition

The MBC results confirmed our hypothesis, that altered microclimatic conditions under PC enhance microbial growth. Generally, increased MBC values mostly correspond either directly to higher soil temperature and soil moisture [3, 5, 57] or to increased root biomass and root exudation triggered by higher soil temperature and soil moisture [1, 34, 71]. Thus, we attribute the strong increase in MBC in both treatments between late-July and late-September to an increased root biomass entry of the growing strawberry plants, which is known to stimulate microbe proliferation [46]. Between late-September and late-November, the MBC still increased under PC while it dropped under NC. We link this drop to the lower temperatures (night frost) under NC, which inhibit the temperature-sensitive plant and microbial biomass growth [36, 50], whereas higher temperatures under PC still maintain plant and microbial biomass growth. Thus, PC can extend the active phase of the microbes. The lower MBC values under PC compared to NC in late-September can be interpreted as an effect of the lower soil moisture under PC but also as a relic of the initial differences between treatments. These initial differences were unexpected but according to the farmer, a possible explanation could be that during strawberry transplanting in mid-July, the field was twice irrigated with an aboveground sprinkler system with circular movement, which presumably caused an uneven irrigation pattern. Furthermore, larger MBN values under PC corroborate our hypothesis of an enhanced microbial biomass under PC. But the MBN values show a different pattern compared with MBC values and lead to increasing MBC:MBN ratios from late-July to late-November in both treatments, which are indicative of larger fungal fractions in the microbial community [12] and thus display a shift in microbial community. Because of increasing C:N ratios during the sampling period and a positive correlation between C:N and MBC:MBN ratios, we suggest that this shift might be caused by low quality SOM entry into soil (SOM with high C:N ratios), which is known to favor fungal growth [9]. But how the fungal community composition changes and how the fungal community gets influenced by the high soil temperature and low soil moisture under PC in the next summer remain unclear. Further research should clarify if the potentially changed fungal communities contain mycotoxigenic fungi genera, which can be triggered by environmental stress conditions to mycotoxin production [55]. Additionally, increasing MBC:MBN ratios are also indicative of long-term decreases in nutrient availability to soil microorganisms [28]. Beside the SOM entry, the crop rotation from winter wheat to strawberries might also have influenced the microbial community structure. It is known that, especially in the rhizosphere, microbial communities are influenced by the plant species, because of different root exudation and rhizodeposition in different root zones [10, 25]. However, as our MBC:MBN ratios were partially markedly wider than the typical ratios of 5–10 for arable soils (reviewed in Joergensen and Emmerling [28]) and showed large standard deviations, the MBC:MBN ratios should be interpreted carefully and require further investigation on the microbial community to confirm the indicated microbial shift.

### 4.4.2 Effects on organic matter input and decomposition

The general increase of SOC in both treatments, especially between late-July and late-September, most likely originated from the additional root biomass entry into soil from the growing strawberry plants after seedling transplantation and the increase in microbial biomass. The higher SOC under PC in late-September and late-November was presumably caused by a favored root growth under PC application [17, 66, 72]. Our results are in contrast to the reduced SOC contents found by Li et al. [39] and several other studies (e.g. [23, 75]) after 1-4 months and 2-4 years of PC application, respectively. Higher decomposition rates were discussed as reason for these SOC declines, which were induced by higher soil temperatures and soil moisture under PC [33, 35, 72]. Since we found reduced soil moisture under PC, this might explain the contrasting results. On the other hand and in agreement with our results, some studies reported that PC has no impact on SOC or even increase the SOC under PC [16, 43, 66]. As PC increases aboveground and belowground biomass [66, 70, 72], it was argued that increased biomass productivity under PC can balance out SOC losses caused by accelerated decomposition [19]. Thus, the root biomass input in our study seemed to be more pronounced and compensates a possibly higher decomposition rate of SOC by larger microbial activity as indicated by the increased soil temperature and MBC values under PC. SOC and MBC are known to correlate positively with DOC because it derives among other from leaf litter, root exudates and decomposition and metabolic by-products [8]. But despite of the increased SOC and MBC values under PC in late-November, our study showed hardly any influence of PC on DOC. Only the high DOC content under NC in the 0–10 cm soil layer in late-November, which we attributed to the leaching of organic substances from decaying plant litter on the soil surface [8], was obviously impeded by the plastic mulch. Similarly to DOC, the C:N ratios revealed no differences between the studied treatments, but the C:N ratios became wider in both treatments during the establishment phase of the strawberries. This might be explained by fresh SOM entering the soil by growing strawberry roots and reduced TN stocks. Wider C:N ratios point to poorly degradable litter and thus, to slower transformation and recycling of SOM [32]. This lower quality of SOM can promote fungal growth [9] and can change the microbial community towards larger fungal fractions [7] as already indicated by our MBC:MBN ratios. The MBC:SOC ratios increased during the sampling period under PC, which is in line with a study by Luo et al. [44]. Under NC, the MBC:SOC ratios increased from late-July to late-September but remained constant afterwards. The large standard deviations challenged the identification of any difference between the treatments. Generally, our MBC:SOC ratios lie within their typical range of 0.7–7% and also showed the typically occurring large seasonal variances [68, 69]. The increasing MBC:SOC are indicative for a higher availability of SOM to soil microorganisms [28]. The initial MBC:SOC ratios below 2% under PC are according to Anderson [2] an indicator for reduced SOM availability and a hint to soil degradation. Thus, it might be an important point to check, if the MBC:SOC drop again below 2% in the next summer.

### 4.4.3 Effects on transformation of different SOM pools

The frSOM and oSOM fractions represent the light fraction organic matter (LFOM), which is mineralized fast and important for the short-term nutrient supply in soil [7, 63]. The LFOM decreased in both treatments during the establishment phase of the strawberries, but only under PC with a significant extent. This possibly indicates that PC enhances the LFOM mineralization, as consequence of soil warming [18], which likely triggers an increased microbial activity as suggested by our MBC results. Despite the reduced LFOM contents, the SOC increased during the establishment phase of the strawberries, which showed that the increasing aSOM fraction (also referred to as heavy fraction organic matter (HFOM)) compensated the decline in LFOM. This might indicate that a part of the LFOM was transformed into the HFOM pool. The HFOM consists of organo-mineral complexes and is responsible for the longterm stabilization of SOM [7, 63]. Organo-mineral formation depends on surface properties of the minerals as well as the organic compounds and it can proceed in various mechanisms. Mainly simple organic compounds undergo organo-mineral formation [7]. Because of this, we assume that the higher HFOM contents were possibly caused by increasing entry of root and microbial exudates and larger amounts of degradation products, resulting from the higher microbial activity. The assumed larger root biomass production under PC might have triggered the significant larger aSOM fractions under PC in the root zone (10–30 cm soil layer) in late-November.

In summary, our results indicate a general shift in SOM composition to more SOC with larger HFOM and lower LFOM fractions, which seems to be more pronounced under PC than under NC. Thus, SOM quality is shifted from a faster mineralizable SOM towards a more stable SOM with a lower nutrient supply but with an improved nutrient sorption, water holding capacity and soil stability [7, 63]. Finally, this confirms our hypothesis of an altered SOM quality under PM. The assumed better nutrient sorption by a more stable SOM under PC might have additionally enhanced our TN and EC values under PC.

### **5 Conclusions**

Our study demonstrated that PC influences various soil processes already within a 4-month period during strawberry establishment in a loamy silt soil in a temperate, humid climate with significant outcomes. These effects were not restricted to the topsoil (0-10 cm) but propagated to deeper soil layers, partially down to 60 cm. The depth- and time-dependent approach of the present study allowed conclusions on transport and transformation processes in soil like leaching and accumulation of dissolved substances, but also on transformation of SOM pools due to processes like aggregation, adsorption, degradation and mineralization. The influence of PC on various soil processes within our 4-month experiment can be interpreted as mainly positive for soil quality. The PC changed the soil microclimate in the strawberry establishment phase by increasing soil temperature and reducing soil moisture. The reduced soil moisture under PC emphasizes that in a temperate, humid climates the impeded rainfall infiltration and higher transpiration losses of plants can compensate the water-saving through reduced evaporation. Reduced leaching processes under PC are indicated by an enhanced EC and TN, which can improve nutrient supply and fertilizer usage. The increased soil temperature under PC extends the microbial growth period and increased microbial biomass at the end of our sampling period, indicating higher SOM losses by microbial decomposition. However, PC also increased SOC content in the establishment phase of strawberries and thus overbalances the potentially higher SOM losses by microbial decomposition with higher biomass inputs resulting from stronger root and microbial growth. Additionally, PC changes SOM quality due to an enhanced shift in SOM towards a higher SOC with larger HFOM and lower LFOM fractions. This indicates a reduced fast mineralizable SOM pool, resulting in a lower nutrient supply, but on the other hand a larger stable SOM pool with larger nutrient sorption, water holding and soil stability. Studies on larger scale are advisable to check if our findings are generalizable on landscape levels. Future long-term studies should assess how PC influence soil processes over a complete season and in the commonly multi-annual application of PC.

Acknowledgements We want to thank C. Steegmüller for providing access to the sampling site and for his expertise on strawberry cultivation and Katharina Lühmann, Maria Olivares and Sirma Scopchanova for valuable assistance during sample analysis. Furthermore, we would like to thank Prof. S. Thiele-Bruhn (University of Trier) for providing the field measuring station.

Funding This work was supported by the Ministry for Education, Sciences, Further Education and Culture of the State of Rhineland-Palatinate (MBWWK) in the frame of the Interdisciplinary Research Group for Environmental Studies (IFG-Umwelt) of University Koblenz-Landau, by the Prof. B. Gedek and W. Gedek foundation and by the research fund of the University of Koblenz-Landau. Open Access funding enabled and organized by Projekt DEAL.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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### **CHAPTER 4**

Agricultural mulching and fungicides – Impacts on fungal biomass, mycotoxin occurrence and soil organic matter decomposition

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Environmental Science and Pollution Research. 28(27): 36535-36550.

#### **RESEARCH ARTICLE**



## Agricultural mulching and fungicides—impacts on fungal biomass, mycotoxin occurrence, and soil organic matter decomposition

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Received: 11 September 2020 / Accepted: 1 March 2021 © The Author(s) 2021

#### **Abstract**

Plastic and straw coverage (PC and SC) are often combined with fungicide application but their influence on fungicide entry into soil and the resulting consequences for soil quality are still unknown. The objective of this study was to investigate the impact of PC and SC, combined with fungicide application, on soil residual concentrations of fungicides (fenhexamid, cyprodinil, and fludioxonil), soil fungal biomass, mycotoxin occurrence, and soil organic matter (SOM) decomposition, depending on soil depth (0–10, 10–30, 30–60 cm) and time (1 month prior to fungicide application and respectively 1 week, 5 weeks, and 4 months afterwards). Soil analyses comprised fungicides, fusarium mycotoxins (deoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, and zearalenone), ergosterol, soil microbial carbon and nitrogen, soil organic carbon, dissolved organic carbon, and pH. Fludioxonil and cyprodinil concentrations were higher under SC than under PC 1 week and 5 weeks after fungicide application (up to three times in the topsoil) but no differences were observed anymore after 4 months. Fenhexamid was not detected, presumably because of its fast dissipation in soil. The higher fludioxonil and cyprodinil concentrations under SC strongly reduced the fungal biomass and shifted microbial community towards larger bacterial fraction in the topsoil and enhanced the abundance and concentration of deoxynivalenol and 15-acetyldeoxynivalenol 5 weeks after fungicide application. Independent from the different fungicide concentrations, the decomposition of SOM was temporarily reduced after fungicide application under both coverage types. However, although PC and SC caused different concentrations of fungicide residues in soil, their impact on the investigated soil parameters was minor and transient (< 4 months) and hence not critical for soil quality.

**Keywords** Plastic mulching · Fenhexamid · Cyprodinil · Fludioxonil · Deoxynivalenol

### Introduction

Mulching techniques such as straw and plastic mulching have become important agricultural practices to improve crop growth conditions in order to increase agronomic productivity (Haapala et al. 2014; Iqbal et al. 2020). Strawberry cultivation typically uses mulching techniques, which improve growth conditions by increasing soil temperature and reducing evaporation, weed growth, and erosion (Iqbal et al. 2020;

Responsible Editor: Kitae Baek

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Published online: 11 March 2021

Steinmetz et al. 2016). The conventional straw mulching is still applied today in matted row systems, particularly in colder regions, because of its low costs and labor intensity (Daugaard 2008; Lille et al. 2003; Poling 2016; Zhou et al. 2016). Because plastic mulching mostly performs better on the aforementioned attributes than straw mulching (e.g., Gao et al. 2019; Li et al. 2018a, b; Qin et al. 2015; Yang et al. 2018), it has become often combined with ridge-furrow systems and subsurface drip irrigation, a widely applied agricultural practice in strawberry cultivation, which has largely replaced straw mulching (Kasirajan and Ngouajio 2012; Zhou et al. 2016). However, plastic mulching can also increase plastic residues and pesticide runoff, reduce SOM, and shift the microbial community towards mycotoxigenic fungi (reviewed in Steinmetz et al. 2016), which has recently raised concerns about the sustainability of the system (Steinmetz et al. 2016). In particular, two aspects are still missing about plastic mulching, which are necessary to evaluate its impact



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on soil quality in the long term: (1) its potential to contaminate soil with microplastics and mycotoxins and (2) a substantial process understanding of its influence on various soil parameters and processes (Accinelli et al. 2020; Steinmetz et al. 2016).

Fungicides are widely applied in agriculture to protect crops against fungal diseases and are frequently combined with plastic and straw mulching. They can reach the soil either by direct application or indirectly by drift during spraying or runoff from sprayed plants after precipitation or irrigation (Arias et al. 2005; Cisar and Snyder 1996; Wainwright 1977). Fungicides are known to impact directly or indirectly on a multitude of soil processes. For example, fungicides reduce fungal population by inhibiting growth and sporulation (Campos et al. 2014). This can result in a reduced microbial activity (Chen et al. 2001a; Chen and Edwards 2001), a shifted microbial community (Munier-Lamy and Borde 2000; Sigler and Turco 2002; Yang et al. 2011), and a reduced genetic diversity of soil bacteria and fungi, leading to restricted enzyme activities in soil (Baćmaga et al. 2016, 2020; Monkiedje 2002; Tu 1993). Thus, relevant soil biogeochemical process can be indirectly affected by the use of fungicides, such as soil organic matter (SOM) decomposition (Chen et al. 2001a; Chen and Edwards 2001; Zaller et al. 2016) or nitrogen mineralization and nitrification (Chen et al. 2001b; Domsch 1964; Monkiedje 2002). Furthermore, filamentous fungi are an integral part of soil microbial communities and well-known producers of secondary metabolites with biological and toxic activities such as mycotoxins (Murphy et al. 2006). Mycotoxins are biosynthesized by several fungal species of the genera Aspergillus spp., Penicillium spp., and Fusarium spp. (Abbas et al. 2009; Murphy et al. 2006), frequently as response to stress conditions (Ponts 2015; Schmidt-Heydt et al. 2008), which can be generated by the action of certain fungicides (Chen et al. 2019; Li et al. 2018a, b). The aforementioned effects of fungicides on soil quality depend on the residual concentration of fungicides in soil, which in turn depend on application rate and frequency as well as on physicochemical and microbial soil properties (Chen and Edwards 2001; Martinez-Toledo et al. 1998; Wainwright 1977). In addition, the soil entry of fungicides and soil residual concentrations are influenced by mulching materials, which can act, depending on the mulching material, as semipermeable or impermeable physical barrier or as a sorbent material (Guo et al. 2020; Nerín et al. 1996).

Fenhexamid, cyprodinil, and fludioxonil are commonly used fungicides in strawberry cultivation to prevent infestation with *Botrytis cinerea* (gray mold) and related fungi like *Monilinia* and *Sclerotinia* (Rosslenbroich and Stuebler 2000; Strand 2008). Their half-life dissipation time (DT<sub>50</sub>) was in soil under aerobic laboratory conditions < 1, 53, and 239 days for fenhexamid, cyprodinil, and fludioxonil, respectively (Agriculture, and Environment Research Unit, University of Hertfordshire 2019).

However, dissipation in soil depends on factors such as soil temperature, moisture, and microbial activity (Borzì et al. 2007; Dec et al. 1997; Roberts et al. 1998), which in turn can be strongly influenced by mulching treatments (Haapala et al. 2014; Li et al. 2020; Steinmetz et al. 2016).

It can be assumed that the impermeable plastic mulch acts as physical barrier for fungicides, which impedes the entries of fungicides into soil, leading to lower residual concentrations when compared to the traditional straw mulching. Because of that, we expected different effects of both mulching types on microbial (fungal) biomass, mycotoxin occurrence, and SOM decomposition, which are important factors, influencing soil quality and fertility and hence in the long-term also productivity and sustainability of the agricultural management (Bünemann et al. 2018; Frac et al. 2018; Kibblewhite et al. 2008).

However, this has not yet been investigated but is important to understand how mulching can impact on fungicide fate and soil quality. Derived from the proceeding information, we hypothesized the following: (i) the impermeable plastic mulch mitigates fungicide entry into soil and reduces residual concentrations of fungicides in soil compared to straw-covered soil; (ii) higher fungicide concentrations in soil will strongly reduce fungal biomass and induce higher stress level to fungi, triggering a higher mycotoxin production; (iii) the fungicide residues decelerate SOM decomposition by inhibiting and reducing soil microbial biomass. In order to close the mentioned research gaps, the objective of this study was to investigate the residual concentrations of the fungicides fenhexamid, cyprodinil, and fludioxonil in soil under plastic and straw coverage in strawberry cultivation in dependence of time (4 months) and soil depth (three soil layers) and to estimate their impact on microbial (fungal) biomass, SOM decomposition, and mycotoxin occurrence.

### Material and methods

### Site description and soil management

This study was conducted in the frame of a triennial field experiment on the influence of plastic mulching on biogeochemical soil properties and processes in strawberry cultivation. The sampling site was a commercial strawberry field in southwestern Germany (49° 11′ N, 8° 10′ E, 130 m a.s.l.), which has a temperate, humid climate with an annual average precipitation of 643 mm a<sup>-1</sup> (weather station of Landau-Wollmesheim, Agrarmeteorologie Rheinland-Pfalz). According to FAO classification, the soil type was a silt loam (Anthrosol) with a texture of  $7 \pm 2\%$  sand,  $83 \pm 5\%$  silt, and  $10 \pm 3\%$  clay (IUSS Working Group WRB 2015) and an average cation exchange capacity of  $1035 \pm 50$  mmol kg<sup>-1</sup> in the 0–60-cm soil layer. Strawberries (*Fragaria* × *ananassa*, 'Malwina') were transplanted in July 2016 (8 plants per m²), after tillage,



fertilization, and establishment of a ridge-furrow system with subsurface drip irrigation and plastic-mulched ridges (black polyethylene, 50 μm) and bare furrows. The furrows were covered with wheat straw in April 2017. Fungicide application was conducted by foliar application with a tractor-mounted field sprayer and application periods were from late-May 2017 until early-June 2017, depending on bloom of the strawberries. First, the fungicide Switch® (37.5% cyprodinil and 25% fludioxonil, application ratio 1.5) was applied together with the acaricide Masai® (tebufenpyrad) with an application rate of respectively 1 and 0.375 kg ha<sup>-1</sup>. Two weeks later, the fungicide Teldor® (50% fenhexamid) was applied with an application rate of 2 kg ha<sup>-1</sup>. The relevant physicochemical properties of the applied fungicides are summarized in Table 1.

### Experimental design and sample collection

A semicontrolled field experiment was designed that reflected current agricultural practice while enabling us to study soil processes in a homogeneous soil type and avoiding masking of treatment effects by landscape variation, edge effects, and variability in agricultural treatment (e.g., in terms of active compounds and application rates). The test field included two experimental areas (21 × 10 m). The plastic mulch was immediately removed in one area after strawberry transplantation (July 2016) and the bare soil was later covered with wheat straw (April 2017), whereas the second area was left plastic-covered. Henceforth, we refer to them as straw-covered (SC) and plastic-covered (PC) areas. Both experimental areas were treated identically regarding fertilization, irrigation, fungicide application, and strawberry transplantation.

Soil samples were taken at four dates: 1 month before fungicide treatments in late-April (25.4) and respectively 1 week, 5 weeks, and 4 months after end of the fungicide treatments in mid-June (19.6), mid-July (18.7), and mid-October (9.10). In

both experimental areas, a composite sample from the ridge (five single soil cores) was collected from a subplot  $(1 \times 1 \text{ m})$  in each of five randomly chosen plots  $(10 \times 1.5 \text{ m})$ : PC (n = 5) and SC (n = 5). Soil samples were taken equidistantly between two plants (20 cm distance to plant) from topsoil layer (0–10 cm), the root layer, representing the main root zone of the strawberries (10–30 cm), and the subsoil layer below the root zone of the strawberries (30–60 cm). In the topsoil layer, soil samples were taken with stainless steel sampling rings (d = 5 cm, h = 5 cm), whereas a boring rod (Pürckhauer) was used for both deeper layers. Soil samples were homogenized and stored at 4 °C for further analyses.

### **General soil parameters**

Respectively one sensor of a field measuring station (ecoTech®, Bonn, Germany) was installed at three soil depths (5, 15, and 35 cm) in both treatments, which recorded hourly soil temperature and moisture. The soil depths were chosen in accordance with the soil layers selected for soil analyses. Air temperature and precipitation data were taken from the weather station Landau-Wollmesheim (Agrarmeteorologie Rheinland-Pfalz). Unless stated otherwise, air-dried and sieved (< 2 mm) soil samples were used for subsequent analyses. Soil pH was measured in 0.01 M CaCl<sub>2</sub> solution, in accordance with DIN EN 15933:2012-11. For total nitrogen (TN) determination, soil samples were oven-dried (105 °C), milled (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany), and finally analyzed with a CHNS Analyzer (vario MicroCUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany).

### Determination of residual concentration of fungicides in soil

Fungicides in soil samples were quantified with liquid chromatography—high-resolution mass spectrometry (LC-HRMS,

Table 1 Physicochemical properties of the fungicides fenhexamid, fludioxonil, and cyprodinil

Fungicide	Water solubility $l$ (mg $L^{-1}$ )	GUS leaching potential index (leachability) <sup>1</sup>	Vapor pressure (volatility) <sup>1</sup> (mPa)	$Log K_{ow}$	Photolysis <sup>1</sup> , DT <sub>50</sub> (days)	Hydrolysis <sup>1</sup> , DT <sub>50</sub> (days)	Soil degradation, DT <sub>50</sub> (field) (days)
Fenhexamid	24	- 0.42 (low)	0.0004 (low)	3.51	0.05	Stable	$\sim 1^{2,3}$
Fludioxonil	1.8	- 1.47 (low)	0.0004 (low)	4.12	10	Stable	$6-21^{1,4,5,6}$
Cyprodinil	13	1.06 (low)	0.51 (low)	4.00	7.5	Stable	2-45 <sup>1,4,5,6</sup>

<sup>&</sup>lt;sup>1</sup> Agriculture, and Environment Research Unit, University of Hertfordshire (2019)



<sup>&</sup>lt;sup>2</sup> Abbate et al. (2007)

<sup>&</sup>lt;sup>3</sup> Borzì et al. (2007)

<sup>&</sup>lt;sup>4</sup> Liu et al. (2011a)

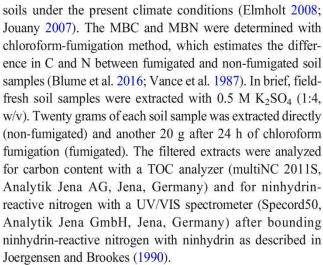
<sup>&</sup>lt;sup>5</sup> Liu et al. (2011b)

<sup>&</sup>lt;sup>6</sup> Zhang et al. (2015)

Thermo Fisher Scientific, Waltham, USA) after solid-liquid extraction. More precisely, 5 g of an air-dried, milled soil sample (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany) was weighed in in 50-mL centrifuge tubes, mixed with 15 mL methanol (LC-MS grade), and shaken for 30 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany). After 10 min of ultrasonic treatment (DT 514H, Bandelin electronics GmbH & Co.KG, Berlin, Germany), the suspension was centrifuged at 3170g for 5 min (Universal 320, Hettich Lab Technology, Tuttlingen, Germany). Subsequently, 2 mL of the supernatant was filtered with a 0.2-µm PTFE syringe filter. Finally, 0.5 mL of the filtered extract was diluted with 0.5 mL deionized water and stored at - 20 °C until LC-HRMS measurement. Chromatographic separation was performed at room temperature in a Hypersil GOLD™ C<sub>18</sub> column (100 × 2.1 mm, 1.9 μm particle size, Thermo Fisher Scientific, Waltham, USA). As mobile phase, a gradient of solvent A (water + 0.1% formic acid and 4 mM ammonium formiate) and solvent B (methanol + 0.1% formic acid and 4 mM ammonium formiate) with a flow rate of 0.2 mL min<sup>-1</sup> was used: 0-1 min 10% B; 1-3 min 10 to 100% B; 3-7 min 100% B; 7-7.5 min 100 to 10% B; and 7.5–10 min 10% B. The fungicides were quantified in the positive ion mode, using the following mass-to-charge ratios: 266.0730, 266.1339, and 302.0709 for fludioxonil, cyprodinil, and fenhexamid, respectively. Fungicide concentration in soil was quantified using a matrix-matched calibration curve (0.1, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 25, and 50  $\mu$ g L<sup>-1</sup>), which consisted of a 1:1 (v/v) mixture of a methanol soil extract (extraction followed the same procedure as samples) and deionized water. Samples were considered positive when concentrations were above the lowest calibration level (LCL) of 0.1  $\mu$ g L<sup>-1</sup>, which corresponds to a soil concentration of 0.6 µg kg<sup>-1</sup>. For preparation of the calibration curve, fungicide standards, purchased by Sigma-Aldrich (Taufkirchen, Germany), were weighted quantitatively and reconstituted in methanol. Furthermore, a mixture of all three fungicides in methanol (conc. 1 mg  $L^{-1}$  each) was prepared and used for spiking and calibration purposes. The method was evaluated in terms of reproducibility at two concentrations of 4 and 40  $\mu$ g kg<sup>-1</sup> (n = 5 for each concentration). Recovery values ranged between 77.7 and 91.5%, with a relative standard deviation (RSD) < 20%.

### Analysis of microbial soil parameters

Soil samples were analyzed for several microbial parameters (microbial biomass carbon (MBC) and nitrogen (MBN), MBC:MBN ratio, and ergosterol as proxy for fungal biomass) and mycotoxin occurrence to describe the impact of fungicide residues on microbial and fungal biomass and microbial community. The focus was set on fusarium mycotoxins, because *Fusarium* spp. are relevant field fungi, occurring ubiquitous in



The ergosterol determination was based on the method of Gong et al. (2001). Briefly, 4 g of air-dried, milled soil (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany) was extracted with 12 mL methanol for 60 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany). The suspension was sonicated for 10 min (DT 514H, Bandelin electronics GmbH & Co.KG, Berlin, Germany), subsequently centrifuged for 10 min at 2000g (Universal 320, Hettich Lab Technology, Tuttlingen, Germany), and finally ultracentrifuged for 3 min at 7270g (Micro centaur, MSE Ltd, London, UK). For ergosterol measurement, 20 µL of the supernatant was injected in a high-performance liquid chromatography system with a UV detector (HPLC-UV, HPLC 1200 series, Agilent technologies, Santa Clara, USA), which was equipped with a C<sub>18</sub> LiChrospher® column (LiChrospher RP-18e, 5  $\mu$ m, 100 Å, 250  $\times$  4.6 mm, Merck KGaA, Darmstadt, Germany) and quantified ergosterol at a wavelength of 282 nm. The applied method had a limit of detection (LOD) of 0.06 mg kg<sup>-1</sup>.

Soil samples were analyzed for the fusarium mycotoxins deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), and zearalenone (ZEN). Principally, mycotoxin analysis was based on Mortensen et al. (2003) with small modification (Muñoz et al. 2017). In brief, 5 g of air-dried, milled soil (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany) was extracted with 15 mL methanol:water mixture (9:1, v/v) for 30 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany) and subsequently for 10 min with ultrasonication (DT 514H, Bandelin electronics GmbH & Co.KG, Berlin, Germany). The suspension was centrifuged for 10 min at 2000g (Universal 320, Hettich Lab Technology, Tuttlingen, Germany). An aliquot of 10 mL was subsequently evaporated to dryness under a nitrogen stream at 50 °C (Evaporatorsystem EVA-EC1-24-S, VLM Korrosions-Prüftechnik, Labortechnik & Dienstleistungen GmbH, Bielefeld, Germany). One milliliter of mobile phase (methanol:water 1:1 v/v with 0.1% formic acid and 4 mM



ammonia formiate) was used to reconstitute the residues. This solution was ultracentrifuged for 5 min at 7270g (Micro centaur, MSE Ltd, London, UK) and subsequently 20 µL of the supernatant was injected the LC-HRMS (Thermo Fisher Scientific, Waltham, USA), using the aforementioned Hypersil GOLD™ column for mycotoxin analysis. The mycotoxins were quantified with a matrix-matched calibration curve (1, 2.5, 5, 10, 25, 50, 75, and 100  $\mu$ g L<sup>-1</sup>), which was prepared in soil extract (extraction followed the same procedure as samples). All calibration standards for mycotoxins were purchased by Romer Labs Deutschland GmbH (Butzbach, Germany). Samples were considered positive when concentrations were above the LCL, which was 2.5  $\mu$ g L<sup>-1</sup> for 15-ADON and 1  $\mu$ g L<sup>-1</sup> for DON, NIV, and ZEN, respectively, corresponding to a soil concentration of 0.75 and 0.3 µg kg<sup>-1</sup>. For DON and NIV, <sup>13</sup>C-labeled internal standards were used as additional confirmation step. All mycotoxins were quantified in the negative ion mode (exception 15-ADON), using the following mass-to-charge ratios: 356.1750 and 272.1701 for <sup>13</sup>C-DON and <sup>13</sup>C-NIV and 341.1240, 357.1195, 339.1430, and 317.1389 for the DON, NIV, 15-ADON, and ZEN, respectively.

### Characterization of soil organic matter

In order to estimate the impact of fungicide residues on SOM and SOM decomposition, we examined soil organic carbon (SOC), dissolved organic carbon (DOC), MBC:SOC ratio, and C:N ratio. The SOC was measured in air-dried, milled soil samples with CHNS Analyzer (vario MicroCUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany). An averaged carbonate content, determined by acid fumigation in accordance with Harris et al. (2001), was used to obtain SOC values from total C content measured by CHNS analyses. The DOC was determined in filtrated soil extracts (0.45  $\mu m$ , 1:5 soil-to-water ratio, w/v) of field-fresh soil samples with a TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany) in accordance with DIN EN 1484:1997-05. The C:N ratio corresponds to the SOC divided by the TN of the same sample.

### **Data analyses**

Correlation between two variables was calculated with Pearson's correlation coefficient or Spearman's rho if normality distribution was not given. For correlation analysis, only positive samples were used, with regard to fungicide and mycotoxin detection. In order to determine significant differences between means, a mixed factorial ANOVA design was used, with time and soil depth as repeated factors and treatment as fixed factor. If significant interaction effects occurred, an additional ANOVA was applied to locate significant differences, with least significance distance (LSD) testing as post hoc test. Normality distribution of data and of residuals was examined

graphically with histograms and quantile-quantile plots. Variance homogeneity was checked by Levene's test. If the probability of error was < 0.05, the differences were termed as statistically significant. Method validation for ergosterol, mycotoxin, and fungicide determination, with regard to repeatability, recovery, linearity, and range, and LOD calculation of ergosterol were based on ICH guideline Q2. The LOD was calculated as  $3.3\sigma/S$ , with  $\sigma$  as the standard deviation of the intercept of the regression line and S as the slope of the regression line calculated from the calibration standards (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use 2005). For fungicide and mycotoxin determination, the LCL of the matrix-matched calibration curve which gives a clear identifiable peak was used as LOQ. This method is based on visual (empirical) evaluation, which has been suggested to provide more realistic values in complex matrices (Şengül 2016). For all results below the LCL, the LCL/2 was used for mean calculation (Ogden 2010). IBM SPSS Statistics 25 and Microsoft Excel 2010 were used for all statistical analysis.

### Results

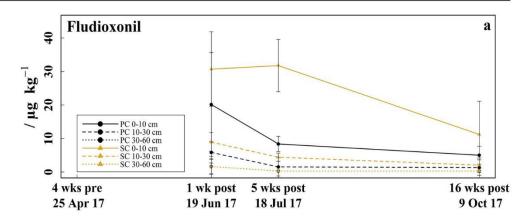
### Residual concentrations of fungicides in soil under plastic and straw coverage

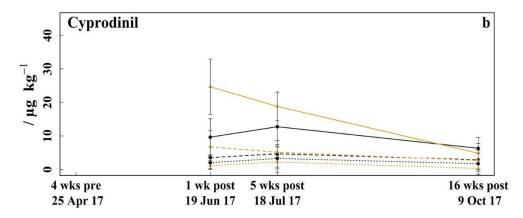
The fungicide soil concentrations measured 1 week, 5 weeks, and 4 months after fungicide application (mid-June, mid-July, and mid-October) were between LCL-31.8 µg kg<sup>-1</sup> for fludioxonil (Fig. 1a) and LCL-24.7 µg kg<sup>-1</sup> for cyprodinil (Fig. 1b). One month (late-April) before fungicide application, neither fludioxonil nor cyprodinil was detected in any soil sample. No fenhexamid was found in any soil sample.

The fludioxonil and cyprodinil concentrations were up to three times higher under SC than under PC in the 0-10 and 10–30 cm soil layer 1 week (significant for cyprodinil in the 0-10 cm soil layer: p = 0.011) and 5 weeks after fungicide application (significant for fludioxonil in the 10–30 cm soil layer: p = 0.002). However, no differences were observed anymore between treatments 4 months after fungicide application (only the fludioxonil concentrations in the 0–10 cm soil layer under SC were still twice as high as under PC). Under SC, the fludioxonil and cyprodinil concentrations decreased by 64-82% and by 61-80%, respectively, in all soil layers from mid-June to mid-October (significant in the 0-10 cm soil layer for fludioxonil and cyprodinil: p <0.030). Under PC, fludioxonil declined also by 75-82% in all soil layers from mid-June to mid-October but the cyprodinil concentrations increased by 32-64% in all soil layers from mid-June to mid-July and decreased by 38–86% to mid-October. The decline of fludioxonil was 2-23 times stronger in all soil layers under both treatments between



**Fig. 1** Fungicide residual concentrations in soil. **a** Fludioxonil concentrations determined 4 weeks before (late-April) and respectively 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC), respectively, shown as mean with standard deviation (*n* = 5). **b** Cyprodinil concentrations





mid-June and mid-July than between mid-July and mid-October (exception: 0–10 cm soil layer under SC). Both the fludioxonil and the cyprodinil concentrations decreased significantly with soil depth in both treatments (p < 0.018 and p < 0.040, respectively).

Despite applied in lower amounts, fludioxonil showed a tendency to higher soil concentrations compared with cyprodinil, especially under SC. In mid-June, the cyprodinil:fludioxonil ratios of both treatments ranged between 0.5 and 1.2 and were thus below the applied ratio of 1.5 (Table 2). Under SC, the cyprodinil:fludioxonil ratios remained below 1.5 in all soil layers in mid-July and mid-October (exception: 30–60 cm soil layer in mid-July), whereas under PC, the cyprodinil:fludioxonil ratios ranged between 1.5 and 10.9 above the applied ratio (exception: 0–10 cm soil layer in mid-October).

### **General soil parameters**

Soil temperature (Fig. 2) was higher under PC than under SC at each soil depth from April to October 2017. The differences in the monthly mean soil temperature ( $\Delta T$ ) between both coverage types were up to 2.1 °C at 5 cm soil depth in April and generally decreased with soil depth and during the sampling period (Table 3). Soil moisture (Fig. 3) was markedly lower under PC than under SC at 5 and 35 cm soil depth, whereas at 15 cm soil depth, soil moisture was partially higher under PC. Soil moisture increased with soil depth in both treatments. The pH (Fig. 4a) was between 0.05 and 0.15 units higher under SC compared to PC in all soil layers from mid-July to mid-October. The pH dropped from 7.4 in late-April to values between 6.6 and 7.0 in mid-June in all soil layers of both treatments (p < 0.001). The TN (Fig. 4b) ranged between

Table 2 Cyprodinil:fludioxonil ratios measured 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC), respectively

	PC			SC			
Date	0–10 cm	10–30 cm	30–60 cm	0–10 cm	10-30 cm	30–60 cm	
Mid-June	0.5	0.6	1.2	0.8	0.8	0.7	
Mid-July	1.5	3.1	10.9	0.6	1.2	7.3	
Mid-October	1.3	2.2	5.6	0.4	1.3	1.0	



**Table 3** Monthly mean, maximum, and minimum soil temperature measured at 5, 15, and 35 cm soil depth by field measuring station under plastic coverage (PC) and straw coverage (SC) during the sampling

period. Values are given as mean with standard deviation (values from 13 May to 8 June are missing due to malfunction of the field measuring station)

Date	Soil depth	PC			SC	$\Delta T$ PC-SC		
		Mean temperature (°C)	Max. temperature (°C)	Min. temperature (°C)	Mean temperature (°C)	Max. temperature (°C)	Min. temperature (°C)	(°C)
Apr. 17	5 cm	$12.6 \pm 2.9$	21.7	5.9	$10.5 \pm 2.2$	16.5	4.5	2.1
	15 cm	$12.2\pm1.7$	16.7	7.6	$10.8\pm1.6$	14.9	6.3	1.4
	35 cm	$12.0\pm1.1$	14.3	9.2	$11.1\pm1.0$	13.4	8.5	0.9
May 17	5 cm	$13.3\pm1.9$	19.3	9.6	$12.2\pm1.6$	16.3	8.5	1.2
	15 cm	$12.8\pm1.1$	15.8	10.7	$12.2\pm1.1$	15.2	9.8	0.6
	35 cm	$12.6 \pm 0.6$	14.3	11.3	$12.2 \pm 0.7$	14.0	11.0	0.4
Jun. 17	5 cm	$20.0\pm1.9$	23.9	15.2	$18.6\pm1.8$	22.4	14.3	1.4
	15 cm	$19.5 \pm 1.4$	22.4	16.0	$18.5\pm1.4$	21.1	15.2	1.0
	35 cm	$19.0 \pm 1.1$	20.7	16.5	$18.2\pm1.1$	19.9	15.8	0.8
Jul. 17	5 cm	$19.4 \pm 1.8$	24.7	16.2	$18.7\pm1.5$	22.1	15.8	0.7
	15 cm	$19.1 \pm 1.3$	22.4	16.5	$18.6\pm1.2$	21.3	16.3	0.5
	35 cm	$18.9 \pm 0.9$	20.7	17.3	$18.5 \pm 0.9$	20.1	17.0	0.4
Aug. 17	5 cm	$19.4 \pm 1.5$	22.8	16.2	$19.0\pm1.4$	21.7	15.7	0.4
	15 cm	$19.1 \pm 1.1$	21.3	16.8	$18.8\pm1.1$	20.9	16.5	0.3
	35 cm	$19.0 \pm 0.8$	20.5	17.2	$18.7 \pm 0.7$	20.1	17.0	0.3
Sep. 17	5 cm	$15.4 \pm 1.8$	19.9	11.6	$15.0 \pm 1.7$	19.3	11.5	0.4
	15 cm	$15.5 \pm 1.7$	20.3	12.6	$15.2\pm1.6$	19.7	12.4	0.3
	35 cm	$16.0 \pm 1.6$	20.3	13.8	$15.7\pm1.6$	19.9	13.5	0.3
Oct. 17	5 cm	$12.6 \pm 1.4$	16.8	7.6	$12.5 \pm 1.4$	16.5	7.0	0.1
	15 cm	$12.8 \pm 1.2$	16.2	8.9	$12.7\pm1.2$	15.8	8.4	0.1
	35 cm	$13.4 \pm 1.0$	16.0	10.7	$13.2\pm1.0$	15.7	10.2	0.2

0.06 and 0.12% but showed no differences between treatments or sampling time.

### Microbial soil parameters

The ergosterol concentrations (Fig. 5a) ranged from 0.09 to 0.38 mg kg<sup>-1</sup> and were by 0.01–0.11 mg kg<sup>-1</sup> higher under SC compared to PC in the 0–10 cm soil layer (significant in mid-June: p = 0.041 and mid-October: p = 0.008). The ergosterol concentrations in all soil layers decreased by 0.01–0.04 mg kg<sup>-1</sup> under PC and by 0.04–0.07 mg kg<sup>-1</sup> under SC from mid-June to mid-July and increased by 0.01–0.05 mg kg<sup>-1</sup> under PC and 0.04–0.15 mg kg<sup>-1</sup> under SC to mid-October. This pattern was most pronounced in the 0–10 cm soil layer under SC. The highest ergosterol concentrations (0.22–0.38 mg kg<sup>-1</sup>) were found in the 0–10 cm soil layer and decreased significantly with soil depth in both treatments (p < 0.012).

The MBC (Fig. 5b) ranged from 158 to 604 mg kg<sup>-1</sup> and was by 247–352 mg kg<sup>-1</sup> higher under PC than under SC in all soil layers in late-April (p < 0.001) and by 100 and 154 mg kg<sup>-1</sup> higher in the 10–30 and 30–60 cm soil layer in mid-

October (30–60 cm soil layer: p = 0.001). In contrast, the MBC in mid-June was by 100–161 mg kg<sup>-1</sup> smaller under PC than under SC in all soil layers (0–10 cm and the 10–30 cm soil layer:  $p \le 0.045$ ). Under PC, the MBC decreased in all soil layers from 357 to 552 mg kg<sup>-1</sup> in late-April to 199–246 mg kg<sup>-1</sup> in mid-June (p = 0.003) and showed less variation to mid-July. From mid-July to mid-October, the MBC under PC increased to 423–586 mg kg<sup>-1</sup> (p < 0.001). In contrast to PC, the MBC under SC increased in all soil layers from 129 to 229 mg kg<sup>-1</sup> in late-April to 299–414 mg kg<sup>-1</sup> in mid-June (p = 0.001) but showed the same pattern as under PC for mid-July and mid-October.

The MBN (Fig. 5c) ranged from 9 to 31 mg kg<sup>-1</sup> and was higher under SC than under PC in the 0–10 cm soil layer, with differences increasing from 2.02 to 6.76 mg kg<sup>-1</sup> during the sampling period. The MBN in all soil layers of both treatments decreased from mid-April to mid-June (p < 0.001), remained almost constant until mid-July, and increased from mid-July to mid-October (p < 0.001).

The MBC:MBN ratios (Fig. 5d) ranged between 6 and 33 and were significantly wider under PC compared to SC in all soil layers in late-April ( $p \le 0.012$ ), whereas in mid-June, the



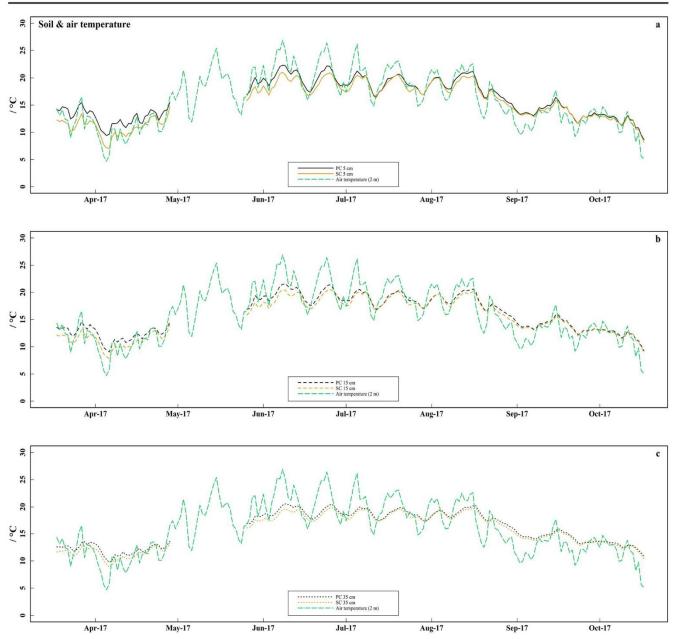
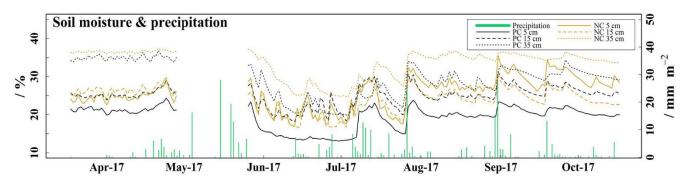


Fig. 2 Soil and air temperature. a-c Daily mean soil temperature in strawberry cultivation, measured at 5, 15, and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC) and daily mean air

temperature measured 2 m above ground. The soil temperature data exhibit a data gap from mid-May (13.5) to early-June (8.6), due to technical malfunction of the measuring station

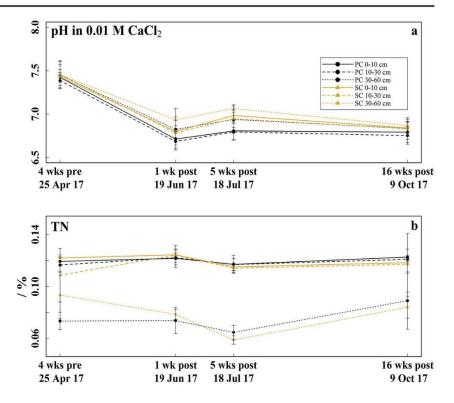


**Fig. 3** Daily mean soil moisture in strawberry cultivation, measured at 5, 15, and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC) and daily precipitation. The soil moisture data exhibit a data gap

from mid-May (13.5) to early-June (8.6), due to technical malfunction of the measuring station

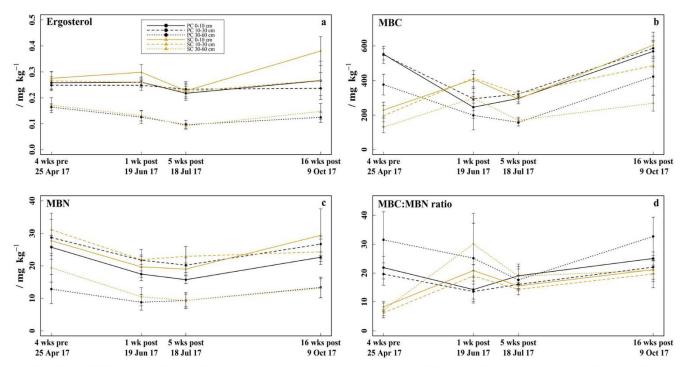


Fig. 4 Physicochemical soil properties. a pH (in 0.01 M CaCl<sub>2</sub>) determined 4 weeks before (late-April) and respectively 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC), respectively, shown as mean with standard deviation (n = 5). b Total nitrogen (TN)



MBC:MBN ratios were by 5–7 units narrower under PC (0–10 and 10–30 cm soil layer:  $p \le 0.019$ ). Furthermore, the MBC:MBN ratios were by 2–4 units wider under PC than under SC in the 0–10 and 10–30 cm soil layer in mid-July and by 2–12 units wider under PC in all soil layers in mid-October (30–60 cm soil layer: p = 0.039).

The fusarium mycotoxins DON and 15-ADON (Fig. 6a, b) were measured in concentrations between LCL–21.8  $\mu$ g kg<sup>-1</sup> and LCL–4.7  $\mu$ g kg<sup>-1</sup> after fungicide application but were not detected in any sample before fungicide application. Both mycotoxins showed a tendency to higher concentrations under SC compared to PC 5 weeks after fungicide application. The

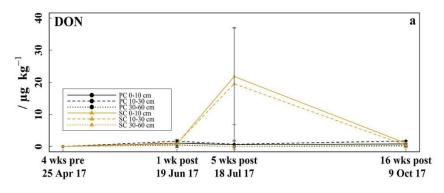


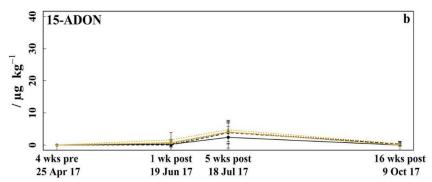
**Fig. 5** Soil microbial biomass. **a** Ergosterol concentrations determined 4 weeks before (late-April) and respectively 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and

straw coverage (SC), respectively, shown as mean with standard deviation (n = 5). **b** Soil microbial biomass carbon (MBC). **c** Soil microbial biomass nitrogen (MBN). **d** MBC:MBN ratio



**Fig. 6** Mycotoxins. **a** Deoxynivalenol concentrations (DON) determined 4 weeks before (late-April) and respectively 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC), respectively, shown as mean with standard deviation (*n* = 5). **b** 15-Acetyldeoxynivalenol concentrations (15-ADON)



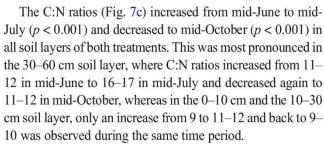


DON concentrations in the 0–10 and 10–30 cm soil layer under SC increased in mid-June from slightly above the LCL to  $\sim 20~\mu g~kg^{-1}$  in mid-July and then decreased again until mid-October to a level comparable with mid-June. The same pattern was found for 15-ADON in all soil layers of both treatments, but with maximum concentrations  $\sim 4~\mu g~kg^{-1}$  in mid-July, the 15-ADON concentrations were markedly lower than the DON values. The mycotoxin NIV was found in none of the soil samples and ZEN was detected only twice in concentrations of 0.6 and 1.1  $\mu g~kg^{-1}$ . Cyprodinil and DON showed a positive correlation ( $r_S = .380, p = 0.029, n = 33$ ).

### Soil organic matter

The SOC (Fig. 7a) ranged between 0.83 and 1.36% and was 0.03–0.15% higher under PC in relation to SC in all soil layers during the sampling period (exception: 30–60 cm soil layer in late-April). The SOC increased by 0.11–0.24% in all soil layers of both treatments from mid-June to mid-July (p = 0.017) and decreased by 0.02–0.23% in all soil layers of both treatments from mid-July to mid-October (p = 0.013).

Because the DOC values (Fig. 7b) in late-April were unrealistically high (494–1024 mg kg $^{-1}$ ) and we cannot exclude an error during DOC measurement (maybe no filtration), we excluded them from statistical analysis. From mid-June to mid-October, the DOC was 3–8 mg kg $^{-1}$  higher under SC compared to PC in the 0–10 cm soil layer (mid-October: p = 0.021). The DOC in all soil layers of both treatments decreased from 11–24 mg kg $^{-1}$  in mid-June to 7–14 mg kg $^{-1}$  in mid-July (p < 0.001) and increased again to 16–28 mg kg $^{-1}$  in mid-October (p < 0.001).



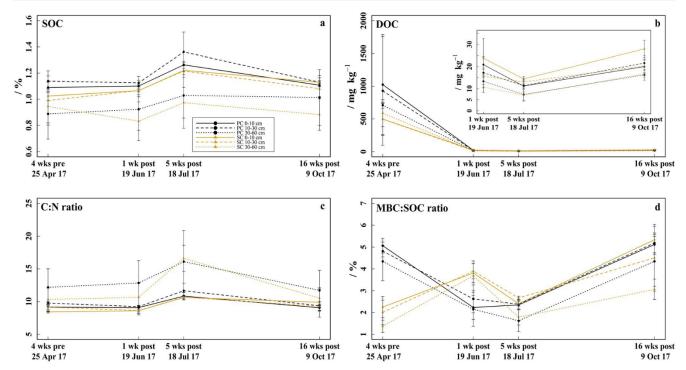
The MBC:SOC ratios (Fig. 7d) were 2.81-2.98% higher under PC than under SC in all soil layers in late-April ( $p \le 0.003$ ), whereas 1.27-1.58% lower MBC:SOC ratios were observed under PC in mid-June ( $p \le 0.030$ ). Under PC, the MBC:SOC ratios were between 4.3 and 5.1% in all soil layers in late-April and decreased to 2.1-2.6% in mid-June (p = 0.004), remained almost constant to mid-July, and increased to 4.6-5.2% in mid-October (p < 0.001). Under SC, the MBC:SOC ratios were between 1.4 and 2.3% in all soil layers in late-April and increased to 3.7-3.9% in mid-June (p = 0.002). Subsequently, the MBC:SOC ratios decreased to 1.8-2.7% in mid-July (p < 0.001) and increased to 3.1-5.3% in mid-October (p < 0.001).

### Discussion

### Influence of plastic and straw coverage on residual concentration and fate of fungicides in soil

The non-detects of fludioxonil and cyprodinil in late-April confirm that the fungicide concentrations measured after fungicide application were not biased by potential fungicide





**Fig. 7** Soil organic matter. **a** Soil organic carbon (SOC) determined 4 weeks before (late-April) and respectively 1 week (mid-June), 5 weeks (mid-July), and 16 weeks (mid-October) after fungicide application in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and

straw coverage (SC), respectively, shown as mean with standard deviation (n = 5). **b** Dissolved organic carbon (DOC). **c** Carbon:nitrogen ratio (C:N ratio). **d** MBC:SOC ratio

residues from previous-year applications. The partly two to three times higher fludioxonil and cyprodinil concentrations under SC than under PC in the 0–10 and 10–30 cm soil layer confirmed our hypothesis 1 that the impermeable plastic mulch reduces fungicide entry in soil. However, a significant fraction of the fungicides also reached the soil under PC, presumably through the planting holes. No fenhexamid was detected in any soil sample, which we explain by the fast microbial degradation of fenhexamid under aerobic conditions in soil, resulting in a low DT<sub>50</sub> of approximately 1 day (Abbate et al. 2007; Borzì et al. 2007).

Although the soil under SC received higher fungicide loads (fludioxonil and cyprodinil), the fungicide concentrations 4 months after fungicide application were similar in both treatments, which point to a faster fungicide degradation under SC, especially in the 0–10 cm soil layer. In average, 27% of the fungicide concentrations measured 1 week after fungicide application were still present in soil 4 months later (without cyprodinil under PC), which point to a slower degradation as reported elsewhere ( $DT_{50} = 6-21$  days for fludioxonil and 2-45 days for cyprodinil) (Agriculture, and Environment Research Unit, University of Hertfordshire 2019; Liu et al. 2011a, b; Zhang et al. 2015). However, the majority of fungicide residues (up to 82%) in soil vanished from mid-June to mid-October, which can be attributed to microbial degradation or non-extractable residue binding to soil matrix and organic matter (Arias et al. 2005; Chen et al. 2018; Dec et al. 1997; Zhang et al. 2015). Because fludioxonil and cyprodinil have both low water solubility and hence a low leachability index (Table 1), leaching processes are unlikely to explain the fungicide declines. According to this, no hints were observed which point to relocation processes of both fungicides with soil depth over time. However, the small fungicide amounts found in the 30–60 cm soil layer 1 week after fungicide application might be explained by preferential flow pathways (Flury 1996; Köhne et al. 2009).

The increase of the cyprodinil concentrations under PC from mid-July to mid-June might be caused by washing off of cyprodinil, which got adsorbed to the plastic film during fungicide application, by the rainfall events between mid-June and mid-July. Many (lipophilic) pesticides have been shown to adsorb to plastic mulches (Guo et al. 2020; Nerín et al. 1996). Thus, an adsorption of fludioxonil and cyprodinil on plastic mulch during spraying seems likely to occur as both are lipophilic (log  $K_{\rm ow} \geq 4$ ) and exhibit a strong adsorption behavior (Agriculture, and Environment Research Unit, University of Hertfordshire 2019; Arias et al. 2005). Arias et al. (2005) described a higher tendency to desorb again for cyprodinil than for fludioxonil, possibly due to its higher water solubility (Table 1), which might explain why the fungicide increase under PC to mid-July was only found for cyprodinil.

Despite its lower application amount, the cyprodinil:fludioxonil ratios below 1.5 one week after fungicide application indicate an in relation higher fludioxonil concentration compared to cyprodinil, which is in contrast to lower  $DT_{50}$  in the field, reported for fludioxonil (6–21 days) than for cyprodinil (2–45 days)



(Agriculture, and Environment Research Unit, University of Hertfordshire 2019; Liu et al. 2011a, b; Zhang et al. 2015). A possible reason might be the lower water solubility of fludioxonil (Table 1) and thus its lesser accessibility to microbial degradation (Arias et al. 2005; Roberts et al. 1998). The successive desorption of cyprodinil from the plastic film might be responsible for the increased cyprodinil:fludioxonil ratios under PC 5 weeks and 4 months after fungicide application. Further studies addressing fungicide runoff from plastic-covered ridges and adsorption/desorption processes to plastic mulches are advisable to fully evaluate the influence of plastic mulches on fungicide fate.

Additionally, differences in microclimate, pH, and soil microbial biomass between treatments could have influenced degradation efficiency of fungicides and hence their residual concentrations in soil. Although available literature regarding the degradation of fludioxonil and cyprodinil residues in soil is scarce, it is assumed that soil temperature and moisture correlate positively with fungicide degradation (Fenoll et al. 2010; Liu et al. 2011a; Marín et al. 2003; Roberts et al. 1998). As fungicide residue degradation was mostly faster under SC than under PC, the higher soil temperature under PC obviously not accelerated degradation or this was probably balanced out by the mostly lower soil moisture under PC. Furthermore, soil microbial biomass and activity as well as SOC can influence the degradation of fludioxonil and cyprodinil in soil (Arias et al. 2005; Beigel et al. 1999; Roberts et al. 1998). Thus, the lower MBC under PC in mid-June might decreased fungicide degradation. Additionally, the lower pH under PC than under SC might have induced larger fractions of positive charged fludioxonil and cyprodinil molecules, which adsorb stronger to the soil matrix and to the larger SOC fraction under PC (Arias et al. 2006; Pose-Juan et al. 2011). This can reduce the accessibility of fungicide residues to degradation processes and thus additionally decelerate their degradation under PC.

# Influence of residual concentration of fungicides in soil under plastic and straw coverage on fungi and mycotoxins

Fungal population decreased from mid-June to mid-July as indicated by declining ergosterol concentrations, which was most likely induced by the fungicide residues (Pal et al. 2005; Tu 1993). The effect of fungicide residues on fungi seems to be concentration-dependent as largest declines in fungal populations were found in the topsoil (0–10 cm) under SC, followed by PC, which coincided with the highest loads of fungicide residues. The strong decline in ergosterol from mid-June to mid-July might be explained by the fact that ergosterol is not immediately degraded after fungal cell dead but only after several days (Zhao et al. 2005). However, fungal population recovers within the experimental period as indicated by ergosterol concentrations in mid-October comparable to the initial ones in late-April. The higher ergosterol concentrations

under SC than under PC in the 0–10 cm soil layer in mid-June and mid-October might result from the wide C:N ratio of the (wheat) straw mulch, which favor fungal growth (Bossuyt et al. 2001; Muñoz et al. 2017).

Because bacterial biomass have smaller C:N ratios ( $\approx 4$ ) than fungal biomass (≈ 10) (Sylvia et al. 2005), decreasing MBC:MBN ratios are indicative for decreasing fungal fractions in microbial communities (Campbell et al. 1991). Thus, the smaller MBC:MBN ratios and the larger MBN under SC in the topsoil, 5 weeks and 4 months after fungicide application, point to a shifted microbial community with larger bacterial and smaller fungal fractions. This could be initiated by a stronger intermediate dying-off of fungi under SC due to higher fungicide residues, which were reported elsewhere to favor strongly bacterial proliferation (Martinez-Toledo et al. 1998; Monkiedje 2002) and thus can shift microbial community (Chen et al. 2001a, b; Sigler and Turco 2002). The initially larger MBC under PC compared to SC in late-April might result from higher soil temperatures (> 2 °C) under PC. The lower MBC in mid-June and mid-July compared to mid-October are eventually a combined result from high soil temperature, low soil moisture, and the impact of the fungicide residues on microbial biomass, especially fungal biomass (Lal 2006; Pal et al. 2005; Smith et al. 1993; Yang et al. 2011).

The highest concentrations of the mycotoxins DON and 15-ADON 5 weeks after fungicide application coincide with the lowest values observed for ergosterol. The higher concentrations of DON and 15-ADON observed after fungicide application might be interpreted as a stress response of certain fungi strains to fungicide residues (Jouany 2007; Magan et al. 2002; Ponts 2015). Although literature is scarce on this topic, an increased mycotoxin production of DON and fumonisin B by certain Fusarium species after application of fungicides such as myclobutanil, azoxystrobin, and prothioconazole have already been reported for some crops (Audenaert et al. 2011; Li et al. 2018a, b; Simpson et al. 2001). The tendency to higher DON and 15-ADON concentrations under SC may point to a fungicide-induced mycotoxin production, which is corroborated by the significant positive correlation between cyprodinil and DON. But as mycotoxins can also result from stress induction by environmental and biological factors such as high temperatures, water or nutrient scarcity and competition (Medina et al. 2015; Reverberi et al. 2010; Schmidt-Heydt et al. 2008, 2011), maybe high soil temperatures and low soil moisture have additionally triggered mycotoxin formation. However, the investigation if the aforementioned conditions can also influence the occurrence of further mycotoxins such as ochratoxin A, fumonisin B1, or even aflatoxins, the latter are more relevant in hotter climates (Moretti et al. 2019; Sweeney and Dobson 1998) was beyond the scope of this study but should be investigated in further studies.

In summary, the higher fungicide concentrations under SC stronger reduced fungal biomass in the topsoil (0–10 cm) and



induced a higher mycotoxin occurrence of DON and 15-ADON 5 weeks after fungicide application. Thus, a fungicide concentration—dependent effect (and thus coverage type—dependent effect), which was proposed in hypothesis 2, was at least to some extent recognizable, especially in the topsoil where the differences between fungicide residues were largest. However, these effects were short-lived and not observed anymore 4 months after fungicide application.

### Influence of residual concentrations of fungicides in soil under plastic and straw coverage on soil organic matter decomposition

The increase in SOC from mid-June to mid-July might result from a reduced SOM degradation due to an inhibited and reduced microbial biomass by the fungicide residues (Chen et al. 2001a, 2001b; Chen and Edwards 2001). This assumption is corroborated by the in parallel occurring increase in C:N ratio and the decrease of DOC. DOC originates mainly from SOM degradation (Bolan et al. 2011) and can thus be used as an indicator for it. Furthermore, higher C:N ratios can be indicative for an accumulation of fresh biomass, which show normally higher C:N ratios and an easy accessibility for degradation by microorganisms (Blume et al. 2016). Especially fungi play an important role in the degradation of fresh biomass, which favor biomass with large C:N ratios because of their higher C demand (Bossuyt et al. 2001; Keiblinger et al. 2010). In mid-July, the MBC:SOC ratio is partly below 2, which is seen as critical for soil fertility (Anderson 2003) and indicates a low conversion of SOM to MBC and a low SOM degradation (Sparling 1992). However, as confirmed by the strongly increased MBC:SOC ratio in mid-October, the low MBC:SOC ratios might indicate a temporary effect of the fungicide residues on microbial biomass and SOM decomposition.

In summary, our results indicate that the fungicide residues temporarily inhibit and reduce microbial biomass and thus decelerate SOM decomposition, which confirms hypothesis 3. However, no concentration-dependent, and thus treatment-dependent, effect of the fungicide residues could be observed. It can be discussed that the reducing effect on microbial biomass by the fungicide residues might have additionally been enhanced by higher soil temperatures and lower soil moisture during the summer months (Andrade et al. 2003; Smith et al. 1993). However, continuous irrigation by the farmer should have kept soil moisture high enough to maintain microbial growth and activities.

### Conclusion

PC reduces the entry of fludioxonil and cyprodinil into soil after fungicide application compared to SC. The higher

fludioxonil and cyprodinil residues under SC strongly reduced the fungal biomass in the topsoil and enhanced the DON and 15-ADON concentrations 5 weeks after fungicide application, which can be interpreted as stress response of the fungal community to the fungicides. Fungal populations were recovered within 4 months, but indications were found that the higher fungicide concentrations under SC had shifted microbial community towards larger bacterial fractions. Furthermore, SOM decomposition was temporarily reduced under both mulching types, presumably by an inhibited and reduced microbial community due to the fungicide residues. In summary, although PC and SC resulted in different amounts of fungicide residues in soil, their effects (partly coverage-dependent) on microbial biomass, fusarium mycotoxin occurrence, and SOM decomposition were short-lived and vanished within 4 months and seem thus not critical for long-term soil fertility and agricultural productivity. However, whether the fungicides can change the structure of the microbial community, especially the fungal community, needs further investigation. Finally, the delayed cyprodinil entry into soil under PC, presumably due to desorption from the plastic film after rainfalls, advises to address the influence of plastic mulching on fungicide adsorption and desorption in further studies.

**Abbreviations** SOM, Soil organic matter; DT<sub>50</sub>, Half-life dissipation time; SC, Straw-covered ridge-furrow system with subsurface drip irrigation; PC, Plastic-covered ridge-furrow system with subsurface drip irrigation; TN, Total nitrogen; LC-HRMS, Liquid chromatography—highresolution mass spectrometry; LCL, Lowest calibration level; RSD, Relative standard deviation; MBC, Microbial biomass carbon; MBN, Microbial biomass nitrogen; HPLC, High-performance liquid chromatography; LOD, Limit of detection; DON, Deoxynivalenol; 15-ADON, 15-Acetyldeoxynivalenol; NIV, Nivalenol; ZEN, Zearalenone; SOC, Soil organic carbon; DOC, Dissolved organic carbon; LSD, Least significance distance

Acknowledgements We want to thank Christof Steegmüller for providing access to the sampling site and for his expertise on strawberry cultivation; Christian Buchmann, Kilian Kenngott, and Zacharias Steinmetz for valuable assistance during soil sampling; and Lea Bosch, Maria Olivares, Johanna Girardi, Collins Ogbeide, and Ken Mauser for valuable assistance during sample analyses. Furthermore, we would like to thank Prof. S. Thiele-Bruhn (University of Trier) for providing the field measuring station.

Author contribution The concept and the experimental design of this paper was developed by MM in cooperation with KM. All soil samplings, laboratory analyses, data evaluations, and statistics were planned and conducted by MM with the help of DD and KM. Soil samplings and laboratory analyses were additionally supported by technical and student staff. Data interpretation and paper writing were done by MM in collaboration with DD, GES, and KM.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by the Ministry for Education, Sciences, Further Education and Culture of the State of Rhineland-Palatinate (MBWWK) in the frame of the Interdisciplinary Research Group for Environmental Studies (IFG-Umwelt) of University Koblenz-Landau, by the Prof. B.



Gedek and W. Gedek foundation and by the research fund of the University of Koblenz-Landau.

**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on a reasonable request.

### **Declarations**

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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### **CHAPTER 5**

Multiannual soil mulching in agriculture: analysis of biogeochemical soil processes under plastic and straw mulches in a 3-year field study in strawberry cultivation

Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Journal of Soils and Sediments. 21:3733-3752

### SOILS, SEC 1 • SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING • RESEARCH ARTICLE



# Multiannual soil mulching in agriculture: analysis of biogeochemical soil processes under plastic and straw mulches in a 3-year field study in strawberry cultivation

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Received: 23 March 2021 / Accepted: 24 July 2021 © The Author(s) 2021

Published online: 04 August 2021

#### Abstract

**Purpose** The application of plastic mulching differs globally as well as climate, soils, crops, and agricultural practices, making it difficult to generalize the reported impacts on soil. Because literature is scarce about the influence of plastic mulching on soil under temperate, humid climate, the objective of this study was to understand how multiannual plastic mulching influences central soil parameters and processes under Central European cultivation conditions to evaluate its impact on soil quality in the long term.

Materials and methods Central soil parameters and processes like leaching, aggregation, soil organic matter (SOM), and microbial biomass were investigated in a strawberry cultivation in Southwestern Germany. The field experiment compared a plastic-covered ridge–furrow system with subsurface drip irrigation (PC) to the same system with straw coverage (SC) in three soil layers (0–10, 10–30, and 30–60 cm) at seven dates within a 3-year period. Soil analyses comprised soil temperature and moisture, pH, bulk density, water-stable aggregates, soil organic carbon, dissolved organic carbon, and microbial biomass carbon and nitrogen.

Results Rainfall infiltration impeded by PC reduces soil moisture but neither reduces leaching nor promotes (macro-)aggregate formation or stability; however, it maintains a loose and friable soil structure in surface soil (0–5 cm), compared to SC. PC promotes SOM accumulation and shifted SOM composition to a more hardly degradable SOM, especially below the topsoil (10–60 cm). Furthermore, PC revealed no indications of an increased microbial biomass or activity accompanied with an enhanced SOM decomposition due to the shifted microclimate. The seasonal, time- and depth-dependent effects, observed in some parameters, emphasize the importance to include them in future studies for a more holistic process understanding. Conclusion Our study showed no indications that multiannual plastic mulching influences soil quality within the 3 years of this study. Further research is advisable to support our findings on a larger scale and longer time periods and across various soil and crop types.

**Keywords** Drip-irrigated ridge-furrow mulching · Humid region · Soil structure · Aggregate stability · Soil organic matter · Soil microbial biomass

	Abbreviations			
	SOM	Soil organic matter		
Responsible editor: Caixian Tang	PC	Plastic-covered ridge–furrow system with subsurface drip irrigation		
☐ Gabriele Ellen Schaumann schaumann@uni-landau.de	SC	Straw-covered ridge-furrow system with sub- surface drip irrigation		
Group of Environmental and Soil Chemistry, iES Landau,	CEC	Cation-exchange capacity		
Institute for Environmental Sciences, University of Koblenz-	<sup>1</sup> H-NMR	Proton nuclear magnetic resonance		
Landau, Landau, Germany	SOC	Soil organic carbon		
Group of Organic and Ecological Chemistry, iES	C:N ratio	Carbon-to-nitrogen ratio		
Landau, Institute for Environmental Sciences, University of Koblenz-Landau, Landau, Germany	MBC	Microbial biomass carbon		



MBN Microbial biomass nitrogen DOC Dissolved organic carbon

### 1 Introduction

During the past decades, plastic mulching has become a worldwide practice, and further expansion is forecasted for the next years (Sintim and Flury 2017; Transparency Market Research 2020). Plastic mulching is applied for a variety of purposes; all aim to improve growth and harvest conditions and thus agronomic productivity (reviewed in Gan et al. 2013; Steinmetz et al. 2016). Different materials are used to produce plastic mulches, which are applied in various thicknesses, mulching arrangements, and periods (Kasirajan and Ngouajio 2012; Liu et al. 2014; Zhao et al. 2018) to various crops, soil types, and climatic conditions. Application and purposes of plastic mulching can differ globally: In China, with 20 million ha of plastic-covered farmland and globally the largest user of plastic mulches (Liu et al. 2014; Mordor Intelligence 2020), mainly film thicknesses < 0.008 mm are used, whereas in Europe and the USA often film thicknesses ≥ 0.020 mm are used (Liu et al. 2014; Ingman et al. 2015). In China, plastic mulching is mainly used to improve water use efficiency to ensure agronomic productivity under water-scarce conditions, as large parts of the farmland are located in arid and semiarid regions (Deng et al. 2006; Han et al. 2014; Zhang et al. 2018; Gao et al. 2019). Conversely, in Europe, plastic mulching is mainly used to improve product quality and to promote earliness or extend harvest periods (Scarascia-Mugnozza et al. 2011; Neri et al. 2012) and is hence often used for premium and seasonal products, such as strawberries, asparagus, and other vegetables (Scarascia-Mugnozza et al. 2011; Steinmetz et al. 2016).

Most current research and scientific literature on plastic mulching and its impacts on soil is based in China, whereas in Europe, scientific literature and research is scarce on this topic, despite of its increasing application (Mordor Intelligence 2020). Since application of plastic mulching can differ globally, as exemplarily described above, as well as climate, soils, crops, and agricultural practices (Farmer et al. 2017; Ma et al. 2018), it is difficult to extrapolate the reported impacts on soil to European cultivation condition. Furthermore, Steinmetz et al. (2016) pointed out that most recent studies focused on individual effects of plastic mulches, particularly on their short-term agronomic benefits, whereas a substantial process understanding of its impact on key soil parameters and processes is still missing but necessary to evaluate the impact of plastic mulching on soil quality in the long term. Additionally, less literature is available on the influence of plastic mulching on soil below the topsoil layer (0–10 cm) and over the temporal course of a multiannual

application, typical in, e.g., strawberry and asparagus cultivation (Steinmetz et al. 2016).

A common plastic mulching application, e.g., in strawberry cultivation, is to cover the ridges of a ridge-furrow system with a black plastic film (mostly black polyethylene), often combined with subsurface drip irrigation (Neri et al. 2012; Poling 2016). The optical properties and the impermeability (physical barrier) of the plastic film influence heat transfer and impede gas and mass exchange between soil surface and surroundings (Ham et al. 1993; Ham and Kluitenberg 1994; Khan et al. 2000). This increases soil temperature; reduces evaporation, rainfall infiltration, and entry of aboveground biomass; suppresses weed growth; and decreases soil erosion by wind and water (reviewed in Gan et al. 2013; Steinmetz et al. 2016). This in turn can impact on biogeochemical soil processes such as aggregation, leaching, soil organic matter (SOM) decomposition, and microbial biomass growth and activity.

The objective of this study was to provide information on the influence of multiannual plastic mulching application on biogeochemical soil processes, governing SOM quality, soil structure, and microbial biomass, under humid Central European cultivation conditions to better estimate the plastic mulching impact on soil quality.

Rainfall increases soil moisture and can induce seepage water flows after infiltration, which can increase nutrient leaching (Cameron et al. 2013). Furthermore, rainfalls can cause aggregate slaking and dispersion at the soil surface, resulting in crusted soil with a higher density and erodibility and lower aeration (Bronick and Lal 2005; Bing So 2006). Consequently, we hypothesized the following: (1) The water-impermeable PC reduces leaching due to impeded rainfall infiltration and hence impeded seepage water flow during rainfall events. (2) PC maintains a loose and friable soil structure in the topsoil, because PC prevents aggregate slaking and dispersion on the soil surface during rainfall and thus crust formation, soil silting, and compaction.

Soil temperature and moisture influence the shoot and root growth of plants (Kumar and Dey 2011; Gan et al. 2013) and growth and activity of microorganisms (Coûteaux et al. 1995; Pietikåinen et al. 2005). Microbial biomass, SOM, roots, and soil moisture influence aggregate formation and stability (Bronick and Lal 2005). Thus, the third hypothesis was as follows: (3) The elevated soil temperature and moisture under PC promotes root and microbial biomass growth and leads to increasing macroaggregate formation and stability. Plant growth affects SOM inputs into soil (Grego and Lagomarsino 2008; Jackson et al. 2017), whereas microbial biomass and activity correlate positively with SOM decomposition (von Lützow and Kögel-Knabner 2009; Stockmann et al. 2013). SOM input and decomposition govern the quantity and composition of SOM (von Lützow et al. 2006). Derived from this, we hypothesized



that (4) PC reduces SOM due to an impeded entry of aboveground biomass into soil and enhanced SOM decomposition, induced by the larger microbial biomass under PC. (5) Furthermore, PC reduces the fresh and fast-mineralizable SOM and thus shifts SOM composition towards an older, hardly degradable SOM.

In order to test this hypotheses, the respective soil processes were investigated in a plastic-covered ridge–furrow system with subsurface drip irrigation (PC) in comparison to the same system with straw coverage (SC) in three soil layers (0–10, 10–30, and 30–60 cm) at seven dates within a 3-year period of strawberry cultivation in Southwestern Germany. With this experiment design, an additional focus was set on how the plastic coverage influences soil in a temporal course and in two soil layers below the topsoil (0–10 cm).

### 2 Material and methods

### 2.1 Site description and field establishment

The sampling site was a commercial strawberry field in Southwestern Germany (49° 11' N, 8° 10' E, 130 m asl), located in a temperate, humid climate with an annual average rainfall of 643 mm year<sup>-1</sup> (weather station of Landau-Wollmesheim, Agrarmeteorologie Rheinland-Pfalz). According to FAO soil classification, the soil type was classified as a silt loam (Anthrosol) (IUSS Working Group WRB 2015) and the soil texture consisted of  $7 \pm 2\%$  sand,  $83 \pm 5\%$  silt, and  $10 \pm 3\%$  clay in the 0–60-cm soil layer. The sampling field was cultivated with winter wheat in the previous season and had neither been cultivated with strawberries nor been mulched with plastic or straw in recent years. After tillage and fertilization (MALTaflor® and NPK (Mg) fertilizer), a raster sampling was conducted on the field (May 2016), which identified no significant gradients or inhomogeneities of soil properties that may interfere with the experiment design. A ridge-furrow system was established in the field (late June 2016) with subsurface drip irrigation and black plastic-covered ridges (polyethylene, 50 µm) and bare furrows. Strawberries (Fragaria × ananassa, 'Malwina') were planted as bare-root plants in mid-July 2016 at the ridges in double rows with a 40-cm distance between plants (8 plants per m<sup>2</sup>). A straw coverage (wheat straw) was applied to the furrows in April 2017 and was renewed every year. Further information about the sampling site can be found in Meyer et al. (2020).

### 2.2 Experimental design and soil sampling

In short, a semi-controlled field experiment was designed that reflected current agricultural practice while enabling us to study soil processes in a homogeneous soil type and avoiding masking of treatment effects by landscape variation and edge effects. Two treatment areas were chosen  $(21 \times 10 \text{ m})$ , respectively, one with plastic-covered and one with straw-covered ridges (PC and SC), in which respectively five sampling plots  $(10 \times 1.5 \text{ m})$  were randomly chosen for soil sampling: PC (n=5) and SC (n=5). To establish the SC treatment area, the plastic film was manually removed from the ridges immediately after field setup (July 2016). As usual in strawberry cultivation, the ridges were covered with wheat straw before the first harvest (April 2017) and the straw cover was yearly renewed.

Ten soil samplings were conducted during a 3-year period of strawberry cultivation from 2016 to 2019 (SI Fig. 1): During the establishment period of strawberries, three samplings in 2-month intervals were conducted after strawberry plantation from late July to late November in 2016 (T0-T2) to identify a potential short-term impact of PC on soil parameters and processes after field setup and strawberry plantation (results described in Meyer et al. (2020)). The results of T0 (25 July 2016) and T1 (26 September 2016) are presented here again to describe the initial field conditions and to draw conclusions over the full 3-year investigation period. For the remaining investigation period, five samplings were conducted in larger time intervals ( $\geq 6$  months) on 25 April 2017 (T3), 9 October 2017 (T6), 3 May 2018 (T7), 11 October 2018 (T8), and 23 July 2019 (T9). In order to give a complete overview over the samplings conducted in the 3-year field study, two further samplings have to be mentioned here: They were conducted on 19 June (T4) and 18 July (T5) in 2017 after fungicide application to estimate the influence of both coverage types (plastic vs. straw) on fungicide residues in soil and their impact on mycotoxin occurrence, microbial biomass, and SOM decomposition (results described in Meyer et al. (2021)). Besides coverage type, the agricultural practice was identical in both treatments: Subsurface drip irrigation (three emitters per meter) was applied from March until September each year depending on weather conditions for 3-4 h a day (7-11 L water per meter). The field was weekly fertilized with a mineral fertilizer (15 kg N, 5 kg P, 30 kg K, 2 kg Mg) via drip irrigation during an 8-week period from March to May each year. The fungicides Switch® (37.5% cyprodinil and 25% fludioxonil) and Teldor® (50% fenhexamid) were yearly applied during strawberry bloom with an application rate of 1 and 2 kg ha<sup>-1</sup>, respectively.

Composite soil samples (five single cores) were taken in the ridges of each sampling plot in the surface, root and subsoil layer (0–10, 10–30, and 30–60 cm) at each sampling date. Soil samples were collected with stainless steel soil sampling rings (0–10 cm) and a boring rod (> 10 cm). Further information about experimental design and soil sampling can be found in Meyer et al. (2020).



Besides the pore size distribution, the same soil parameters were determined in this study as in Meyer et al. (2020) with identical methods. Therefore, these methods are subsequently only briefly outlined.

### 2.3 Soil temperature and moisture

A field measuring station (ecoTech®, Bonn, Germany) recorded hourly soil temperature and moisture in both treatment areas at the 5-, 15-, and 35-cm soil depths, according to the investigated soil layers in the field experiment. Air temperature and precipitation data were received from the weather station of Landau-Wollmesheim (Agrarmeteorologie, Rheinland-Pfalz). The differences in soil temperature and moisture between SC and PC were calculated for each soil depth based on daily means. The data were smoothened by calculating for each day a 60-day mean (30 days before and after this day).

### 2.4 Soil physicochemical parameters

The cation-exchange capacity (CEC) was determined at the first (T0) and last sampling (T9), according to DIN ISO 11,260:1997–05. In short, field-fresh soil was threefold extracted with 0.1 M BaCl<sub>2</sub> solution and subsequently with 0.02 M MgSO<sub>4</sub> solution. Both extracts were analyzed with inductively coupled plasma-optical emission spectrometry (Agilent 720 Series, Thermo Fisher Scientific, Karlsruhe, Germany). Soil pH in 0.01 M CaCl<sub>2</sub> solution and electrical conductivity in deionized water were determined with airdried soil, based on DIN EN 15,933:2012–11 and DIN CEN/TS 15,937:2013–08, respectively. Total N was measured in milled, oven-dried soil with a CHNS analyzer (vario Micro-CUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany).

### 2.5 Soil structure indicators

Bulk density, pore size distribution, and water-stable aggregates are interconnected soil structure parameters, which can be used to assess the influence of agricultural practices on soil structural stability, aeration, water-holding capacity, and water movement (Bronick and Lal 2005; Lal 2006). The determination of the bulk density and the pore size distribution was restricted to the 0–5-cm soil layer, since the impact of coverage type was expected mainly in the surface soil. The dry bulk density was determined gravimetrically, according to DIN ISO 11,272:2014–06. The pore size distribution was determined with the method described in Meyer et al. (2018). In brief, soil cores were sampled with plastic rings (d = 3.64 cm, h = 5 cm) in triplicates at one sampling plot in both treatment areas, respectively. Samples

were completely saturated with water, and subsequently, a matric potential of -0.3 kPa was adjusted with a sandbox (08.01 sandbox, Eijkelkamp, Zeitz, Germany). Samples were weighted at matric potential of -0.3 kPa and subsequently measured with proton nuclear magnetic resonance (<sup>1</sup>H-NMR) relaxometry, using a Bruker Minispec MQ, version 2.2 (Bruker BioSpin, Rheinstetten, Germany). After <sup>1</sup>H-NMR measurement, a replicate was centrifuged for 20 min at 2000 g with a Universal 320 centrifuge (Hettich Lab Technology, Tuttlingen, Germany) to remove bulk solution, using centrifuge tubes with 2-µm filter inserts. The bulk solution was afterwards measured with <sup>1</sup>H-NMR relaxometry to receive the bulk relaxation time. Two replicates were dried at 105 °C to determine the gravimetrical water content at matric potential -0.3 kPa. The <sup>1</sup>H-NMR device measured the transverse relaxation time with a Carr-Purcell-Meiboom-Gill pulse sequence at a magnetic field strength of 0.176 T and the following instrument settings: 256 scans were conducted and the echo time was set to 300 µs. Gain was adjusted to 80–90% signal intensity. The repetition time and the number of 180° pulses were set individually such that complete magnetization decay of the prior measurement was enabled. The Butler, Reeds, and Dawson algorithm (Butler et al. 1981) was used in MAT-LAB 7.7.0 (R2008b) to convert <sup>1</sup>H-NMR data into relaxation time distributions. Each relaxation time constant of the relaxation time distributions was bulk-corrected with the bulk relaxation time and subsequently transformed with the "common calibration curve" into pore sizes. The amplitudes associated with the relaxation time constants were transformed with the gravimetric water content of a sample into pore volume (the sum of all amplitudes is equal to the gravimetric water content of a sample). The volumes of the macropore, medium pore, and micropore domains (> 10, 0.2–10, and  $< 0.2 \mu m$ ) were calculated from the pore size distributions (Blume et al. 2016) by cumulating the volumes of the single pore sizes belonging to the pore range of the respective pore domains. The water-stable aggregates (soil aggregates > 0.2 mm) of the 1-2-mm aggregate fraction was determined by the wetsieving procedure (Buchmann et al. 2015). The 1-2-mm aggregate fraction was separated from air-dried, sieved (<2-mm) soil samples with a 1-mm sieve, and its weight percent of the total soil fraction was determined.

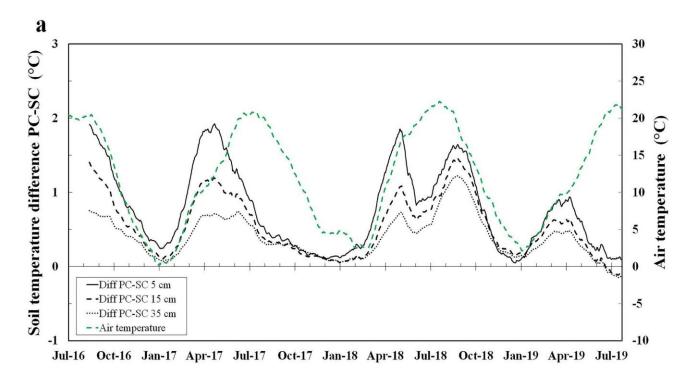
### 2.6 Characterization of SOM

We used the soil organic carbon (SOC) to quantify SOM and the C:N ratio as an indicator for degradability and transformation velocity of SOM (Kindler et al. 2011). Soil microbial carbon (MBC) and nitrogen (MBN) were used to quantify soil microbial biomass. The MBC:MBN



ratio was used to estimate the microbial community composition (Moore et al. 2000). Because of their rapid turnover rates, MBC and dissolved organic carbon (DOC) can be used as indicators for changes in SOM due to management practices (Haynes 2005). In order to identify potential changes in different SOM pools,

the SOM was separated depending on density into free, aggregate-occluded, and mineral-associated SOM fraction. According to their turnover rates, these SOM fractions are associated with the active, slow (or intermediate), and passive (or inert) SOM pool, respectively (von Lützow et al. 2007).



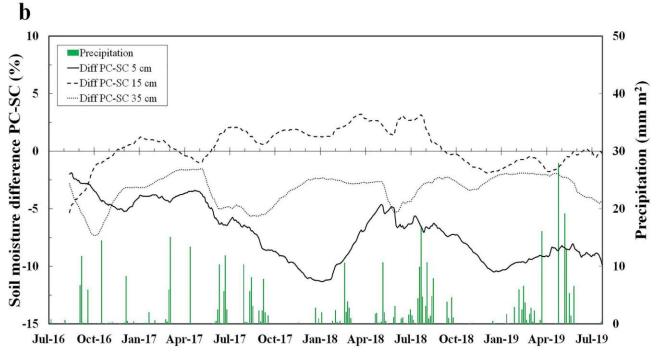


Fig. 1 Soil temperature and moisture of the 3-year field experiment in strawberry cultivation (*Fragaria*×*ananassa*, 'Malwina'). a Smoothened soil temperature differences, based on daily means, between plastic coverage (PC) and straw coverage (SC) measured at 5-, 15-,

and 35-cm soil depths and daily mean air temperature measured 2 m above ground. **b** Smoothened soil moisture differences, based on daily means, between plastic coverage (PC) and straw coverage (SC) measured at 5-, 15-, and 35-cm soil depths and daily precipitation



### 2.6.1 Analysis of SOC and DOC

The SOC was measured in accordance with Harris et al. (2001). After carbonate removal with HCl fumigation, the oven-dried soil samples were analyzed for carbon content with a CHNS analyzer (vario MicroCUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany). In accordance with DIN EN 1484:1997–05, the DOC was measured in filtrated soil extracts (0.45 µm) from field-fresh soil with a TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany).

# 2.6.2 Separation of SOM into free, aggregate-occluded, and mineral-associated SOM fractions by density fractionation

The density fractionation was conducted through an adapted method, principally based on the methods described by Cerli et al. (2012) and Ontl et al. (2015): In brief, air-dried, sieved soil (2 mm) was carefully extracted with sodium polytungstate solution (1.6 g cm<sup>-3</sup>). The centrifuged supernatant was filtered (20 µm), and filter residues were quantified gravimetrically (free SOM) after cleaning (deionized water) and drying (60 °C). In a second step, soil was re-suspended with sodium polytungstate solution and treated ultrasonically (350 J mL<sup>-1</sup>) for aggregate disruption. Again, the centrifuged supernatant was filtered (20 µm) and filter residues were quantified gravimetrically (aggregate-occluded SOM). The mineral-associated SOM fraction was obtained by subtracting free and aggregate-occluded SOM from the total SOM. Total SOM was obtained by multiplying the SOC values by a factor of 2 (Blume et al. 2016).

### 2.6.3 Analysis of MBC and MBN

The MBC and MBN were determined by the chloroform-fumigation method (Vance et al. 1987; Blume 2000): Respectively, a chloroform-fumigated and a non-fumigated sample of field-fresh soil were extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> solution. The filtered soil extracts were analyzed for carbon content with a TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany) and for ninhydrin-reactive nitrogen (Joergensen and Brookes 1990) with a photometer (Specord50, Analytik Jena GmbH, Jena, Germany).

### 2.7 Statistical analyses

The correlation of two variables was estimated with Pearson's correlation coefficient or Spearman's rho if data were not normally distributed. The normality distribution of data was examined graphically with histograms and quantile—quantile plots. Mixed factorial ANOVAs with coverage time and soil depth as repeated factors and treatment

**Fig. 2** Physicochemical soil properties. **a** pH (in 0.01 M CaCl<sub>2</sub>) deter-▶ mined in the 0–10-, 10–30-, and 30–60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experiment in strawberry cultivation (*Fragaria*×*ananassa*, 'Malwina'), respectively, shown as mean with standard error (*n*=5). **b** Electrical conductivity. **c** Total nitrogen

as fixed factor were applied to determine significant differences between means. If significant interaction effects were determined, additional ANOVAs, with least significance distance testing as post hoc test, were applied to locate significant differences. Variance homogeneity was confirmed with Levene's test. Differences were reported as statistically significant if the probability of error was < 0.05. All statistical analyses were done with IBM SPSS Statistics 25.

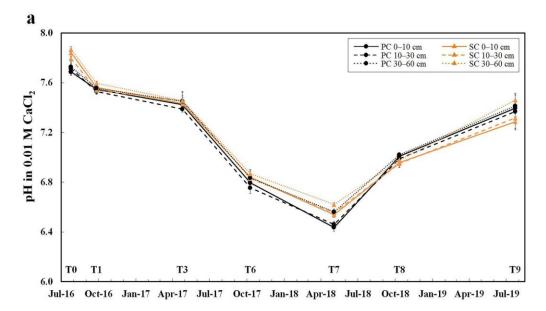
### 3 Results

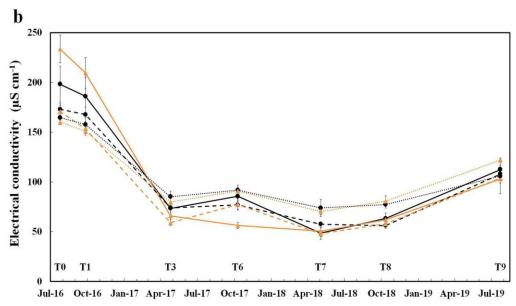
### 3.1 Soil temperature and moisture

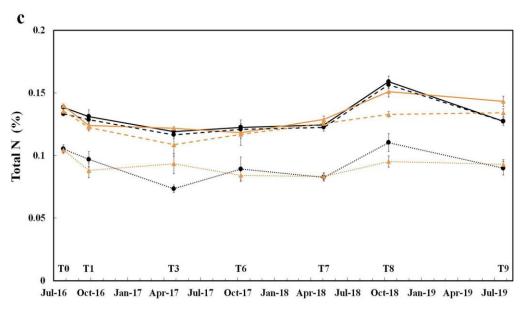
Soil temperature differences between PC and SC (Fig. 1a, SI Fig. 2) showed that soil temperature was higher under PC during the 3-year sampling period. Maxima of up to 2 °C higher soil temperature under PC were yearly found in spring (March–June) and additionally in late summer and autumn (August–October) in 2016 and 2018. During these periods, soil depth had a clear effect on soil temperature difference (differences of > 0.5 °C between soil depths), which decreased with increasing soil depth. No or only small differences (<0.2 °C) were observed between soil depths in the remaining autumn and winter periods. The hourly recorded data revealed up to 6.5 °C higher soil temperatures under PC than under SC at 5-cm soil depth during midday on sunny days in the summer season (data not shown).

The differences in soil moisture between PC and SC (Fig. 1b, SI Fig. 3) demonstrated that soil moisture under SC was about 5-10 and 2-5% higher at the 5- and 35-cm soil depths, respectively (with maxima of > 10% in winter 2018 and 2019 at 5 cm and of  $\geq$  5% in summer and autumn at 35-cm depth). In contrast, the 15-cm soil depth showed partly higher soil moisture under PC (mainly from June 2017 to September 2018) and, generally, the smallest differences in soil moisture between treatments (mostly < 2%). The 20 largest daily rainfall events during the sampling period (15.1 to 39.8 mm) showed that the increase in soil moisture after rainfalls was clearly lower and mostly delayed at 5-cm soil depth under PC in comparison to SC (SI Fig. 4a-p). In contrast, soil moisture increases were greater after rainfalls under PC than under SC at the 35-cm depth and partly at the 15-cm soil depth. Generally, the increase in soil moisture after rainfalls decreased and was temporally delayed with increasing

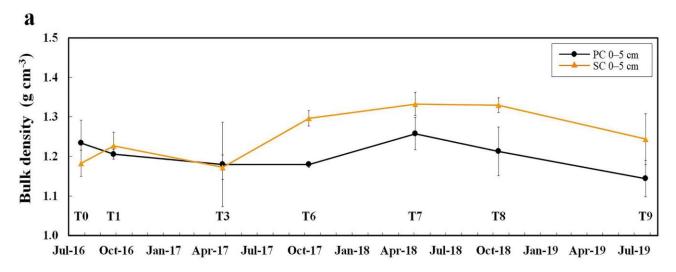












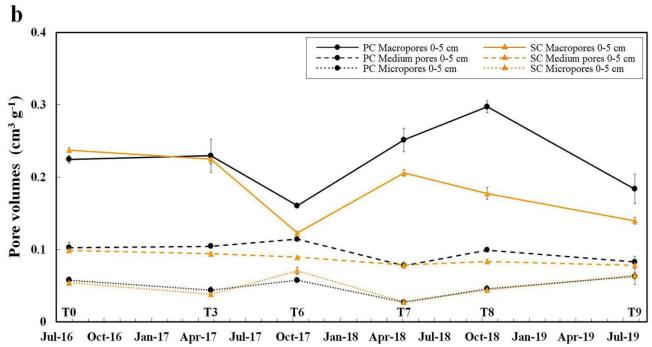


Fig. 3 Soil structural indicators. **a** Bulk density determined in the 0–5-cm soil layer under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experiment in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=3). **b** Pore volumes of the macropore,

medium pore, and micropore domains (derived from quantitative pore size distributions) determined in the 0–5-cm soil layer under plastic coverage (PC) and straw coverage (SC) at six dates within the 3-year field experiment in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=3)

soil depth under SC, whereas under PC, the lowest and most delayed increase in soil moisture was observed at 5-cm soil depth.

#### 3.2 Physicochemical soil parameters

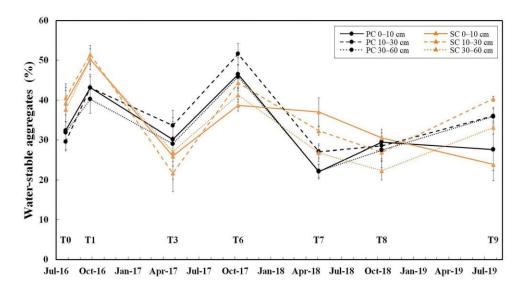
The CEC (Table 1) was between 97 and 107 cmol kg<sup>-1</sup> at T0 and increased in all soil layers under SC (7–18 cmol kg<sup>-1</sup>) and PC (6–12 cmol kg<sup>-1</sup>) during the sampling period (p<0.001).

The pH, electrical conductivity, and total N (Fig. 2a–c) showed a similar pattern in their temporal fluctuations

during the sampling period. The pH was around 7.8 in all soil layer under both treatments at T0 and decreased to T7 by ~ 1.3 units (p < 0.001) and increased to T9 by ~ 0.9 units (p < 0.001). Similar to pH, the electrical conductivity was between 160 and 234  $\mu$ S cm<sup>-1</sup> at T0 and strongly declined by 79–168  $\mu$ S cm<sup>-1</sup> in all soil layers to T3 (p < 0.001), remained between 50 and 90  $\mu$ S cm<sup>-1</sup> from T3 to T8, and increased again by 29–52  $\mu$ S cm<sup>-1</sup> to T9 (p < 0.001). The total N was between 0.07 and 0.14% from T0 to T6 and showed a strong increase between T7 and T8 (p < 0.001) followed by a decrease to T9 (p < 0.001). This effect was stronger under PC (~0.03%) than under



Fig. 4 Water-stable aggregate fractions of the 1–2-mm aggregate fraction determined in the 0–10-, 10–30-, and 30–60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experiment in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=5)



SC (~0.01%), such that total N was higher under PC at T8 (10–30-cm soil layer, p < 0.001) and lower at T9 (0–10-cm soil layer, p = 0.015) compared to SC. Mostly small differences and no clear trends were observed between treatments and soil layers for pH (<0.15 units), electrical conductivity (<15  $\mu$ S cm<sup>-1</sup>), and total N (<0.005%). Only total N in the 30–60-cm soil layer of both treatments was on average 0.04% lower than in both upper layers (p < 0.001).

#### 3.3 Soil structural indicators

The bulk density (Fig. 3a) was comparable in both treatments from T0 to T3 (1.17–1.23 g cm<sup>-3</sup>) but increased under SC to 1.33 g cm<sup>-3</sup> at T7 (p = 0.037) and was 0.08–0.12 g cm<sup>-3</sup> higher under SC than under PC from T6 to T9 (T6, p = 0.016). The bulk density under PC did not change significantly during the experiment, but as a trend, a slight decrease was revealed from T0 (1.23 g cm<sup>-3</sup>) to T9 (1.14 g cm<sup>-3</sup>).

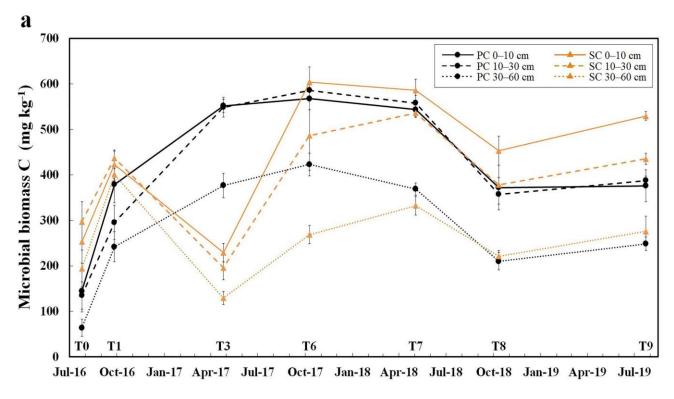
**Table 1** Cation-exchange capacity (CEC) determined in the 0–10-, 10-30-, and 30-60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at the beginning (T0) and end (T9) of the 3-year field experiment in strawberry cultivation (*Fragaria*×*ananassa*, 'Malwina'), respectively, shown as mean with standard error (n=5)

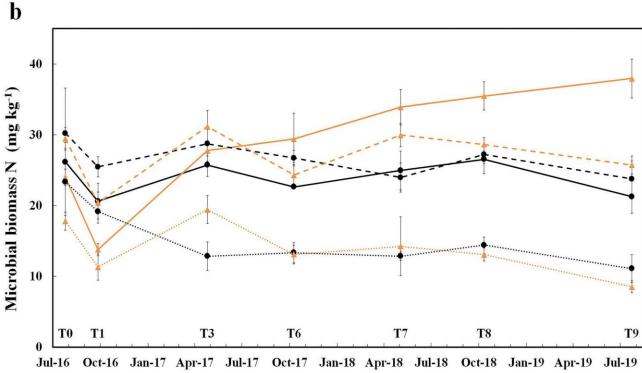
Sampling	Soil layer	PC	SC
		CEC (cmol kg <sup>-1</sup> )	CEC (cmol kg <sup>-1</sup> )
T0 (July 2016)	0–10 cm	106±2	97 ± 1
	10-30 cm	$106 \pm 2$	$102 \pm 3$
	30-60 cm	$107 \pm 2$	$103 \pm 1$
T9 (July 2019)	0-10 cm	$114 \pm 5$	$115\pm2$
	10-30 cm	$118 \pm 1$	$112 \pm 1$
	30-60 cm	$113 \pm 4$	$110\pm4$

Treatment-related differences in the pore volumes (Fig. 3b, SI Fig. 5) in the 0-5-cm soil layer increased with increasing pore size and time from T0 to T8. Starting with comparable values at T0, the PC maintained a 0.04–0.12 cm<sup>3</sup> g<sup>-1</sup> larger macropore volume from T6 to T9 (T8, p = 0.001) and a 0.02–0.03 cm<sup>3</sup> g<sup>-1</sup> larger medium pore volume at T6 and T8 (T8, p = 0.038) compared to SC, whereas the micropore volume remained unaffected. During the sampling period, the macropore volumes declined by 0.04 and 0.1 cm<sup>3</sup> g<sup>-1</sup> under PC and SC, respectively (SC, p < 0.001), whereas the medium pore volumes decreased by  $0.02 \text{ cm}^3 \text{ g}^{-1}$  in both treatments (p = 0.011). The results of T6 were excluded from statistical analyses and should be carefully interpreted as three samples have to be discarded due to accidentally captured earthworms, which destroyed the samples during water saturation.

The weight percentage of the 1–2-mm aggregate fraction in soil (SI Fig. 6) showed no clear differences between treatments or soil layers but decreased from 47 to 58% at T0 to 22–32% at T7 (p < 0.001) and increased strongly to 71–80% at T8 (p < 0.001). The water-stable aggregates (Fig. 4) showed large seasonal fluctuations (up to 30%), which occurred simultaneously but with different extents in all soil layers of both treatments from T0 to T7. A seasonal pattern was observed (exception T8) with a low water-stable aggregate fraction in spring (22–32%), an intermediate water-stable aggregate fraction in summer (28-41%), and a high water-stable aggregate fraction in autumn (39-52%). The effects of treatment and soil layers were mostly < 10% and hardly interpretable because of the large standard errors. However, as a trend, the water-stable aggregate fractions under PC were larger at T3 and T6 and smaller at T1 and T7 compared to SC. The largest water-stable aggregate fractions were (mostly) found in the 10-30-cm soil layer of both treatments. The







**Fig. 5** Soil microbial biomass. **a** Soil microbial biomass carbon determined in the 0–10-, 10–30-, and 30–60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year

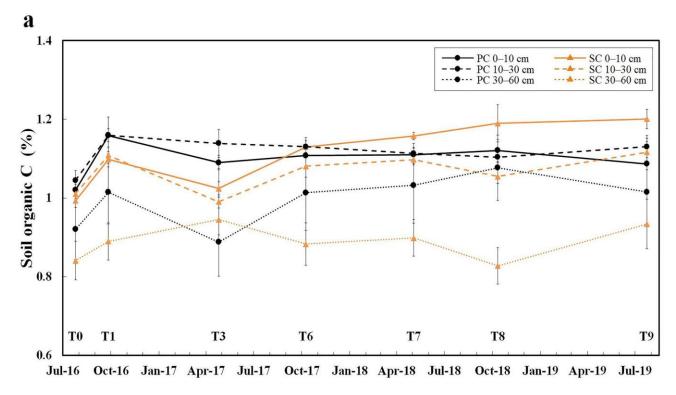
field experiment in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=5). **b** Soil microbial biomass nitrogen

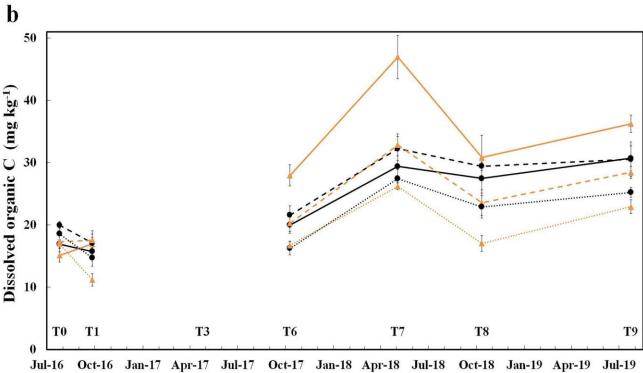
water-stable aggregates correlated positively with CEC ( $r_S$  = 0.264, p = 0.041, n = 60), pH ( $r_S$  = 0.188, p = 0.006, n = 210), electrical conductivity (r = 0.341, p < 0.001, n = 210), MBC (r = 0.231, p = 0.001, n = 210), and DOC (r = 0.335, p < 0.001, n = 180).

#### 3.4 Soil organic matter

Generally, MBC (Fig. 5a) increased by 2–7 times to 269–604 mg kg<sup>-1</sup> in all soil layers of both treatments from T0 to T6/7 (p < 0.006), whereas the MBC at T8 and T9







**Fig. 6** Soil organic matter. **a** Soil organic carbon determined in the 0–10-, 10–30-, and 30–60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experi-

ment in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=5). **b** Dissolved organic carbon

was 210–530 mg kg<sup>-1</sup> lower than at T6/7, but still 1.5–4 times higher than at T0 (p < 0.024). At T3, the MBC under SC strongly decreased in all soil layers, leading to a 247–352 mg kg<sup>-1</sup> lower MBC under SC compared to PC (p < 0.001), but MBC fully recovered to T6. In the 0–10-cm

soil layer, the MBC under SC was higher than under PC from T6 to T9 (T9, p=0.010) and showed gradually increasing differences between treatments (36–154 mg kg<sup>-1</sup>).

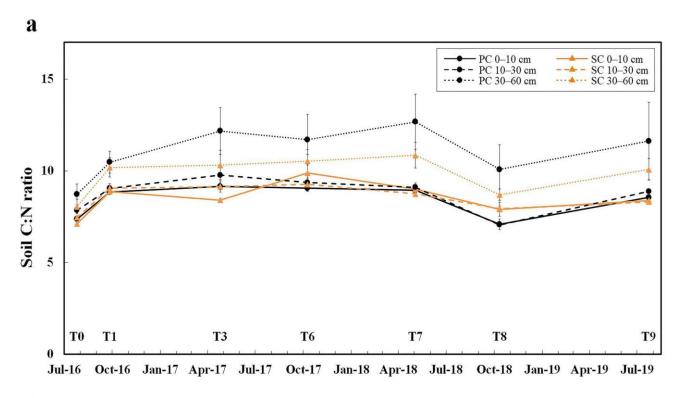
The MBN (Fig. 5b) was 18–30 mg kg<sup>-1</sup> at T0 and decreased during the sampling period by 3.7–12.2 mg kg<sup>-1</sup>

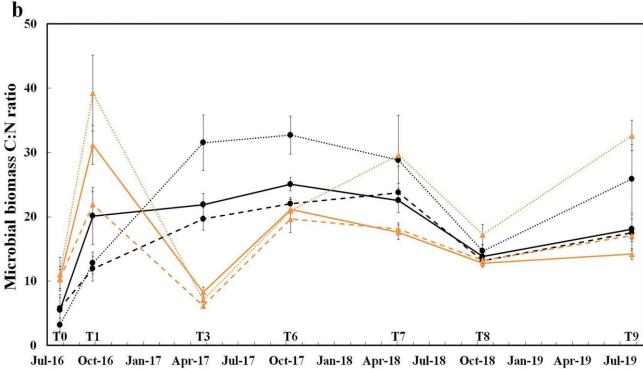


in all soil layers of both treatments (p < 0.001, with a stronger effect under PC and with increasing soil depth), with exception of the 0–10-cm soil layer under SC, where MBN increased by 14 mg kg<sup>-1</sup> (p < 0.001). Similar to MBC, the increase in MBN in the 0–10-cm soil layer led to a higher

MBN under SC compared to PC (T7, T8, and T9, p < 0.043) with gradually increasing differences between the treatments (2.0–16.7 mg kg<sup>-1</sup>).

The SOC (Fig. 6a) ranged from 0.83 to 1.20% and was higher under PC compared to SC in all soil layers during





**Fig. 7** Soil organic matter ratios. **a** C:N ratio determined in the 0–10-, 10–30-, and 30–60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experiment

in strawberry cultivation ( $Fragaria \times ananassa$ , 'Malwina'), respectively, shown as mean with standard error (n=5). **b** MBC:MBN ratio



the sampling period with largest differences between treatments in the 30–60-cm soil layer (up to 0.25%). However, this trend changed in the 0–10-cm soil layer during the sampling period, and from T6 to T9, higher SOC values were observed under SC (0.02–0.11%), with gradually increasing differences between treatments (T3, T7, and T9, p < 0.045). The SOC showed a strong increase (0.05–0.14%) in all soil layers of both treatments from T0 to T1 (p < 0.001). From T1 until T9, the SOC revealed a trend to increase under SC and to decrease under PC in all soil layers (exception, 30–60-cm soil layer under PC). These effects decrease with increasing soil depth. The SOC correlated positively with CEC ( $r_S$ =0.283, p=0.028, n=60).

Because the DOC at T3 (SI Fig. 7) was unrealistically high with values of 494–1024 mg kg $^{-1}$  (> 2000 mg kg $^{-1}$  in some replicates) and we did not find a valid explanation for the high values nor can exclude a mistake during the determination process (no filtration maybe), the DOC at T3 was excluded from interpretation and statistical analyses. The DOC (Fig. 6b) ranged from 7 to 47 mg kg<sup>-1</sup> and was 1-18 mg kg<sup>-1</sup> higher under SC than under PC in the 0-10cm soil layer from T1 to T9 (T6 and T7, p < 0.007), whereas in both soil layers below the topsoil, a tendency to the opposite was observed. During the sampling period, the DOC increased by 6-21 mg kg<sup>-1</sup> in all soil layers (effect decrease with increasing soil depth) of both treatments (p < 0.001). The DOC showed a tendency to have a seasonal pattern with a high DOC in April, an intermediate DOC in July, and a low DOC in October, which is the opposite of what was observed with water-stable aggregates.

Generally, the C:N ratios (Fig. 7a) were between 7.1 and 16.1 during the sampling period. After an initial increase from T0 to T1 in all soil layers of both treatments, the C:N ratios in the 30–60-cm soil layer were by 1.2–1.9 units higher under PC than under SC from T3 to T9. In this period, the C:N ratios were by 0.9–2.9 units higher than at T0 (except at T8) in all soil layers of both treatments (p < 0.002). In both treatments, the largest C:N ratios were found in the 30–60-cm soil layer (p < 0.005).

The MBC:MBN ratios (Fig. 7b) were between 3 and 39 and were by 10–26 units lower in all soil layers under PC than under SC at T1 (p<0.021). However, the MBC:MBN ratios were 14–28 units wider in all soil layers under PC from T0 to T3, which led to mostly wider MBC:MBN ratios under PC from T3 to T9, with differences between treatments of up to 25 units (T3, p<0.004; 30–60-cm soil layer at T6, p=0.013; 10–30-cm soil layer at T7, p=0.014). Generally, the MBC:MBN ratios became wider during the sampling period (p<0.001); however, this effect was stronger in all soil layers under PC (13, 12, and 23) than under SC (4, 7, and 22).

All three SOM fractions (Fig. 8a-c) revealed temporal fluctuations in all soil layers of both treatments and partly

large data scattering, which makes it difficult to identify any treatment- or depth-specific differences. However, as a trend, the free SOM in the 0–10-cm soil layer increased by 0.9 g kg<sup>-1</sup> under SC and decreased by 1.8 g kg<sup>-1</sup> under PC during the sampling period, which resulted in a higher free SOM under SC compared to PC from T6 to T9 (0.4–1.3 g kg<sup>-1</sup>). The mineral-associated SOM showed a tendency to higher values under PC than under SC in all soil layers (exception, 0–10-cm soil layer from T7 to T9).

#### 4 Discussion

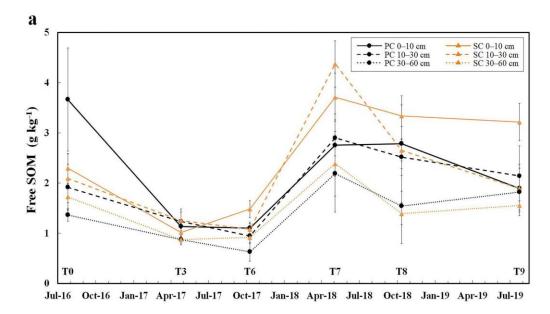
# 4.1 Influence of soil coverage on rainfall infiltration and leaching

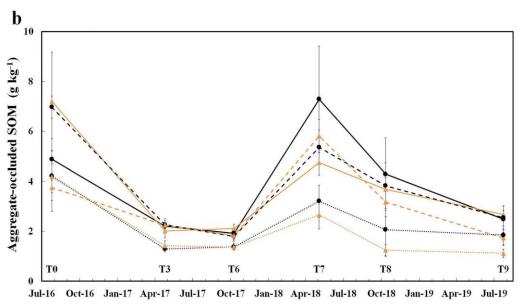
The delayed and smaller increases in soil moisture after rainfalls and the considerably lower soil moisture at 5-cm soil depth under PC compared to SC indicated that PC strongly reduces rainfall infiltration and excess water at the soil surface and thus seepage water flows, which was expected to reduce leaching under PC (Lal 2006). In contrast to the downward-directed seepage water flows, hints were observed to lateral and ascending water flows after rainfalls under PC, presumably induced by lateral pressure heads between ridges and furrows (Ruidisch et al. 2013). This points to an upward and laterally directed particle and substance relocation, which can counteract leaching. However, clear indications for the hypothesized reduced leaching under PC were observed neither in total N nor in electrical conductivity during the sampling period. However, Meyer et al. (2020) described reduced leaching under PC compared to bare soil in the establishment period of strawberries (the first 4 months of the experiment). Thus, we conclude that leaching might also been mitigated under SC due to a decelerated rainwater infiltration by the straw mulch (applied in April 2017) and rainfall interception by the densely grown plant canopy (Gan et al. 2013). Additionally, a stronger nitrogen (nutrient) uptake under PC (Subrahmaniyan et al. 2006; Kumar and Dey 2011), because of stronger plant growth (Kumar and Dey 2011; Gan et al. 2013; Zhang et al. 2017), could have compensated the nitrogen (nutrient) gains due to omitted leaching losses. The yearly fertilization from March until May seem to have no influence on pH, electrical conductivity, and total N, which might indicate a good fertilizer use efficiency.

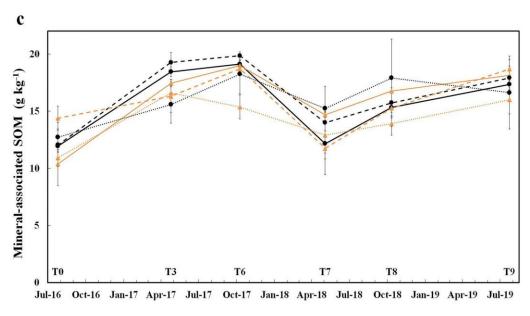
## 4.2 Influence of soil coverage on pore volumes and soil structure

Under PC, a lower bulk density and a higher macropore volume became observable in the surface soil (0–5 cm) after 1 year, which was presumably governed by two processes:











**<Fig. 8** Soil organic matter composition. **a** Free soil organic matter (SOM) determined in the 0-10-, 10-30-, and 30-60-cm soil layers under plastic coverage (PC) and straw coverage (SC) at seven dates within the 3-year field experiment in strawberry cultivation (*Fragaria* × *ananassa*, 'Malwina'), respectively, shown as mean with standard error (n = 5). **b** Aggregate-occluded SOM. **c** Mineral-associated SOM

In contrast to SC, the water-impermeable PC avoids rapid soil wetting after rainfalls, which prevents aggregate slaking, physicochemical dispersion, and particle relocation and thus soil crusting and compaction (Bing So 2006; Le Bissonnais 2006). Soil crusting and compaction reduce water infiltration, increase runoff, and consequently make soils more prone to erosion (Le Bissonnais 2006). Secondly, the bulk density slightly decreased under PC during the sampling period, which was not reflected in increasing macropore volumes and thus might be caused by larger root growth under PC (Fernandez et al. 2001; Kumar and Dey 2011). Thus, PC maintained a more loose and friable soil structure in the surface soil compared to SC, which made soil less prone to erosion and promotes good aeration, soil warming, rooting, and water retention and, thus, soil quality and agronomic productivity (Bronick and Lal 2005; Le Bissonnais 2006). However, no increase in the (water-)stability of macroaggregates was observed in the topsoil under PC compared to SC, despite the impeded (disruptive) raindrop impact on aggregates at the soil surface and the lower soil moisture under PC (Bing So 2006; Blanco-Canqui and Lal 2006). Presumably, the straw mulch and the dense plant canopy reduced raindrop impact on aggregate stability also under SC; therefore, the physical impact of raindrops on surface soil seems to have only a minor role for the aggregate stability in both treatments. As Sintim et al. (2021) found a higher aggregate stability in the topsoil after 4 years of continuous plastic mulching, we cannot exclude that effects on aggregate stability would not appear during an extended experiment period.

# 4.3 Influence of soil coverage on soil microbial biomass and aggregate formation and stability

As described in numerous studies, PC increased soil temperature (reviewed in Gan et al. 2013; Steinmetz et al. 2016), which is generally attributed to the optical properties of the mulch and its impact on the heat transfer between soil surface and surroundings (Tarara 2000). In contrast to the higher soil moisture under plastic mulching, attributed to a reduced evaporation in semiarid and arid regions (e.g., Gan et al. 2013; Wang et al. 2015), our results showed a predominantly lower soil moisture under PC compared to SC, especially in the topsoil. This suggests that under humid climate, impeded rainfall infiltration strongly outweighs the effect of reduced evaporation (Meyer et al. 2020). Similarly,

a lower soil moisture under PC was also found in a study conducted by Schirmel et al. (2018) under humid conditions in Southwestern Germany. The seasonal effects on soil temperature presumably depend on seasonal changes in solar radiation but additionally also on wind, cloudiness, and density of plant canopy. In winter, the missing irrigation combined with impeded rainfall infiltration under PC might have led to the increasing soil moisture differences between PC and SC in the topsoil (0–10 cm). Although the subsoil under PC received more water after rainfalls compared to SC, the soil moisture decreased more strongly afterwards and led especially in summer and autumn to a clearly lower soil moisture under PC. This might be explained by a higher water uptake of the plants and ascending water flows to the drier upper soil layers (Tarara 2000; Wang et al. 2014).

Several studies found a higher MBC under PC, which was mainly attributed to the elevated soil temperature and moisture under PC (e.g., Gan et al. 2013; Luo et al. 2015; Yang et al. 2018). In spite of partly up to 6.5 °C higher soil temperatures under PC, a clearly larger MBC under PC was only found at T3 (April 2017) and partly at T6 (October 2017) in the 3-year study. Maybe the partly lower soil moisture under PC compensated for the expected positive effect of soil temperature on MBC. However, as differences in soil temperature and moisture between coverage types were soiland depth-specific but were not reflected in MBC, it can be concluded that these differences were not large enough to shape different MBC under the investigated coverage types. The higher MBC and MBN in the topsoil under SC compared to PC from T6 to T9 were presumably caused by the incorporation of straw and aboveground biomass into soil (Bünemann et al. 2006; Huo et al. 2017). This implies, in contrast to the differences in soil temperature and moisture, a strong and gradually increasing treatment effect on microbial biomass.

Wang et al. (2017) described a 16–28% increase in the water-stable macroaggregate fraction under PC, which they attributed to an increased root growth and microbial activity under PC. In contrast to Wang et al. (2017), no clear positive effects of PC on aggregation and aggregate stability was found in this study. This is in accordance with the results of microbial biomass, which was hypothesized as main driver for an increased aggregate formation and stability under PC. However, a strong influence of microorganisms on aggregate stability was confirmed by the highly significant positive correlation between MBC and water-stable aggregates. Generally, aggregation and aggregate stability are influenced by a multitude of factors such as soil moisture, SOM, root growth, fungal hyphae, exudation of roots and microbes, and wetting-drying cycles (Bronick and Lal 2005). This is reflected in the observed positive correlation between waterstable aggregates and pH, electrical conductivity, MBC, and DOC. Thus, the increasing aggregate stabilities from spring



to autumn and their strong variations between sampling time points and treatments are likely the result of several processes (co-)occurring during the sampling period. The largest water-stable aggregate fraction found in the root layer (10–30 cm) and increasing aggregate stabilities from spring to autumn point to a large influence of root growth and exudation (Tisdall and Oades 1982; Bronick and Lal 2005). However, more detailed studies are necessary to break down the individual processes that govern aggregate stability and elucidate how they are influenced by soil coverage and how much they contribute to the observed variations.

# 4.4 Influence of soil coverage on entry and decomposition of soil organic matter

The general increase in SOC under both treatments after strawberry establishment was attributed to the strong root growth of the plants (Meyer et al. 2020). This increase in SOC was stronger under PC, presumably because of a larger root growth and exudation under PC due to the higher soil temperature (Fernandez et al. 2001; Kumar and Dey 2011; Yin et al. 2013). This results in a larger SOC under PC than under SC (especially in the 30-60-cm soil layer), even though the aboveground biomass entry under PC was impeded by the impermeable plastic mulch. Similarly, Luo et al. (2015) described higher SOC stocks in the 20–40-cm soil layer under plastic mulching, which they attributed to improved rooting systems and increased root biomass. However, this effect was compensated for in the topsoil (0–10 cm) after 1 year (T6), and from T6 to T9, the SOC was higher under SC compared to PC, which resulted most likely from the SOM entry under SC due to decaying straw mulch and plant residues. The higher DOC in the topsoil under SC supports this assumption as DOC mainly derives from fresh plant residues or decomposition by-products (Bolan et al. 2011). In contrast, the partially higher DOC in the 10–30and 30-60-cm soil layers under PC might originate from higher root exudation or small organic molecules transported from straw-covered furrows to ridges by lateral water flows under PC (Bolan et al. 2011; Ruidisch et al. 2013). Former studies found reduced SOM under PC, which was attributed to an accelerated SOM decomposition due to changes in microclimate and microorganisms (e.g., Li et al. 2004, 2007; Zhang et al. 2015). However, despite higher soil temperatures, increasing MBC, and an impeded aboveground biomass entry under PC, no increased SOM losses were found in this 3-year study under temperate, humid climate conditions. This suggests that an accelerated SOM decomposition under PC, which would result in reduced SOM stocks, can either be excluded or be compensated for by other processes like, e.g., biomass entry due to root growth and exudation under the present conditions (Fernandez et al. 2001; Kumar and Dey 2011; Yin et al. 2013). Reduced SOM stocks could be critical for soil quality and productivity and would question the sustainability of the management practice (Haynes 2005; Steinmetz et al. 2016). It can be speculated that in arid and semiarid regions, where microbial activity and thus SOM decomposition are usually strongly restricted by the low soil moisture (Coûteaux et al. 1995; Butenschoen et al. 2011), elevated soil moisture under plastic mulching (Gan et al. 2013) might strongly trigger SOM decomposition. In contrast to that, soil moisture under humid conditions is not elevated by plastic mulching and generally high enough to enable decomposition.

# 4.5 Influence of soil coverage on quality and composition of soil organic matter

The still rarely investigated influence of soil coverage on SOM composition and hence SOM quality (Steinmetz et al. 2016) as estimated by density fractionation revealed a positive effect of SC on free SOM (topsoil) and of PC on mineral-associated SOM. The free SOM fraction refers to the active SOM pool (turnover time of 1–2 years) and represents (often combined with aggregate-occluded SOM) the light SOM fraction (von Lützow et al. 2006, 2007). The light SOM fraction consists mainly of a fresh and fast-mineralizable SOM (e.g., plant residues, microbial biomass), which is important for the nutrient supply in soil (Wander 2004; von Lützow et al. 2006). Thus, the lower free SOM in the topsoil under PC from T6 to T9 corroborated our assumption of an impeded aboveground SOM entry into soil compared to SC and point to a lower short-term nutrient supply under PC (Wander 2004). Similarly, Wang et al. (2016) found decreased light SOC fraction under multiannual PC use compared to nonmulched plots and an increased light SOC fraction after straw incorporation. The mineral-associated SOM fraction refers to the passive SOM pool (turnover time of 100 to < 1000 years), which consists of older, more degraded, recalcitrant SOM, often bound in organo-mineral complexes (von Lützow et al. 2006, 2007). The passive SOM pool is of great relevance for long-term SOM stabilization, soil structure, nutrient sorption, and water-holding capacity (Wander 2004; Grego and Lagomarsino 2008). Thus, the tendency for larger mineral-associated SOM fractions under PC might indicate an improved nutrient sorption, water-holding capacity, and soil structural and SOM stability under PC (Wander 2004; Grego and Lagomarsino 2008). According to Jackson et al. (2017), markedly larger amounts of belowground biomass (45%) are stabilized in SOM compared to aboveground biomass (8%) and mainly simple organic compounds undergo organo-mineral formation (Blume et al. 2016). Thus, it was assumed that the larger mineral-associated SOM fractions under PC were caused by temperature-induced larger root growth



accompanied by larger root and microbial exudation under PC (Fernandez et al. 2001; Kumar and Dey 2011; Yin et al. 2013). Similarly, the wider C:N ratios in the 30–60-cm soil layer under PC point to a more hardly degradable SOM (Kindler et al. 2011), which could decelerate microbial SOM mineralization and thus preserve SOM and nitrogen stocks in the subsoil (Jia et al. 2006).

The MBC:MBN ratios under PC indicate an increasing and larger fungal fraction compared to SC, pointing to a shift in microbial community (Campbell et al. 1991). At the beginning of the experiment (T0–T2), the MBC:MBN ratios indicated larger fungal fractions under bare soil compared to PC, which were attributed to aboveground biomass entry, promoting fungal growth (Meyer et al. 2020). However, during the annual fungicide application (in flowering period), the soil under SC received larger fungicide loads than under PC, which reduced fungal growth or rather promoted bacterial growth under SC (Meyer et al. 2021). The larger soil temperature under PC might have additionally favored fungal growth (Pietikåinen et al. 2005; Drenovsky et al. 2010). The influence of PC on microbial community and potential shifts towards mycotoxigenic fungi was already discussed before (Buyer et al. 2010; Muñoz et al. 2015, 2017). However, more detailed investigations are required to verify these indications on microbial community shifts under PC, which additionally seem to vary in dependence of the pesticide treatment and the duration of the PC application.

# 4.6 Influence of seasonality, time, and soil depth on coverage effects

Several investigated soil parameters confirmed the assumption of a seasonal, time- and depth-dependent impact of soil coverage on soil parameters and processes. The differences between coverage types in soil temperature and partly in soil moisture, the main drivers of PC effects (Gan et al. 2013), exhibit strong seasonal and depth-dependent effects, which have already been observed for soil temperature by Wang et al. (2015). However, these differences were not reflected in temperature- and moisture-sensitive soil parameters such as MBC and MBN (Pietikåinen et al. 2005; Bárcenas-Moreno et al. 2009). Additionally, seasonal effects were observed to some extent also in DOC and water-stable aggregates. The influence of soil coverage was partly also observed below the topsoil layer (e.g., for soil temperature and moisture, DOC, and MBC) or only below the topsoil layer (C:N ratios) or differed between soil layers (SOC). The effects of soil coverage on pore volumes, bulk density, and MBC became only observable in the second year of the experiment, whereas the effects on SOC, MBN, and MBC:MBN ratios changed during the sampling period, indicating several co-occurring processes which compensate for and overbalance each other after a certain time. Furthermore, the restriction of the present study to 3 years of observation may hide some long-term effects that become significant only after a longer application. For example, Liu et al. (2021) showed that in particular changes in the microbial community structure were observed after 10 years of continuous plastic mulching in a semiarid climate and Sintim et al. (2021) found a higher aggregate stability in the topsoil after 4 years of continuous plastic mulching. Thus, changes in soil properties after 3 years cannot be excluded. But a longer period was beyond the scope of this study because we wanted to test the effects of the current agricultural practices in strawberry cultivation, which is mainly restricted to 2 years or 3 years before the plastic covers and plants are removed and the ridge-furrow system is renewed. In summary, these findings on seasonal, time- and depth-dependent effects of coverage types on soil pronounce their importance and recommend to include them also in future studies for a holistic process understanding.

#### 5 Conclusion

The impeded rainfall infiltration under plastic mulching reduces soil moisture under temperate, humid climate, especially when no drip irrigation is applied. Thus, the positive effects of plastic mulching on plant growth and yields are mainly induced by the seasonally elevated soil temperature. Plastic mulching neither reduces leaching nor promotes (macro-)aggregate formation or stability; however, it maintains a loose and friable soil structure in surface soil (0-5 cm), which can improve aeration and rooting. Furthermore, it promotes SOM accumulation and shifted SOM composition to a more hardly degradable SOM, especially below the topsoil (10-60 cm), which can also improve soil structure, serve as a nutrient reservoir, and enhance sorption and water-holding capacity. As from the second year, straw mulch provides fresh and fast-mineralizable substrate in the topsoil (0–10 cm), which enhances microbial biomass and fostering larger bacterial fractions. Our study under temperate, humid climate reveals no indications of an increased microbial biomass or activity accompanied with an accelerated SOM decomposition under plastic mulching. The study revealed no significant adverse effects of the plastic coverage on the investigated soil parameters and processes and does not point to a decreased soil quality in the investigated time period. However, the partly seasonal, time- and depthdependent effects of our study emphasize the importance of including soil depth, temporal course, and season in future studies to gain a more holistic process understanding. Additionally, further research is necessary to prove our findings on a larger scale and longer time periods and to various soil and crop types.



**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11368-021-03037-3.

Acknowledgements We want to thank Christof Steegmüller for providing access to the sampling site and for his expertise in strawberry cultivation; Mathilde Knott, Christian Buchmann, Kilian Kenngott, and Zacharias Steinmetz for valuable assistance during soil samplings; and Lea Bosch, Katharina Lühmann, Camilla More, and Ken Mauser for valuable assistance during sample analyses. Furthermore, we would like to thank Prof. S. Thiele-Bruhn (University of Trier) for providing the field measuring station.

Author contribution The concept of this paper was developed by Maximilian Meyer together with Prof. Dr. Gabriele Ellen Schaumann and Dr. Katherine Andrea Muñoz Sepúlveda. Maximilian Meyer planned and conducted the experimental design and all soil samplings, data evaluations, and statistics. Maximilian Meyer had support from colleagues and technical staff during soil samplings and from technical and student staff for laboratory analyses. Data interpretation and paper writing was done by Maximilian Meyer with the support of Prof. Dr. Gabriele Ellen Schaumann, Dr. Katherine Andrea Muñoz Sepúlveda, and Dr. Dörte Diehl.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by the Ministry for Education, Sciences, Further Education and Culture of the State of Rhineland-Palatinate (MBWWK) in the frame of the Interdisciplinary Research Group for Environmental Studies (IFG-Umwelt) of the University of Koblenz-Landau and by the research fund of the University of Koblenz-Landau.

**Availability of data and material** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### **Declarations**

Competing interests The authors declare no competing interests.

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# **CHAPTER 6**

How does multiannual plastic mulching in strawberry cultivation influence soil fungi and mycotoxin occurrence in soil?

Meyer, Maximilian; Schaumann, Gabriele Ellen; Muñoz, Katherine (2022):

Mycotoxin Research. 38(2):93-105

#### **ORIGINAL ARTICLE**



# How does multiannual plastic mulching in strawberry cultivation influence soil fungi and mycotoxin occurrence in soil?

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Received: 11 August 2021 / Revised: 3 February 2022 / Accepted: 17 February 2022 / Published online: 22 March 2022 © The Author(s) 2022

#### **Abstract**

The production of mycotoxins is often interpreted as fungal response to cope with unfavorable growth conditions induced by toxic substances, environmental and biological factors. Soil covers influence soil environment, which consequently can change the abundance and composition of microbial communities. We investigated how plastic coverage (PC) influence soil fungi and mycotoxin occurrence (deoxynivalenol, nivalenol and zearalenone) compared to the traditional straw coverage (SC) in dependence of soil depth and time in a 3-year field experiment in strawberry cultivation. In total, 300 soil samples, resulting from two treatments, three soil layers, and ten sampling dates (n=5), were analyzed for mycotoxins and ergosterol (proxy for soil fungal biomass) with liquid chromatography high resolution mass spectrometry and high-performance liquid chromatography with UV-detection, respectively. The modified microclimate under PC had no significant influence on fungal biomass, whereas SC promoted fungal biomass in the topsoil due to C-input. Mycotoxins were detected under both cover types in concentrations between 0.3 and 21.8  $\mu$ g kg<sup>-1</sup>, mainly during strawberry establishment period and after fungicide application. Deoxynivalenol had the highest detection frequency with 26.3% (nivalenol: 8.3%, zearalenone: 8.7%). This study confirmed the in situ production of mycotoxins in soil, which seems mainly triggered by field treatment (fungicide application) and plant growth stage (establishment period) rather than on mulching type. Further investigations are necessary to better understand the influence of different agricultural practices and soil types on the production and fate of mycotoxins.

 $\textbf{Keywords} \ \ Plastic \ mulching \cdot Soil \ fungi \cdot Ergosterol \cdot Deoxynival en ol \cdot Nival en ol \cdot Zearal en one$ 

Abbreviat	ions
DON	Deoxynivalenol
NIV	Nivalenol
ZEN	Zearalenone
SOM	Soil organic matter
PC	Plastic-covered ridge-furrow system with
	subsurface drip irrigation
SC	Straw-covered ridge-furrow system with sub-
	surface drip irrigation
SOC	Soil organic carbon
TN	Total nitrogen
C:N ratio	Carbon-to-nitrogen ratio

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MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
LOD	Limit of detection

RSD Relative standard deviation LCL Lowest calibration level

#### Introduction

Mycotoxins are secondary metabolites produced by several fungal species and can exert a wide array of toxic effects to humans and animals (Vanhoutte et al. 2016). The frequent mycotoxin contamination of food and feed commodities, exceeding the maximum limits set in many countries to reduce mycotoxin exposure, can result in large economical losses (Robens and Cardwell 2003; Wilson et al. 2018). Mycotoxins are primarily produced by the genera *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. (Sweeney and Dobson 1998). *Aspergillus* spp. and *Penicillium* spp. infest food and feed mainly during storage, whereas the *Fusarium* spp. are field fungi, which can grow in soils and



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infest various food crops during different growth stages (Elmholt 2008). Deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN) are frequently produced mycotoxins by *Fusarium* spp. in temperate climates (Table 1) (Elmholt 2008). Although the role of mycotoxins in the fungal life cycle is not yet completely understood, mycotoxin production of *Fusarium* spp. is often seen as stress response to unfavorable growth conditions (Reverberi et al. 2010), including fungicides, high temperatures, water and nutrient scarcity, and competition (Magan et al. 2002; Schmidt-Heydt et al. 2008; Reverberi et al. 2010; Venkatesh and Keller 2019).

Plastic mulching has become a widely applied agricultural practice to improve growth and harvest conditions of many food crops, because it increases soil temperature and moisture, suppresses weed growth, and reduce nutrient (fertilizer) leaching by an impeded rainfall infiltration (reviewed by Steinmetz et al. 2016). Elevated soil temperature and moisture can enhance fungal growth (Dighton 2003; Pietikåinen et al. 2005; Bárcenas-Moreno et al. 2009) and shift microbial community (Castro et al. 2010), which might stress soil fungi due to competition and nutrient scarcity (Reverberi et al. 2010; Venkatesh and Keller 2019). This assumption is emphasized by decreasing soil organic matter (SOM) under plastic mulching (reviewed by Steinmetz et al. 2016), which can result in a limited substrate for fungal growth and hence stronger competition for nutrients (Reverberi et al. 2010; Swer et al. 2011). The high soil temperatures under plastic mulching in the summer season can exceed the growth optima and even the growth maxima of Fusarium spp. (Sweeney and Dobson 1998), which might additionally stress soil fungi (Schmidt-Heydt et al. 2008; Muñoz et al. 2015). The impeded rainfall infiltration under plastic mulching decrease leaching (Subrahmaniyan et al. 2006) but might lead to an accumulation of mycotoxins in surface soil, which can become susceptible to leaching after plastic mulch removal. First indications that plastic mulching can affect mycotoxin occurrence and shift fungal community toward mycotoxigenic fungi were reported by Muñoz et al. (2015, 2017). But it is largely unknown how mycotoxin levels and fungal biomass can change during the multiannual use of plastic mulching due to its effects on the soil environment.

However, this is an important information, as studies by Schenzel et al. (2012b) and by Kolpin et al. (2014) showed that DON, NIV, and ZEN have the potential to contaminate running and groundwaters due to runoff and leaching from contaminated fields. Especially, DON and NIV have a high water solubility (Table 1) and can thus remain in the soil solution and possibly undergo plant uptake (Rolli et al. 2018) or microbial degradation (Vanhoutte et al. 2016). In contrast to the well-documented mycotoxin occurrence in food and feed (Elmholt 2008), the available literature is very scarce about the occurrence, production, and fate of mycotoxins in agricultural soil and how this is linked to agricultural practices.

The objective of this study was to investigate the influence of plastic coverage (PC) on soil fungi and occurrence of the mycotoxins DON, NIV, and ZEN in soil compared to the traditional straw coverage (SC) in dependence of soil depth and time in a 3-year field experiment in strawberry

Table 1 Main producer, synthesis conditions and physicochemical properties of relevant mycotoxins in temperate climates

Mycotoxin	Main producer <sup>1,2,3</sup>	Structure <sup>2,4</sup>	Water solubility <sup>5</sup>	Heat stability <sup>1,2,3</sup>	Log Koc6	Chemical stability <sup>7,8</sup>
Deoxynivalenol	Fusarium graminearum F. culmorum	16 10 H 10	283 mg L <sup>-1</sup>	stable at 120 °C moderately stable at 180 °C	< 0.7	very stable
Nivalenol	F. poae F. cerealis F. graminearum F. culmorum	H <sub>3</sub> C 0 10 H 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1275 mg L <sup>-1</sup>	stable up to > 150 °C	< 0.7	very stable
Zearalenone	F. graminearum F. culmorum F. equieseti F. cerealis	OH O 99 7	31.8 mg L <sup>-1</sup>	stable up to > 150 °C	3.42	very stable

<sup>&</sup>lt;sup>1</sup>EFSA (2004a)

<sup>&</sup>lt;sup>8</sup>Pitt et al. (2012)



<sup>&</sup>lt;sup>2</sup>EFSA (2013)

<sup>&</sup>lt;sup>3</sup>EFSA (2011)

<sup>&</sup>lt;sup>4</sup>EFSA (2004b)

<sup>5</sup>HGED 4 (2012)

<sup>&</sup>lt;sup>5</sup>USEPA (2012)

<sup>&</sup>lt;sup>6</sup>Schenzel et al. (2012a)

<sup>&</sup>lt;sup>7</sup>Lauren and Smith (2001)

cultivation. We hypothesize that (1) elevated soil temperature and moisture under PC compared to SC promote fungal growth and expand fungal community, and (2) the adaption of the soil fungi to the modified microclimate under PC will trigger a higher mycotoxin occurrence under PC compared to SC.

#### Material and methods

This study was part of a 3-year field experiment on the influence of plastic mulching on various soil parameters and biogeochemical soil processes, investigated on the example of a commercial strawberry cultivation (Meyer et al. 2020, 2021a, b). This paper repeats the relevant information to retrace experiment and methods and shows new data about mycotoxins (sampling T0–T2 and T7–T9), ergosterol (sampling T0–T2 and T7–T9), MBC:SOC ratios (sampling T7–T9), and MBN:TN ratios (sampling T0–T9).

#### Site description and field establishment

The study was conducted on a commercial strawberry field in southwestern Germany (49°11′N, 8°10′E, 130 m a.s.l.) from 2016 to 2019. The soil was a silt loam (Anthrosol), according to FAO soil classification (IUSS Working Group WRB 2015), with a texture of  $7\pm2\%$  sand,  $83\pm5\%$  silt, and  $10\pm3\%$  clay in the 0–60 cm soil layer. A ridge-furrow system was established in the field in late-June 2016 with subsurface drip irrigation and black plastic-covered ridges (polyethylene, 50 µm). Strawberry seedlings (*Fragaria*×*ananassa*, "Malwina") were planted in double rows at the ridges in mid-July 2016. The initially bare furrows were yearly covered with wheat straw, starting in April 2017.

#### Experimental design and soil sampling

A semi-controlled field experiment with a homogeneous soil type was used, which represents current agricultural practice and avoids masking of treatment effects by landscape variation and edge effects. One treatment area with plasticcovered (PC) and one with straw-covered ridges (SC) were chosen (both  $21 \times 10$  m), in which respectively five sampling plots  $(10 \times 1.5 \text{ m})$  were randomly chosen for soil sampling: PC (n=5) and SC (n=5). In the SC treatment area, plastic coverage was manually removed after field setup in July 2016 and the bare soil ridges were yearly covered with wheat straw, starting in April 2017. The agricultural practices (irrigation, fertilization and pesticide application) were identical in both treatments during sampling period. In brief, subsurface drip irrigation was applied yearly from March until September depending on weather conditions and fertilization (15 kg N, 5 kg P, 30 kg K, 2 kg Mg) was weekly conducted via drip irrigation during an 8-week period from March to May each year. Fungicides (1 kg ha<sup>-1</sup> Switch and 2 kg ha<sup>-1</sup> Teldor) were applied yearly during bloom of strawberries.

Ten soil samplings were conducted during a 3-year period of strawberry cultivation: during the establishment period of strawberry plants, three samplings in 2-month intervals were conducted after planting from late-July to late-November in 2016 (T0-T2) to identify a potential short-term impact of PC on soil parameters and processes after field setup and strawberry plantation (Meyer et al. 2020). In order to investigate the influence of PC on soil parameters and processes during multiannual application in strawberry cultivation, five further samplings were conducted on 25 April 2017 (T3), 9 October 2017 (T6), 3 May 2018 (T7), 11 October 2018 (T8), and 23 July 2019 (T9) (Meyer et al. 2021b). Additionally, two further samplings were conducted on 19 June (T4) and 18 July (T5) in 2017 after fungicide application to estimate the influence of both coverage types (PC and SC) on fungicide residues in soil and their impact on mycotoxin occurrence, microbial biomass and SOM decomposition (Meyer et al. 2021a).

Composite soil samples (five single cores) were taken in the ridges of each sampling plot in the surface, root and subsoil layer (0–10, 10–30, and 30–60 cm) with stainless steel soil sampling rings (0–10 cm) and a boring rod (> 10 cm).

#### **General soil parameters**

Soil organic carbon (SOC) and C:N ratio can influence fungal growth (Bossuyt et al. 2001; Swer et al. 2011) as well as soil temperature, moisture and pH can influence both fungal growth and mycotoxin biosynthesis (Marin et al. 1995; Sweeney and Dobson 1998; Ramirez et al. 2006; Schmidt-Heydt et al. 2008). For this reason, the temperature and moisture data of the 3-year field study published in Meyer et al. (2021b) are presented in the results. Soil temperature and moisture were measured at the 5, 15, and 35 cm soil depth under PC and SC with a field measuring station (ecoTech®, Bonn, Germany), while the air temperature and precipitation data were received from the weather station Landau-Wollmesheim (Agrarmeteorologie, Rheinland-Pfalz). The data of soil pH (0.01 M CaCl<sub>2</sub>), SOC and C:N ratio, published in Meyer et al. (2020, 2021a, b), was summarized in respectively one figure and added to the supplementary information (SI Figs. 1–3).

#### Analysis of microbial soil parameters

The microbial biomass carbon (MBC) to nitrogen (MBN) ratio, the ratios of MBC and MBN to SOC and total nitrogen (TN), respectively, and ergosterol were used as indicators to describe the impact of coverage type on soil fungi and microbial community (Anderson 2003; Joergensen



and Emmerling 2006). The MBC:MBN, MBC:SOC and MBN:TN ratios were calculated from the MBC (chloroformfumigation, TOC analysis), MBN (chloroform-fumigation, ninhydrin reaction, UV/VIS spectrometry), SOC (HCl fumigation, CHNS analysis), and TN (CHNS analysis) values published in Meyer et al. (2020, 2021a, b). The ergosterol determination was based on the method of Gong et al. (2001). Four grams of air-dried, milled soil were mixed with 12 mL methanol (1:3, w/v) and subsequently shaken for 60 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany) and afterwards ultrasonically treated for 10 min (DT 514H, Bandelin electronics GmbH & Co. KG, Berlin, Germany). Then, the suspension was centrifuged for 10 min at 2000 g (Universal 320, Hettich Lab Technology, Tuttlingen, Germany), and ultracentrifuged for 3 min at 7270 g (Micro centaur, MSE Ltd, London, UK). Finally, 20 µL of the supernatant were analyzed with a high-performance liquid chromatography system with UV detection at 282 nm (HPLC 1200 series, Agilent technologies, Santa Clara, USA), equipped with a C<sub>18</sub> LiChrospher® column (LiChrospher RP-18e, 5 μm, 100 Å, 250×4.6 mm, Merck KGaA, Darmstadt, Germany). For method validation, the soil was in advance spiked with ergosterol at concentrations of 0.5 and 5 mg kg<sup>-1</sup> (n=5 for each concentration). Recovery ranged between  $96 \pm 1$  and  $115 \pm 2\%$  and showed a relative standard deviation (RSD) of < 2%. Regression analysis of eight ergosterol standards in the range from 0.05 to 10 mg L<sup>-1</sup> showed an excellent linear fit  $(R^2 = 1)$  and a limit of detection (LOD) < 0.06 mg kg<sup>-1</sup>. For more detailed information on method validation see the supplementary information (SI Table 1 and SI Fig. 4).

#### Analysis of mycotoxins in soil

Soil samples were analyzed for DON, NIV, and ZEN, based on a method by Mortensen et al. (2003), modified by Muñoz et al. (2017). Five grams of air-dried, milled soil (Planetary micro mill PULVERISETTE 7 premium line, Fritsch GmbH, Idar-Oberstein, Germany) were shaken with 15 mL methanol/ water mixture (9:1, v/v) for 30 min on a horizontal shaker (Kreisschüttler 3015, GFL, Burgwedel, Germany) and subsequently treated for 10 min with ultrasonication (DT 514H, Bandelin electronics GmbH & Co.KG, Berlin, Germany). The suspension was 10 min centrifuged at 2000 g (Universal 320, Hettich Lab Technology, Tuttlingen, Germany). An aliquot of 10 mL was subsequently evaporated to dryness under a nitrogen stream at 50 °C (Evaporatorsystem EVA-EC1-24-S, VLM Korrosions-Prüftechnik, Labortechnik & Dienstleistungen GmbH, Bielefeld, Germany), and the residues were reconstituted with 1 mL of mobile phase (methanol/water 1:1 v/v with 0.1% formic acid and 4 mM ammonia formiate). The solution was ultracentrifuged for 5 min at 7270 g (Micro centaur, MSE) Ltd, London, UK), and 20 µL of the supernatant were analyzed

for mycotoxins with liquid chromatography-high-resolution mass spectrometer (LC-HRMS, Thermo Fisher Scientific, Waltham, USA), using a Hypersil GOLD™ C<sub>18</sub> column (100×2.1 mm, 1.9 μm, Thermo Fisher Scientific, Waltham, USA). The mycotoxins were quantified with a matrix-matched calibration curve  $(1, 2.5, 5, 10, 25, 50, 75, \text{ and } 100 \,\mu\text{g L}^{-1})$ prepared in soil extract (same procedure as for samples). All calibration standards for mycotoxins were purchased by Romer Labs Deutschland GmbH (Butzbach, Germany), Samples were considered positive when concentrations were above the lowest concentration level (LCL), which was 1  $\mu$ g L<sup>-1</sup> for DON, NIV, and ZEN, respectively, and corresponds to a soil concentration of 0.3 µg kg<sup>-1</sup>. For DON and NIV, <sup>13</sup>C labelled internal standards were used as additional confirmation step. All mycotoxins were quantified in the negative ion mode, using the following mass-to-charge ratios: 356.1750 and 272.1701 for <sup>13</sup>C-DON and <sup>13</sup>C-NIV and 341.1240, 357.1195, and 317.1389 for the DON, NIV, and ZEN, respectively. For method validation, the soil was in advance spiked with DON, NIV, and ZEN at concentrations of 5, 15, and 50 μg kg<sup>-1</sup>. Recovery values ranged between  $117 \pm 11$  and  $144 \pm 29\%$  for DON,  $119 \pm 10$  and  $139 \pm 22\%$  for NIV, and  $67 \pm 7$  and  $126 \pm 10\%$ for ZEN. The RSD was ≤20% for all mycotoxins. Regression analyses of nine mycotoxin standards in the range from 0.5 to 100  $\mu$ g L<sup>-1</sup> showed a good linear fit (DON:  $R^2 = 0.9725$ , NIV:  $R^2$ =0.9888 and ZEN:  $R^2$ =0.9937). For more detailed information on method validation see the supplementary information (SI Table 2, SI Figs. 5–7).

#### Statistical analyses

Normality distribution of data was examined graphically with histograms and quantile-quantile plots. Mixed factorial ANOVAs with coverage time and soil depth as repeated factors and treatment as fixed factor were applied to determine significant differences between means. If significant interaction effects were occurring, additional ANOVAs, with least significance distance testing as post hoc test, were applied to locate significant differences. Variance homogeneity was confirmed with Levene's test. Differences were reported as statistically significant if the probability of error was < 0.05. Method validation for ergosterol and mycotoxin analysis were based on ICH guideline Q2 (except LCL determination of mycotoxins). The LOD was calculated as  $3.3\sigma/S$ , with  $\sigma$  as the standard deviation of the intercept of the regression line and S as the slope of the regression line calculated from the calibration standards (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use 2005). For mycotoxin determination, the LCL was used as LOQ. The LCL determination based on the visual evaluation (empiric) methods, which has been suggested to provide more realistic values in complex matrices (Sengül 2016). According to

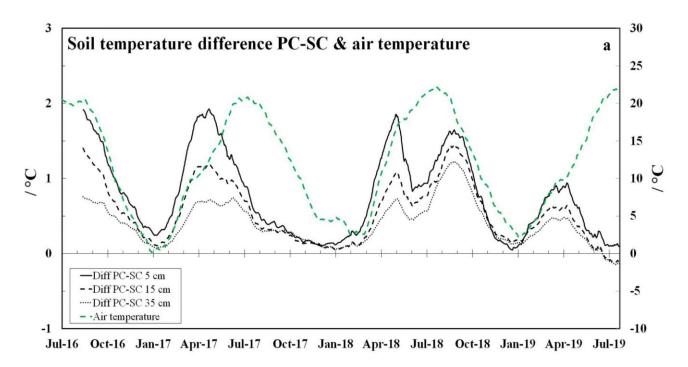


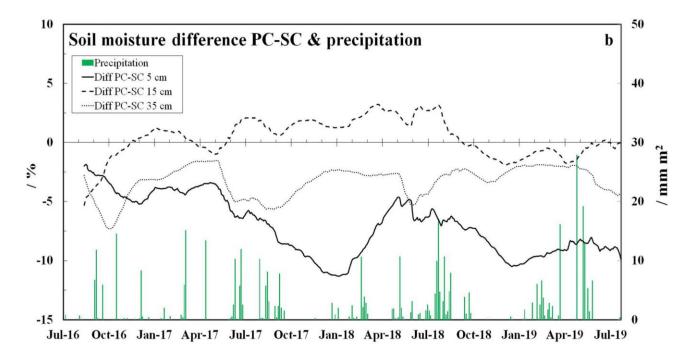
this, the smallest standard of the matrix-matched calibration curve that gives a clear peak (signal-to-noise ratio > 10) was used to assess the LCL. For all results below the LCL, the LCL/2 was used for mean and standard deviation calculation (Ogden 2010). All statistical analysis was done with IBM SPSS Statistics 25.

#### Results

### General soil properties under plastic and straw coverage

Soil temperature (Fig. 1a) was up to 2 °C (daily mean) or rather 6.5 °C (hourly) higher under PC than under SC,





**Fig. 1** Soil temperature and moisture within the 3-year field experiment. **a** Smoothen soil temperature differences, based on daily means, between plastic coverage (PC) and straw coverage (SC) measured at 5, 15, and 35 cm soil depth and daily mean air temperature measured

2 m above ground. **b** Smoothen soil moisture differences, based on daily means, between plastic coverage (PC) and straw coverage (SC) measured at 5, 15, and 35 cm soil depth and daily precipitation (published in Meyer et al. (2021b))



especially in spring (March–June) and in late-summer (August–October). The highest soil temperatures (hourly) reached 34.2 and 34.6 °C under PC in summer 2016 and 2019, whereas the highest soil temperatures under SC reached only 29.2 and 25.9 °C in the same periods (SI Table 4). In contrast, the soil moisture (Fig. 1b) was about 5–10 and 2–5% lower under PC compared to SC at the 5 and 35 cm soil depth, respectively, whereas the soil moisture at the 15 cm soil depth was partly higher under PC but the differences between treatments were generally small (mostly < 2%).

# The influence of plastic and straw coverage on microbial soil properties

The ergosterol values (Fig. 2) ranged from 0.09 to  $0.40 \text{ mg kg}^{-1}$  and were by  $0.09-0.16 \text{ mg kg}^{-1}$  higher under SC compared to PC in the 0–10 cm soil layer from T6 to T9  $(p \le 0.041)$ . The lowest ergosterol values in the respective soil layers of both treatments (except 0-10 cm soil layer under SC at T9) were found at T5 (July 2017) and T9 (July 2019). The MBC:MBN ratios (Fig. 3a) were between 3 and 39 and showed wider ratios under PC than under SC at T2, T3 (all soil layers:  $p \le 0.004$ ), T6 (30–60 cm soil layer: p = 0.013), and T7 (10–30 cm soil layer: p = 0.014). Conversely, narrower MBC:MBN ratios were observed under PC at T0 (30–60 cm soil layer: p = 0.034), T1 (all soil layers:  $p \le 0.021$ ), and T4 (0–10 and 10–30 cm soil layer:  $p \le 0.019$ ). The MBC:MBN ratios became wider in both treatments during the sampling period ( $p \le 0.038$ ); however, the increase was stronger under PC (3.0-8.2 times) compared to SC (1.4–2.9 times). The MBC:SOC (Fig. 3b)

and the MBN:TN ratios (Fig. 3c) were between 0.8–5.3 and 0.6–3.0%, respectively. The MBC:SOC ratios were higher under PC than under SC at T3 (all soil layers:  $p \le 0.001$ ), whereas the opposite was observed at T0 (10–30 and 30–60 cm soil layer:  $p \le 0.048$ ), T1 (10–30 and 30–60 cm soil layer:  $p \le 0.018$ ), T4 (all soil layers:  $p \le 0.010$ ), T8 and T9 (0–10 cm soil layer: p = 0.036). The MBN:TN ratios were by 0.3–1.0% higher under PC than under SC in the 0–10 cm soil layer from T5 to T9 (T5, T8 and T9:  $p \le 0.008$ ).

# Mycotoxin occurrence in soil under plastic and straw coverage

The investigated mycotoxins DON, NIV and ZEN (Tables 2, 3, and 4) were detected in respectively 26.3, 8.3, and 8.7% of the samples and occurred primarily in the establishment period of the strawberries (T0-T2) and regarding DON also in the four months after fungicide application (T4-T6). The DON concentrations ranged from 0.3 (LCL) to  $21.8 \,\mu g \, kg^{-1}$  and occurred mainly in the 0-10and 10-30 cm soil layer at T1, T2, T4, T5, and T6. The DON concentrations were higher under SC than under PC in the 0-10 and 10-30 cm soil layer at T1 (6.4-7.7 vs.  $1.4-1.6 \,\mu g \, kg^{-1}$ ) and at T5 (19.6–21.8 vs. 0.6–0.7  $\mu g \, kg^{-1}$ ). DON was only sporadically detected in the 30-60 cm soil layer and at T0, T3, T7, T8, and T9. The NIV concentrations ranged from 0.3 (LCL) to 10.5 µg kg<sup>-1</sup> and occurred primarily at T1 and T2. At T2, NIV showed a tendency to higher concentrations under PC compared to SC in all soil layers. ZEN was mainly detected at T0 and T2 in low concentrations ( $\leq 0.5 \,\mu g \, kg^{-1}$ ) and showed no differences between treatments.

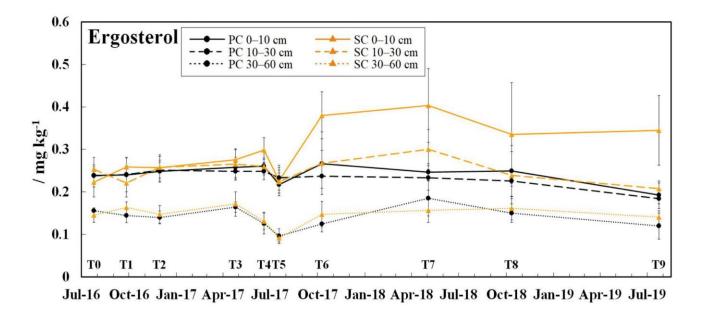


Fig. 2 Ergosterol concentrations determined in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates within the 3-year field experiment, respectively, shown as mean with standard deviation (n=5)



#### Discussion

# How do the soil conditions under plastic and straw coverage influence soil fungi and microbial community?

The PC had no positive effect on fungal growth, despite of the elevated soil temperatures compared to SC (especially in the warmer season), which usually increases fungal growth (Pietikåinen et al. 2005; Bárcenas-Moreno et al. 2009). This is in contrast to former studies, which found an increased fungal biomass under plastic mulching (Subrahmaniyan et al. 2006; Muñoz et al. 2015). But as found that a temperature elevation of 4 °C for several years had no effect on microbial biomass, it was assumed that the temperature elevation under PC was possibly not large or long enough to affect fungal biomass. The larger fungal biomass in the topsoil (0–10 cm) under SC from T6 (October 2017) to T9 (July 2019) was attributed to the higher soil moisture and C-inputs under SC during that period (SI Fig. 2), which favor fungal growth (Dighton 2003; Swer et al. 2011). SOC particularly promote fungal growth if the biomass is fresh and have wide C:N ratios (Bossuyt et al. 2001) such as the applied wheat straw under SC, which usually have C:N ratios between 50 and 100 (Blume et al. 2016). Because the higher soil moisture under SC was already observed before October 2017 and also in the subsoil layer, the biomass entry might be the main driver for the increased fungal biomass. The low ergosterol values in July 2017 and July 2019 might reflect a reduced fungal biomass due to fungicide application in May/June (D'Mello et al. 1998; Magan et al. 2002).

The microbial community under PC showed, derived from MBC:MBN ratios, a stronger increasing and temporally higher fungal fraction (T3, T6, and T7) during the sampling period compared to SC. As fungal biomass has usually wider C:N ratios ( $\approx 10$ ) than bacterial biomass ( $\approx 4$ ) (Sylvia et al. 2005), wider MBC:MBN ratios indicate larger fungal fractions in microbial communities (Campbell et al. 1991). Because fungal biomass (ergosterol) under PC remained almost constant throughout the sampling period, the changed microbial community composition most likely results from a reduced bacterial fraction, which was possibly suppressed by drier soil condition and the mostly higher SOC under PC (SI Fig. 2; Allen et al. 1995; Bailey et al. 2002). The fungal biomass under SC was temporarily stronger reduced after fungicide treatments, because the soil under SC received larger fungicide loads due to the permeability of the straw cover to fungicides in contrast to the impermeable plastic covers (Meyer et al. 2021a). This in turn can temporally favor bacterial growth under SC (Martinez-Toledo et al. 1998; Monkiedje 2002) and hence may additionally shift

microbial community composition. Generally, the high MBC:MBN ratios (>20) during the sampling period point to a strong C entry into soil (Joergensen and Emmerling 2006), presumably due to root growth and exudation of the strawberry plants and aboveground biomass entry (only SC) (Meyer et al. 2021b).

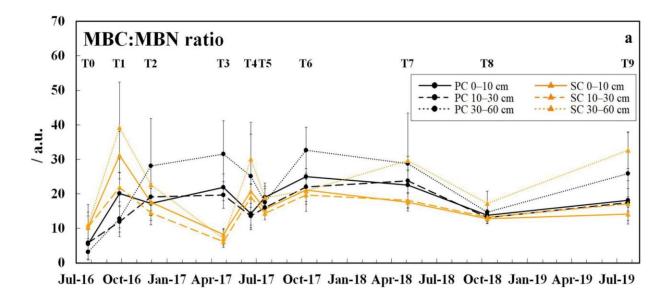
The MBC:SOC and MBN:TN ratios are sensitive for changes in SOM (e.g., due to soil management or environmental changes) and can correlate positively with biomass inputs, soil C and N conversion to microbial biomass (microbial growth), and the degradability/availability of SOM for microorganism (Anderson 2003; Joergensen and Emmerling 2006). The larger MBC:SOC and MBN:TN ratios under SC from T6 (October 2017) to T9 (July 2019), primarily in the surface soil (0–10 cm), indicate a larger conversion of soil C and N resources to microbial biomass and hence a better available soil C and N for microorganism compared to PC (Anderson 2003; Joergensen and Emmerling 2006). This corresponds to the larger labile SOM fraction under SC (Meyer et al. 2021b), which is usually considered as fast-mineralizable and easily available substrate for microorganisms (Wander 2004), derived from the fresh biomass input under SC (Powlson et al. 1987; Meyer et al. 2021b).

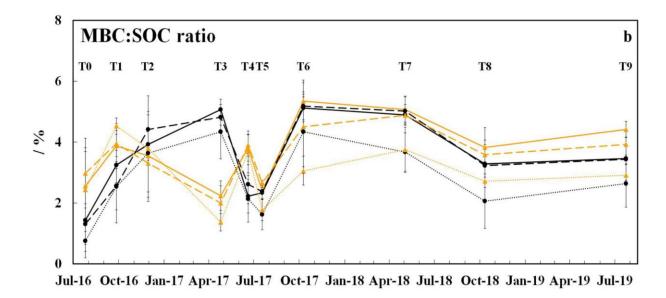
# How do the soil conditions under plastic and straw coverage influence mycotoxin occurrence?

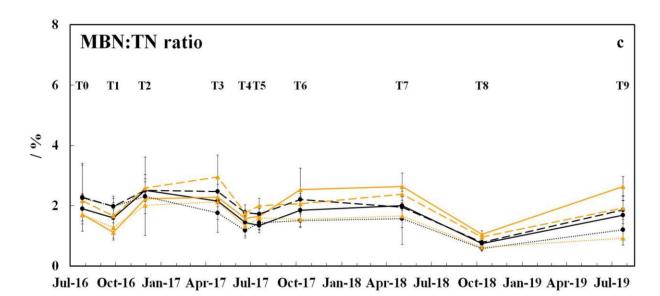
Solely the NIV concentrations at T2 support hypothesis 2 that the adaption of soil fungi to the modified microclimate under PC will trigger a higher mycotoxin occurrence. In contrast to hypothesis 2 and previous studies by Muñoz et al. (2015, 2017), the DON concentrations were particularly at T1 and T5 markedly higher under SC than under PC. The DON and NIV concentrations under PC (21.8 and 10.5 µg kg<sup>-1</sup>) were higher than those measured by Muñoz et al. (2017) in strawberry cultivation (3.0 and 1.1 µg kg<sup>-1</sup>). As the impervious plastic mulch impedes mycotoxin leaching from infested and contaminated plant materials into soil, the results provide a clear evidence that mycotoxins can be produced in situ in soil.

The results suggest that mycotoxin occurrence was stronger influenced by specific field treatment (fungicide application) and by the strawberry growth stage (establishment period) than by the mulching treatments. The biosynthesis of mycotoxins has been suggested as fungal adaptation to stress induced by a multitude of unfavorable growth conditions such as temperature and pH extremes, competition, water, and nutrient scarcity (Wheeler et al. 1991; Schmidt-Heydt et al. 2008; Reverberi et al. 2010; Venkatesh and Keller 2019) and the presence of fungicides (Magan et al. 2002). Thus, the mycotoxin occurrence in the strawberry establishment period (T0–T2) might be interpreted as fungal











**<**Fig. 3 Elemental and eco-physiological ratios. a Microbial biomass carbon to nitrogen ratio (MBC:MBN ratio) determined in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates within the 3-year field experiment, respectively, shown as mean with standard deviation (*n* = 5). **b** Microbial biomass carbon to soil organic carbon ratio (MBC:SOC ratio). **c** Microbial biomass nitrogen to total nitrogen ratio (MBN:TN ratio)

response to the interspecific competition between plants and microorganisms for available N-nutrients (Inselsbacher et al. 2010), induced by the strong plant (root) growth after strawberry plantation (Kumar and Dey 2011; Meyer et al. 2020). Particularly, the adaption of the microbial community to the crop change (Berg and Smalla 2009) induces changes and competition in fungal community (Garbeva et al. 2004; Qin et al. 2017), which can trigger fungi to mycotoxin production (Venkatesh and Keller 2019). This assumption is supported by the fact that mycotoxins were primarily detected in both upper soil layers (0-10 and 10-30 cm), representing the root zone of strawberries. The DON occurrence from T4 to T6 was interpreted as stress response to the fungicide applications (Magan et al. 2002), which was stronger under SC because of the larger fungicide loads received compared to PC (Meyer et al. 2021a). Beside competition and fungicide effects, soil parameters such as temperature, moisture and pH are known to influence Fusarium growth and mycotoxin biosynthesis in soil (Marin et al. 1995; Sweeney and Dobson 1998; Ramirez et al. 2006; Schmidt-Heydt et al. 2008). Soil pH remained neutral (6.5–7.9) during sampling period (SI Fig. 1; Meyer et al. 2021b) and hence had no effect on fungal growth and on mycotoxin biosynthesis (Marin et al. 1995; Sweeney and Dobson 1998). But the temperature maxima and minima for Fusarium growth (above 31–37 °C or below 5 °C) were respectively exceeded in topsoil under PC in summer 2016 and 2019 and in all soil layers of both treatments in each winter 2016-2019 (SI Table 4). As DON and NIV biosynthesis is mainly regulated by temperature (Llorens et al. 2004), the cold temperatures in winter might enhanced mycotoxin production and hence mycotoxins occurrence at T2.

Beside factors influencing mycotoxins occurrence at 12.

Beside factors influencing mycotoxin production in soil, additionally, the fate of mycotoxins must be considered when evaluating mycotoxin concentrations in soil. Literature about the fate of DON, NIV, and ZEN in soil is very limited, but some indications were found for microbial degradation and leaching into running waters (Elmholt 2008; Schenzel et al. 2012b; Kolpin et al. 2014). DON, NIV, and ZEN are stable against transformation by abiotic parameters such as temperature, pH, and hydrolysis (Lauren and Smith 2001; Pitt et al. 2012). The DON and NIV concentrations increased in both mulching treatments during establishment period of strawberries (T0–T2), which implies that mycotoxin production exceeds mycotoxin reduction due to degradation and leaching processes, leading to an accumulation of NIV and DON. No indications were found that the chemically stable and

Table 2 Deoxynivalenol (DON) concentrations determined in the 0-10, 10-30, and 30-60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates in the 3-year field experiment, respectively. DON concentrations are shown as mean with standard deviation (n=5). In brackets, the number of detects above the lowest concentration level (LCL) of 0.3 µg kg<sup>-1</sup>

Treatment	Treatment Soil layer	Sampling									
		To	T1	T2	T3	T4	TS	T6	T7	T8	T9
		25.07.16	26.09.16	29.11.16	25.04.17	19.06.17	18.07.17	09.10.17	03.05.18	11.10.18	23.07.19
		DON	DON	DON	DON	DON	DON	DON	DON	DON	DON
	(cm)	$(\mu g \ kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g \; k g^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g \; k g^{-1})$	$(\mu g kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g kg^{-1})$
PC	0-10 cm	<lcl< th=""><th><math>1.6\pm 3.1</math> (1)</th><th><math>3.4\pm2.9</math> (4)</th><th><pcl< th=""><th><math>1.1 \pm 0.9</math> (3)</th><th><math>0.7 \pm 1.3(1)</math></th><th><math>0.9 \pm 0.7</math> (3)</th><th><tct< th=""><th><pct< th=""><th><pct< th=""></pct<></th></pct<></th></tct<></th></pcl<></th></lcl<>	$1.6\pm 3.1$ (1)	$3.4\pm2.9$ (4)	<pcl< th=""><th><math>1.1 \pm 0.9</math> (3)</th><th><math>0.7 \pm 1.3(1)</math></th><th><math>0.9 \pm 0.7</math> (3)</th><th><tct< th=""><th><pct< th=""><th><pct< th=""></pct<></th></pct<></th></tct<></th></pcl<>	$1.1 \pm 0.9$ (3)	$0.7 \pm 1.3(1)$	$0.9 \pm 0.7$ (3)	<tct< th=""><th><pct< th=""><th><pct< th=""></pct<></th></pct<></th></tct<>	<pct< th=""><th><pct< th=""></pct<></th></pct<>	<pct< th=""></pct<>
	10-30 cm	<pcl< th=""><th><math>1.7 \pm 3.5</math> (1)</th><th><math>3.5\pm1.7</math> (5)</th><th><pcl< th=""><th><math>1.7 \pm 0.6</math> (5)</th><th><math>0.8 \pm 0.9 (2)</math></th><th><math>1.7 \pm 0.1</math> (5)</th><th><math>0.2 \pm 0.2</math> (1)</th><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<></th></pcl<>	$1.7 \pm 3.5$ (1)	$3.5\pm1.7$ (5)	<pcl< th=""><th><math>1.7 \pm 0.6</math> (5)</th><th><math>0.8 \pm 0.9 (2)</math></th><th><math>1.7 \pm 0.1</math> (5)</th><th><math>0.2 \pm 0.2</math> (1)</th><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<>	$1.7 \pm 0.6$ (5)	$0.8 \pm 0.9 (2)$	$1.7 \pm 0.1$ (5)	$0.2 \pm 0.2$ (1)	<pcl< th=""><th><pcl< th=""></pcl<></th></pcl<>	<pcl< th=""></pcl<>
	30-60 cm	<pcl< th=""><th><pcl< th=""><th><pct< th=""><th><pcl< th=""><th><math>0.6\pm0.6(2)</math></th><th>&lt; TCT</th><th><math>0.5\pm0.7(1)</math></th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pct<></th></pcl<></th></pcl<>	<pcl< th=""><th><pct< th=""><th><pcl< th=""><th><math>0.6\pm0.6(2)</math></th><th>&lt; TCT</th><th><math>0.5\pm0.7(1)</math></th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pct<></th></pcl<>	<pct< th=""><th><pcl< th=""><th><math>0.6\pm0.6(2)</math></th><th>&lt; TCT</th><th><math>0.5\pm0.7(1)</math></th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pct<>	<pcl< th=""><th><math>0.6\pm0.6(2)</math></th><th>&lt; TCT</th><th><math>0.5\pm0.7(1)</math></th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<></th></pcl<>	$0.6\pm0.6(2)$	< TCT	$0.5\pm0.7(1)$	<pcl< th=""><th><pcl< th=""><th><pcl< th=""></pcl<></th></pcl<></th></pcl<>	<pcl< th=""><th><pcl< th=""></pcl<></th></pcl<>	<pcl< th=""></pcl<>
SC	0-10 cm	$1.9 \pm 2.9 (2)$	$6.4 \pm 3.2$ (5)	$3.8 \pm 1.2$ (5)	<pcl< th=""><th><math>0.9 \pm 0.7</math> (3)</th><th><math>21.8 \pm 15.0</math> (5)</th><th><math>1.1 \pm 0.7</math> (4)</th><th><pcc< th=""><th><pcl< th=""><th><ccl< th=""></ccl<></th></pcl<></th></pcc<></th></pcl<>	$0.9 \pm 0.7$ (3)	$21.8 \pm 15.0$ (5)	$1.1 \pm 0.7$ (4)	<pcc< th=""><th><pcl< th=""><th><ccl< th=""></ccl<></th></pcl<></th></pcc<>	<pcl< th=""><th><ccl< th=""></ccl<></th></pcl<>	<ccl< th=""></ccl<>
	10-30 cm	<pcl< td=""><td><math>7.7 \pm 2.3</math> (5)</td><td><math>0.5\pm0.5(2)</math></td><td><pcl< td=""><td><math>1.1 \pm 0.8 (3)</math></td><td><math>19.6 \pm 17.5 (4)</math></td><td><math>0.5 \pm 0.5</math> (2)</td><td><pcl< td=""><td><math>0.3 \pm 0.2 (1)</math></td><td><pcl< td=""></pcl<></td></pcl<></td></pcl<></td></pcl<>	$7.7 \pm 2.3$ (5)	$0.5\pm0.5(2)$	<pcl< td=""><td><math>1.1 \pm 0.8 (3)</math></td><td><math>19.6 \pm 17.5 (4)</math></td><td><math>0.5 \pm 0.5</math> (2)</td><td><pcl< td=""><td><math>0.3 \pm 0.2 (1)</math></td><td><pcl< td=""></pcl<></td></pcl<></td></pcl<>	$1.1 \pm 0.8 (3)$	$19.6 \pm 17.5 (4)$	$0.5 \pm 0.5$ (2)	<pcl< td=""><td><math>0.3 \pm 0.2 (1)</math></td><td><pcl< td=""></pcl<></td></pcl<>	$0.3 \pm 0.2 (1)$	<pcl< td=""></pcl<>
	30–60 cm	<pcl< td=""><td><math>1.5 \pm 1.8 (2)</math></td><td><pcl< td=""><td><pcl< td=""><td><math>0.7 \pm 0.9</math> (2)</td><td><pct< td=""><td><pcl< td=""><td><lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<></td></pcl<></td></pct<></td></pcl<></td></pcl<></td></pcl<>	$1.5 \pm 1.8 (2)$	<pcl< td=""><td><pcl< td=""><td><math>0.7 \pm 0.9</math> (2)</td><td><pct< td=""><td><pcl< td=""><td><lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<></td></pcl<></td></pct<></td></pcl<></td></pcl<>	<pcl< td=""><td><math>0.7 \pm 0.9</math> (2)</td><td><pct< td=""><td><pcl< td=""><td><lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<></td></pcl<></td></pct<></td></pcl<>	$0.7 \pm 0.9$ (2)	<pct< td=""><td><pcl< td=""><td><lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<></td></pcl<></td></pct<>	<pcl< td=""><td><lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<></td></pcl<>	<lcl< td=""><td><pcl< td=""><td><lcl< td=""></lcl<></td></pcl<></td></lcl<>	<pcl< td=""><td><lcl< td=""></lcl<></td></pcl<>	<lcl< td=""></lcl<>



LCL all five samples below LCI

Table 3 Nivalenol (NIV) concentrations determined in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates in the 3-year field experiment, respectively. NIV concentrations are shown as mean with standard deviation (n=5). In brackets, the number of detects above the lowest concentration level (LCL) of 0.3 µg kg<sup>-1</sup>

TO T1   T2   T3   T4   T5   T4   T5   T6   T7   T8   T8   T6   T7   T8   T8   T6   T7   T8   T8   T8   T8   T8   T8   T8	Treatment	Soil layer	Sampling									
1.0   1.0			To	T1	T2	T3	T4	T5	T6	T7	T8	T9
NIV			25.07.16	26.09.16	29.11.16	25.04.17	19.06.17	18.07.17	09.10.17	03.05.18	11.10.18	23.07.19
(cm) (µg kg <sup>-1</sup> ) (			NIV	NIV	NIV	NIV	NIV	NIV	NIV	NIV	NIV	NIV
0-10 cm <lcl (1)="" (4)="" 1.7±3.5="" 7.9±4.4="" <lc<="" <lcl="" th=""><th></th><th>(cm)</th><th><math display="block">(\mu g \; kg^{-1})</math></th><th><math display="block">(\mu g \ kg^{-1})</math></th><th><math>(\mu g kg^{-1})</math></th><th><math>(\mu g kg^{-1})</math></th><th><math display="block">(\mu g \; k g^{-1})</math></th><th><math display="block">(\mu g \; k g^{-1})</math></th><th><math display="block">(\mu g \; kg^{-1})</math></th><th><math>(\mu g kg^{-1})</math></th><th><math display="block">(\mu g \; k g^{-1})</math></th><th><math display="block">(\mu g \; kg^{-1})</math></th></lcl>		(cm)	$(\mu g \; kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g \; k g^{-1})$	$(\mu g \; k g^{-1})$	$(\mu g \; kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g \; k g^{-1})$	$(\mu g \; kg^{-1})$
10-30 cm <lcl< td="">         1.8±3.6 (1)         10.5±1.8 (5)         <lcl< td=""> <l< th=""><th>PC</th><th>0-10 cm</th><th><pcl< th=""><th><math>1.7 \pm 3.5 (1)</math></th><th><math>7.9 \pm 4.4 (4)</math></th><th><pcl< th=""><th><lcl< th=""><th><tct< th=""><th><pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<></th></tct<></th></lcl<></th></pcl<></th></pcl<></th></l<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<>	PC	0-10 cm	<pcl< th=""><th><math>1.7 \pm 3.5 (1)</math></th><th><math>7.9 \pm 4.4 (4)</math></th><th><pcl< th=""><th><lcl< th=""><th><tct< th=""><th><pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<></th></tct<></th></lcl<></th></pcl<></th></pcl<>	$1.7 \pm 3.5 (1)$	$7.9 \pm 4.4 (4)$	<pcl< th=""><th><lcl< th=""><th><tct< th=""><th><pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<></th></tct<></th></lcl<></th></pcl<>	<lcl< th=""><th><tct< th=""><th><pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<></th></tct<></th></lcl<>	<tct< th=""><th><pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<></th></tct<>	<pcl< th=""><th><lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<></th></pcl<>	<lcl< th=""><th><pcl< th=""><th><pct< th=""></pct<></th></pcl<></th></lcl<>	<pcl< th=""><th><pct< th=""></pct<></th></pcl<>	<pct< th=""></pct<>
30-60 cm <lcl< td=""> <lcl< td="">         3.7±4.9 (2)         <lcl< td=""> <lcl< td=""></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<></lcl<>		10-30 cm	<pcl< td=""><td><math>1.8 \pm 3.6 (1)</math></td><td><math>10.5 \pm 1.8 (5)</math></td><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<>	$1.8 \pm 3.6 (1)$	$10.5 \pm 1.8 (5)$	<pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><pcl< td=""><td><cc< td=""></cc<></td></pcl<></td></pcl<>	<pcl< td=""><td><cc< td=""></cc<></td></pcl<>	<cc< td=""></cc<>
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		30-60 cm	<pcl< th=""><th><pcl< th=""><th><math>3.7 \pm 4.9(2)</math></th><th><pcl< th=""><th>&lt; CCL</th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<>	<pcl< th=""><th><math>3.7 \pm 4.9(2)</math></th><th><pcl< th=""><th>&lt; CCL</th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<>	$3.7 \pm 4.9(2)$	<pcl< th=""><th>&lt; CCL</th><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<></th></pcl<></th></pcl<></th></pcl<>	< CCL	<pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<></th></pcl<></th></pcl<>	<pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<></th></pcl<>	<pcl< th=""><th><pcl< th=""><th><pcc< th=""></pcc<></th></pcl<></th></pcl<>	<pcl< th=""><th><pcc< th=""></pcc<></th></pcl<>	<pcc< th=""></pcc<>
	SC	0-10 cm	<pcl< th=""><th><lcl< th=""><th><math>5.5 \pm 4.9(3)</math></th><th><pct< th=""><th><lcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<></th></pcl<></th></lcl<></th></pct<></th></lcl<></th></pcl<>	<lcl< th=""><th><math>5.5 \pm 4.9(3)</math></th><th><pct< th=""><th><lcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<></th></pcl<></th></lcl<></th></pct<></th></lcl<>	$5.5 \pm 4.9(3)$	<pct< th=""><th><lcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<></th></pcl<></th></lcl<></th></pct<>	<lcl< th=""><th><pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<></th></pcl<></th></lcl<>	<pcl< th=""><th><pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<></th></pcl<>	<pcl< th=""><th><pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<></th></pcl<>	<pcl< th=""><th><math>0.2 \pm 0.2</math> (1)</th><th><ccc< th=""></ccc<></th></pcl<>	$0.2 \pm 0.2$ (1)	<ccc< th=""></ccc<>
C  C  C  C  C  C  C  C  C  C  C  C  C		10-30 cm	<pcl< td=""><td><math>3.8 \pm 5.1 (2)</math></td><td><math>7.6\pm4.2(4)</math></td><td><pct <<="" td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><math>0.4 \pm 0.5 (1)</math></td><td><pcl< td=""><td><ccc< td=""></ccc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pct></td></pcl<>	$3.8 \pm 5.1 (2)$	$7.6\pm4.2(4)$	<pct <<="" td=""><td><pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><math>0.4 \pm 0.5 (1)</math></td><td><pcl< td=""><td><ccc< td=""></ccc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<></td></pct>	<pcl< td=""><td><pcl< td=""><td><pcl< td=""><td><math>0.4 \pm 0.5 (1)</math></td><td><pcl< td=""><td><ccc< td=""></ccc<></td></pcl<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><pcl< td=""><td><math>0.4 \pm 0.5 (1)</math></td><td><pcl< td=""><td><ccc< td=""></ccc<></td></pcl<></td></pcl<></td></pcl<>	<pcl< td=""><td><math>0.4 \pm 0.5 (1)</math></td><td><pcl< td=""><td><ccc< td=""></ccc<></td></pcl<></td></pcl<>	$0.4 \pm 0.5 (1)$	<pcl< td=""><td><ccc< td=""></ccc<></td></pcl<>	<ccc< td=""></ccc<>
		30–60 cm	<lcl< td=""><td><lcl< td=""><td><lcl< td=""><td><ccl< td=""><td><lcl< td=""><td><ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<></td></lcl<></td></ccl<></td></lcl<></td></lcl<></td></lcl<>	<lcl< td=""><td><lcl< td=""><td><ccl< td=""><td><lcl< td=""><td><ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<></td></lcl<></td></ccl<></td></lcl<></td></lcl<>	<lcl< td=""><td><ccl< td=""><td><lcl< td=""><td><ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<></td></lcl<></td></ccl<></td></lcl<>	<ccl< td=""><td><lcl< td=""><td><ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<></td></lcl<></td></ccl<>	<lcl< td=""><td><ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<></td></lcl<>	<ccl< td=""><td><ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<></td></ccl<>	<ccl< td=""><td><lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<></td></ccl<>	<lcl< td=""><td><math>0.2 \pm 0.2</math> (1)</td><td><ccl< td=""></ccl<></td></lcl<>	$0.2 \pm 0.2$ (1)	<ccl< td=""></ccl<>

<LCL all five samples below lowest concentration level</p>

**Table 4** Zearalenone (ZEN) concentrations determined in the 0–10, 10–30, and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates in the 3-year field experiment, respectively. ZEN concentrations are shown as mean with standard deviation (n=5). In brackets, the number of detects above the lowest concentration level (LCL) of 0.3 µg kg<sup>-1</sup>

Treatment	Soil layer	Sampling									
		TO	T1	T2	T3	T4	TS	9L	T7	T8	T9
		25.07.16	26.09.16	29.11.16	25.04.17	19.06.17	18.07.17	09.10.17	03.05.18	11.10.18	23.07.19
		ZEN	ZEN	ZEN	ZEN	ZEN	ZEN	ZEN	ZEN	ZEN	ZEN
	(cm)	$(\mu g \ kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g \; kg^{-1})$	$(\mu g kg^{-1})$	$(\mu g \ kg^{-1})$	$(\mu g kg^{-1})$
PC	0-10 cm	<lcl< td=""><td><pct< td=""><td><math>0.2\pm0.1(2)</math></td><td><math>0.2 \pm 0.1 (1)</math></td><td><pcl< td=""><td><pct< td=""><td><pcl< td=""><td><pre></pre></td><td><pct< td=""><td><pcl< td=""></pcl<></td></pct<></td></pcl<></td></pct<></td></pcl<></td></pct<></td></lcl<>	<pct< td=""><td><math>0.2\pm0.1(2)</math></td><td><math>0.2 \pm 0.1 (1)</math></td><td><pcl< td=""><td><pct< td=""><td><pcl< td=""><td><pre></pre></td><td><pct< td=""><td><pcl< td=""></pcl<></td></pct<></td></pcl<></td></pct<></td></pcl<></td></pct<>	$0.2\pm0.1(2)$	$0.2 \pm 0.1 (1)$	<pcl< td=""><td><pct< td=""><td><pcl< td=""><td><pre></pre></td><td><pct< td=""><td><pcl< td=""></pcl<></td></pct<></td></pcl<></td></pct<></td></pcl<>	<pct< td=""><td><pcl< td=""><td><pre></pre></td><td><pct< td=""><td><pcl< td=""></pcl<></td></pct<></td></pcl<></td></pct<>	<pcl< td=""><td><pre></pre></td><td><pct< td=""><td><pcl< td=""></pcl<></td></pct<></td></pcl<>	<pre></pre>	<pct< td=""><td><pcl< td=""></pcl<></td></pct<>	<pcl< td=""></pcl<>
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	30–60 cm	$0.4\pm0.5(1)$	<pcl< td=""><td><math>0.3 \pm 0.1 (3)</math></td><td><pct< td=""><td><pcc< td=""><td><pcl< td=""><td><pcc< td=""><td><lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<></td></pcc<></td></pcl<></td></pcc<></td></pct<></td></pcl<>	$0.3 \pm 0.1 (3)$	<pct< td=""><td><pcc< td=""><td><pcl< td=""><td><pcc< td=""><td><lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<></td></pcc<></td></pcl<></td></pcc<></td></pct<>	<pcc< td=""><td><pcl< td=""><td><pcc< td=""><td><lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<></td></pcc<></td></pcl<></td></pcc<>	<pcl< td=""><td><pcc< td=""><td><lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<></td></pcc<></td></pcl<>	<pcc< td=""><td><lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<></td></pcc<>	<lcl< td=""><td><cct< td=""><td><pcc <<="" td=""></pcc></td></cct<></td></lcl<>	<cct< td=""><td><pcc <<="" td=""></pcc></td></cct<>	<pcc <<="" td=""></pcc>

< LCL all five samples below lowest concentration level



water-soluble DON and NIV (Table 1) were relocated into deeper soil layers during sampling period, which would indicate leaching. In general, leaching under PC seems unlikely because of the impeded rainfall infiltration and the partially occurring lateral and ascending water flows (Ruidisch et al. 2013; Meyer et al. 2021b). Nevertheless, the DON and NIV concentrations at T2 and T5 vanished (almost) completely to the next sampling, which was presumably induced by microbial degradation (Elmholt 2008) because the aforementioned abiotic transformation and leaching can be excluded. If plant uptake of the water-soluble DON and NIV can occur and hence be a possible fate pathway was not yet investigated.

To the best of our knowledge, this was the first study which investigated the influence of plastic mulching on fungi and mycotoxins in soil in the temporal course of a multiannual plant cultivation. Clear evidence was shown that mycotoxins can be biosynthesized in situ in soil. Regarding the investigated mulching treatments, plastic mulching had no clear positive effects on soil fungi and mycotoxin occurrence compared to the more traditional straw mulching, presumably because its effects on soil temperature, moisture, pH, and SOM were mostly small under the present humid, temperate climate (Meyer et al. 2020, 2021a, b). However, as usually stronger effects on soil conditions were reported under warmer and more arid climates (Gan et al. 2013; Steinmetz et al. 2016), further studies under different climate conditions are necessary to better assess the influence of plastic mulching on mycotoxin occurrence. As mycotoxin occurrence in soil was influenced by plant growth stage and fungicide application, further research is required with different crops and fungicides to gain a better understanding of their impact on mycotoxin biosynthesis.

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1007/s12550-022-00451-5.

Acknowledgements We thank Christof Steegmüller for providing the sampling site, conducting the agricultural management of the sampling site, and his expertise on strawberries cultivation. Furthermore, we would like to thank Mathilde Knott, Christian Buchmann, Kilian Kenngott, and Zacharias Steinmetz for valuable assistance during soil sampling and Lea Bosch, Maria Olivares, Johanna Girardi, Camilla More, and Ken Mauser for valuable assistance during sample analyses. Additionally, we want to thank Prof. S. Thiele-Bruhn (University of Trier) for providing the field measuring station.

Author contribution The paper concept was developed by myself in collaboration with Dr. Katherine Muñoz. Experimental design, all soil samplings, data evaluations, and statistics were planned and conducted by myself. Colleagues and technical staff supported me during soil samplings. Laboratory analyses were conducted by myself with assistance of technical and student staff. Data interpretation and paper writing was done by myself with support of Dr. Katherine Muñoz and Prof. Dr. Gabriele Ellen Schaumann.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was supported by the Ministry for Education,

Sciences, Further Education and Culture of the State of Rhineland-Palatinate (MBWWK) in the frame of the Interdisciplinary Research Group for Environmental Studies (IFG-Umwelt) of University Koblenz-Landau, by the Prof. B. Gedek and W. Gedek foundation and by the research fund of the University of Koblenz-Landau.

Availability of data and material The datasets used and/or analyzed during the current study are available from https://doi.org/10.6084/m9.figshare.14447127.v2.

#### **Declarations**

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest The authors declare no competing interests.

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# **CHAPTER 7**

**Synthesis and Conclusions** 

# 7.1 Summarized findings and conclusions: How plastic mulching influences soil processes under temperate, humid climate in Central Europe

In this chapter, the findings and conclusions of the individual studies were interlinked and discussed in a more general context to explain how multiannual plastic mulching influences soil processes under the present temperate, humid climate in Central Europe. Plastic mulching modified the soil microclimate to higher soil temperatures and lower soil moistures compared to straw mulching under the present climate (Chapter 5) due to its optical properties and impermeability which changed heat, gas and water exchange at the soil surface (Ham et al., 1993; Ham & Kluitenberg, 1994; Tarara, 2000). The soil temperature increases due to the changed energy flows under plastic mulching reached until a soil depth of 35 cm and were largest in spring and summer (up to 6.5 °C). Furthermore, the shading of the plant canopy mitigated the increase in soil temperature under plastic mulching and this effect increased with increasing density of the plant canopy (Chapter 3 & 5). The lower soil moisture under plastic mulching showed that under the present climate with higher precipitation and lower evaporation rates than in semiarid and arid climates, the impeded rainfall infiltration via soil surface had a stronger effect on water balance than the reduced evaporation. The impeded rainfall infiltration under plastic mulching shifted the water cycling in soil from the downward directed seepage water flows during and after rainfalls to lateral water flows from furrows to ridges and partially even upward directed water flows in the ridges, when the topsoil in the ridges was very dry. However, only a fraction of the rainwater reached the ridges due to lateral water flows from the straw-covered furrows, whereas the remaining rainwater presumably percolates from the furrows into deeper soil layers and become unattainable for plants (Chapter 5). From that, I concluded that the increased precipitation use efficiency under plastic mulching shown for arid and semiarid areas where evaporation is high and precipitation is low (Gan et al., 2013), is not applicable in humid areas with higher precipitation and lower evaporation rates. Thus, accelerated biochemical soil reactions due to an elevated soil temperatures (Lal, 2004; Dominati et al., 2010; Blume et al., 2016) can be interpreted as the key factor for the enhanced plant growth and harvest yields under plastic mulching in the present climate.

The shifted water cycling under plastic mulching from downward directed seepage water flows to more lateral and upward directed water flows reduced nutrient leaching and hence had an impact on nutrient cycling. This increased the nitrogen content under plastic mulching in the establishment period (Chapter 3). However, no further impacts on nutrient status were observed

during the remaining sampling period (Chapter 5). I assumed that the gains of the reduced nutrient leaching were exceeded by a higher nutrient uptake of plants under plastic mulch, as shown by Kumar & Dey (2011) and Wang *et al.* (2014). Additionally, I suggest that the straw mulch (applied after the establishment period) and the dense plant canopy might also mitigate leaching due to a decelerated rainwater infiltration and an increased rainfall interception and cause thus a similar 'covering effect' as the plastic mulches.

Plastic mulching maintained a stable soil structure in the surface layer (0–5 cm) with a low bulk density and a high macropore volume, which might mitigate soil erosion after mulch removing. In the surface layer, the water-impermeable plastic mulches avoided rapid soil wetting and excess water during rainfalls (Chapter 5). This is known to prevent aggregate breakdown and particle relocation (Bing So, 2006; Le Bissonnais, 2006; Shah *et al.*, 2017) and hence mitigates soil crusting and compaction under plastic mulching. In contrast to my assumption, the expected larger root growth and exudation of strawberry plants under plastic mulching (Fernandez *et al.*, 2001; Kumar & Dey, 2011) was not large enough to increase aggregation or macroaggregate stability within the sampling period. Thus, I conclude that plastic mulching can reduce aggregate disruption processes at the soil surface but seems not to promote aggregation or stabilization processes within the observed time period.

Plastic mulching changed carbon fluxes and transformation so that the total SOM increased and the SOM composition was partially shifted to a more stable SOM during the three-year sampling period. In contrast to my assumptions, the higher belowground biomass productivity under plastic mulch can compensate the expected SOM losses by the impeded aboveground biomass input and the accelerated SOM decomposition due to the increased soil temperature under plastic mulching (Chapter 3 & 5). Thus, in contrast to the SOM losses reported from arid and semiarid climates, attributed to an accelerated SOM decomposition (Gan et al., 2013; Steinmetz et al., 2016), I found under temperate, humid climate no evidence for an accelerated C and N cycling or increased SOM losses under plastic mulching. Because the former studies, which described SOM losses under plastic mulching, also reported an increased soil moisture (e.g. Li et al., 2004; Zhang et al., 2015), soil moisture is probably the main factor for the accelerated SOM decomposition under plastic mulching. Because low soil moisture is known as limiting factor for microbial activity and consequently SOM decomposition (Coûteaux et al., 1995; Butenschoen et al., 2011; Nannipieri et al., 2017), I assume that the low soil moisture in arid and semiarid climates strongly inhibits microbial activity and thus SOM decomposition, which then gets offset by the increased soil moisture under plastic mulching. In contrast to that, the soil moisture under humid climate is in general high enough to enable SOM decomposition and gets not increased by plastic mulching. The partially larger stable SOM fractions indicated that plastic mulching might stabilize the SOM (Chapter 5). I attributed this to an enhanced organo-mineral complex formation caused by the elsewhere described larger root and microbial exudation under plastic mulching (Kumar & Dey, 2011; Yin *et al.*, 2013; Dignac *et al.*, 2017; Jackson *et al.*, 2017).

In contrast to plastic mulching, the fresh biomass input from aboveground under straw mulching increased with time the labile SOM and the total SOM pool in the topsoil and thus promoted the fungal and total microbial growth and finally exceeds the respective levels of SOM and microbial biomass under plastic mulching in the topsoil after one year (Chapter 5). I assume that the aforementioned effects on SOM stocks and microbial community under straw mulching continue with time and might reach later on also the deeper soil layers due to the incorporation of the fresh biomass through preferential flow and the soil fauna.

Plastic mulching reduced fungicide entry into soil compared to straw mulching and reduced consequently the effects on fungal biomass and on DON occurrence in soil (Chapter 4). This reduces soil contamination with fungicides and mycotoxins and maintains soil functions and quality (Mendes *et al.*, 2016; Bünemann *et al.*, 2018). In contrast to my expectations, the modified microclimate under plastic mulching did not promote mycotoxin biosynthesis and thus a higher occurrence of the mycotoxins DON, NIV and ZEN at the investigated time points. However, the soil conditions during the establishment period and after the fungicide treatments promoted the biosynthesis of the investigated mycotoxins and confirmed that mycotoxins are relevant soil contaminants (Chapter 6). I interpreted the increased mycotoxin biosynthesis as stress response of the soil fungi to unfavorable growth conditions, respectively induced by the cultivation change, the strong strawberry growth and nutrient competition and the fungicide residues, as described elsewhere (Magan *et al.*, 2002; Berg & Smalla, 2009; Inselsbacher *et al.*, 2010; Venkatesh & Keller, 2019).

The field study demonstrated that type and extent of the plastic mulching impacts on soil properties and processes can vary depending on the time, season or soil depth (Chapter 3–6). This emphasized the importance of investigating different soil layers and time points to obtain a more complete process understanding of the plastic mulching effects. For example, the statement whether plastic or straw mulching had a higher SOM depended on soil layer and time because the initially larger SOM increase under plastic mulching due to the stronger belowground biomass input was compensated with time in the topsoil layer under straw mulch

due to the continued aboveground biomass input. The protective effects of plastic mulching against aggregate disruption or the promoting effect of straw mulching on microbial growth and the SOM pool were also time-dependent and became only observable in the pore volumes and the bulk density or rather the SOM and the microbial biomass in the second year of the experiment. Furthermore, it should be considered in future studies that soil temperature and moisture, the main drivers of plastic mulching effects (Gan et al., 2013), can exhibit strong climatically, seasonal and depth-dependent effects and thus their impacts on biochemical soil reactions can strongly differ depending on geographic location, season and soil depth. The seasonal and depth-dependent effects of plastic mulching on soil temperature and moisture were meanwhile confirmed by further studies (Xiukang et al., 2015; Li et al., 2016, 2017; Ma et al., 2018). In contrast to later periods of the experiment, the strawberry establishment period showed as expected rapid changes in several soil properties (e.g., pH, electrical conductivity, total nitrogen, microbial biomass and SOM). I interpreted this as consequences of the strong root growth and the high nutrient uptake of the strawberry plants, growing from bare-root plants to full-grown plants (Fernandez et al., 2001; Kumar & Dey, 2011; Mo et al., 2020), and the field set-up (tillage, fertilization and establishing the ridge-furrow system). Therefore, it seems justified to pay particular attention to the establishment period when investigating the impact of plastic mulching on soil processes and this should also be included in future studies.

On basis of this field study, I conclude that generalizing the plastic mulching effects on soil over different climate zones is hardly possible because the plastic mulching impacts on soil processes can strongly differ in type and extent depending on climate. Thus, the dominating soil process, influencing a certain soil property, can differ between climates. For example, plastic mulching decreases soil moisture under temperate, humid climate because the water losses due to the impeded precipitation infiltration dominates over the water gains due to the reduced evaporation, whereas it is the opposite in arid and semiarid regions. Therefore, a differentiated consideration becomes necessary to evaluate the plastic mulching effects on soil under different climates. In contrast to the plastic mulching effects reported from arid and semiarid regions, the impacts of plastic mulching on the investigated soil properties and processes were primarily small, in another way or even absent (e.g., microbial biomass, aggregate stability) under the present temperate, humid climate. As the main reasons for that, I state the fact that plastic mulching did not increase but reduce soil moisture and that soil moisture was under the present climate due to rainfall and irrigation anyway large enough to be not a limiting factor for microbial growth and activity and the microbial-mediated soil processes (e.g. SOM transformation). Furthermore, the 'covering effect' of the plastic mulches (influencing e.g., soil structure in soil surface) was in a similar manner also overtaken by the straw mulch and the dense canopy of the strawberry plants. Thus, crop type and growth stage of the plants should be considered as an important impact factor, especially when comparing plastic mulching to uncovered treatments.

# 7.2 The consequences of plastic mulching for soil quality in terms of a sustainable agriculture

In this chapter, I discuss briefly what consequences plastic mulching under temperate, humid climate could have for soil quality and a sustainable agricultural development on the basis of the aforementioned impacts of plastic mulching on soil processes.

Nowadays, agriculture suffers from increasing water scarcities and lowered groundwater levels due to climate change and increasing irrigation needs (Foley *et al.*, 2005; Piao *et al.*, 2010) and requires an improved water use efficiency (WUE) to use the available water resources more efficient (Mueller *et al.*, 2012; Piñeiro *et al.*, 2020). Drip irrigation combined with plastic mulching is known as an efficient strategy to increase the WUE of irrigation water (Vázquez *et al.*, 2006; Wang *et al.*, 2019). However, the impeded rainfall infiltration under plastic mulches led to a strong decline in soil moisture, especially when no irrigation was applied (Chapter 5), and hence reduces the WUE of the naturally occurring rainfalls and increases the irrigation necessity under plastic mulching. Thus, plastic mulching under the humid climate has the potential to further increase WUE when precipitation would be used more efficiently, which possibly could be achieved by increasing the planting holes or adding percolation holes to the plastic mulch.

An improved nutrient use efficiency (NUE) is crucial for a sustainable agricultural development as in modern agriculture large quantities of fertilizers are lost unused from the agricultural system, for example, 60–70 % of the applied nitrogen fertilizers (Oenema & Pietrzak, 2002; Mueller *et al.*, 2012; Van Grinsven *et al.*, 2013; Mohanty *et al.*, 2020). Plastic mulching reduces nutrient leaching and sustains nitrogen stocks, compared to cultivation conditions with bare soil and no dense plant canopy (Chapter 3), and thus mitigates nutrient and fertilizer losses and groundwater contamination (e.g., with nitrate). This can help to save nutrient resources and reduce fertilizer application, costs and environmental damage of fertilizers and hence improves the NUE.

Soil erosion and compaction are both frequent soil degradation processes, which can e.g. decrease aeration, infiltration and hydraulic conductivity and increase runoff, SOM depletion and nutrient losses and are thus detrimental to soil quality (Pimentel & Burgess, 2013; Vereecken *et al.*, 2016; Shah *et al.*, 2017). The stable soil structure in the surface soil under plastic mulches (Chapter 5) made the soil less prone to erosion and prevents soil compaction (Le Bissonnais, 2006). Furthermore, the loose and friable soil structure sustains a good aeration, soil warming, rooting and water infiltration of the soil, which are essential to enable plant growth and maintain agronomic productivity (Bronick & Lal, 2005; Le Bissonnais, 2006). However, plastic mulching can increase runoff into furrows or to the field surrounding (Wauchope, 1996; Rice *et al.*, 2001, 2002, 2007). Thus, it has to be considered that depending on the conditions of the furrows and the field surrounding (e.g., inclination, vegetation, straw cover) plastic mulching might enhance erosion risk elsewhere (Rice *et al.*, 2004, 2007).

SOM is the most central soil component for soil quality and regulates, for example, soil moisture and structure, nutrient supply rates and microbial activity (McLauchlan, 2006; Lal, 2013). Thus, SOM depletion exacerbates soil degradation in agricultural soils (Lal, 2004). Under the present temperate, humid climate, I observed no indications for an increased SOM depletion under plastic mulching due to an accelerated decomposition or a decreased biomass entry. However, straw mulching increased the SOM with time in the topsoil (Chapter 5). Therefore, the straw mulch alone or its combination with plastic mulch might be a good practice to refill the depleted SOM stocks in agricultural soils to regain higher SOM stocks (Huo et al., 2017; Yang et al., 2018). This might be a good strategy to increase water holding capacity, nutrient supply and structural stability and thus soil quality and agronomic sustainability (McLauchlan, 2006; Lal, 2009). Furthermore, larger SOM stocks improve carbon storage and reduce greenhouse gas emission, which can mitigate climate change (Lal, 2008, 2013). Plastic mulching had partially a more stabilized SOM (Chapter 5), which can reduce nutrient loss, decomposition and erosion (Wander, 2004; Lal, 2004; Grego & Lagomarsino, 2008) and helps thus to increase NUE and WUE and to reduce SOM depletion and greenhouse gas emission (Powlson et al., 2011; Mueller et al., 2012).

Agrochemicals like fungicides can affect soil microorganisms, which regulate 80–90 % of soil processes (Nannipieri *et al.*, 2017). Agrochemicals can thus impair microbial functions and biochemical processes, which in turn can reduce plant growth and development (Nannipieri *et al.*, 2017; Meena *et al.*, 2020). Therefore, soil contamination with agrochemicals can reduce soil functions and quality (Mendes *et al.*, 2016; Bünemann *et al.*, 2018). Plastic mulching

reduced fungicide entry into soil (Chapter 4) and thus mitigates the effects of soil contamination on soil functions and quality. More precisely, the reduced fungicide concentrations under plastic mulching can help to alleviate the negative impacts on soil microorganisms, primarily soil fungi, and maintain their action in important soil processes such as SOM decomposition and nutrient cycling (Stockmann *et al.*, 2013; Frac *et al.*, 2018). Furthermore, the lower fungicide concentrations under plastic mulching (Chapter 4), together with the aforementioned reduced leaching under plastic mulching (Chapter 3 & 5), can reduce the risk of fungicide leaching into the groundwater. Plastic mulching did not promote mycotoxin occurrence in soil (Chapter 6). It may therefore be no risk for soil quality due to soil contamination with mycotoxins or for food or water quality due to a potential mycotoxin uptake by plants or mycotoxin leaching to ground- and running water.

I conclude on basis of my field study that plastic mulching under humid climate might contribute to reduce soil degradation processes such as SOM depletion, erosion, nutrient leaching, soil compaction and soil contamination and hence could help to enable a sustainable agricultural intensification. However, plastic mulching has also critical aspects such as the inefficient precipitation use, the reduced fresh biomass input from aboveground, the soil contamination with macro- and microplastics and the disposal of the plastic mulches, which have to be considered for a complete evaluation (Gao *et al.*, 2019a; Huang *et al.*, 2020). The critical aspects might be eliminated by implementing optimizations, aiming at an improved percolation of rainwater, additional organic inputs, a complete plastic mulch removing and recycling strategies for the mulching waste (Kasirajan & Ngouajio, 2012; Huo *et al.*, 2017; Gao *et al.*, 2019a). On condition that the necessary optimizations are made, an appropriate use of plastic mulching under temperate, humid climate might support modern agriculture to increase agricultural production in a sustainable way without compromising soil quality. Nevertheless, there are still some open questions about how plastic mulching impacts on soil that have to be addressed in future studies.

#### 7.3 Outlook and open questions

There are several aspects and open questions which were beyond the scope of this PhD thesis but need to be addressed in further research to complement the results of this PhD thesis and to obtain a more comprehensive assessment of plastic mulching:

First of all, the findings of this field study are confined to the used crop and soil type. In a next step, it is necessary to prove on a larger scale how generalizable the results are for different soils, crops and agricultural techniques. As in my study the dense strawberry canopy reduced the soil temperature increase under plastic mulching and the belowground biomass input under plastic-mulched strawberry cultivation stronger increased the SOM (Chapter 3 & 5), I expect that other cultivars with different foliage and root systems might have a different impact on the respective soil parameters. For example, a cultivar with a sparser plant canopy or more open space between the plants will presumably have a higher soil temperature under the plastic mulch. Soil texture is known to strongly influence water flows and aggregate stability (Bronick & Lal, 2005) and consequently soils with a different texture might show a different impact of plastic mulching on water flows and aggregate stability. For example, the protective effect of plastic mulching on the soil structure observed in my study (Chapter 5) will most likely not appear in sand soils with a low SOM, which usually have no aggregate structure (Blume *et al.*, 2016). Thus, a differentiated consideration for other cultivation systems and soil types seems necessary.

Based on my experiences, I recommend to cooperate with professional strawberry farmers to conduct the respective field studies under the most recent and realistic cultivation conditions in the respective agricultural field. However, I want to emphasize that a fast and detailed information exchange and a clear definition what are the aspired aims and the tasks to fulfill of each party is absolutely necessary to implement a scientific study in a commercial agricultural production.

With regard to fungicide residues and their effects on soil, it needs to be evaluated how the effects change if application rate, amount and time as well as fungicide type are varied. Furthermore, how plastic mulching can influence the fate of fungicides is almost entirely unknown, especially regarding the adsorption/desorption behavior to the plastic mulches or the potential runoff from plastic-covered ridges to furrows. Thus, it remains unknown how much of the applied fungicides got adsorbed to the plastic mulches (and for how long) and how much runs off to the furrows or the field margins. First studies showed that plastic mulches can adsorb significant amounts of pesticides (Nerín *et al.*, 1996; Guo *et al.*, 2020) and also increase pesticide runoff to the field margins (Rice *et al.*, 2001, 2002). If this also applies for the fungicides used in my study needs to be addressed in future studies. The recycling or disposal of plastic mulches got may hampered if a significant fraction of the applied fungicides endures

absorbed to the mulch during the whole application period of the plastic mulch (Kasirajan & Ngouajio, 2012).

I found indications that the microbial community was shifted and temporally inhibited by the fungicide residues (Chapter 4). Thus, more detailed information whether the structure of microbial community was changed (e.g., with Phospholipid-derived fatty acids analysis) and how long microbial activity was inhibited (e.g., with respiration and enzyme activity measurements) are advisable to fully estimate the fungicide effects on soil microbes and the potential consequences for C, N & P cycles (Bünemann *et al.*, 2006). It should be of primary interest to investigate, whether the potential changes in the structure of the fungal communities are directed toward higher fractions of mycotoxigenic or pathogenic fungi, which could increase the potential for mycotoxins biosynthesis and pest infestation of plants. This might increase mycotoxin occurrence and yield losses and may leads to increased fungicide needs (Tirado *et al.*, 2010). Additionally, it remains unknown whether the effects of fungicide residues on soil decrease in the consecutive years due to an adaption of the microbial community to the yearly repeated fungicide application.

This PhD thesis confirmed that mycotoxins are also relevant soil contaminants, additionally to their well-known and well-investigated occurrence in food and feed during harvest and storage (Jouany, 2007; Elmholt, 2008). I revealed that mycotoxins occur in certain periods of plant development and agricultural treatment. Therefore, a better temporal resolution of samplings might help to better identify potential mycotoxin hotspots. Furthermore, the impact of plastic mulching on other mycotoxins, such as fumonisins or aflatoxins (*Aspergillus spp.* may become relevant in the warmer soil microclimate under plastic mulching), remain a relevant open question (Paterson & Lima, 2010). Generally, the mycotoxin fate in soil (especially plant uptake) as well as the soil conditions triggering mycotoxin biosynthesis were scarcely examined until yet and remains as an important research task due to the toxic character of mycotoxins (Murphy *et al.*, 2006). First studies showed that individual mycotoxins can be leached out into running water (Schenzel *et al.*, 2012a; b; Kolpin *et al.*, 2014) or can be uptaken by certain plants (Mantle, 2000; Rolli *et al.*, 2018), which underlines that further effort is necessary to estimate the mycotoxin fate in soils and identify potential human exposure routes.

Furthermore, it remains an open question to what extent the lateral water flows from straw-covered furrows to plastic-covered ridges after rainfalls (Chapter 5) can influence the transport of nutrients or herbicides (often applied to furrows for weed suppression) from furrows to ridges and thus impact on nutrient balance and microbial community in the ridges.

In recent years, plastic mulching has also received attention because its incomplete removing and improper disposal from agricultural fields can increase macroplastic concentrations in soils to such a degree that plant growth was impaired and yields decreased (Kasirajan & Ngouajio, 2012; Liu *et al.*, 2014; Gao *et al.*, 2019a; Huang *et al.*, 2020). Additionally, the plastic remnants may represent a substantial source for microplastic due to plastic disintegration (Astner *et al.*, 2019; Huang *et al.*, 2020; Li *et al.*, 2020b). Microplastics are able to adsorb toxic substances such as pesticides and heavy metals and can undergo transport through the soil column by bioturbation and agricultural practices (Zhu *et al.*, 2019; Xu *et al.*, 2020). Thus, microplastics might be hotspots for agrochemicals or a vector for agrochemicals or mycotoxins though the soil column, which may enter the human food chain via groundwater or plant uptake. Generally, microplastics in terrestrial ecosystems is an emerging research field which still has to address numerous questions to asses and evaluate the interactions, fate and effects of microplastic in agroecosystems (Ng *et al.*, 2018; Zhu *et al.*, 2019; Xu *et al.*, 2020).

Nowadays, biodegradable plastic mulches are increasingly used as substitute for the conventional non-degradable plastic mulches (Brodhagen *et al.*, 2015; Briassoulis & Giannoulis, 2018; Ghimire *et al.*, 2018). However, whether they can compete with conventional plastic mulches in terms of performance and costs is seen critically (Cowan *et al.*, 2013; Martin-Closas *et al.*, 2016; Touchaleaume *et al.*, 2016; Ghimire *et al.*, 2018). Furthermore, more research is necessary to elucidate whether biodegradable plastic mulches can fully degrade in a sufficient time period under the various cultivation conditions with different soils and climates (Brodhagen *et al.*, 2017; Sintim & Flury, 2017; Bandopadhyay *et al.*, 2018). Research is only at the beginning to investigate whether the mulch residues can migrate from the agricultural systems to other environmental compartments and how the respectively used polymers and additives influence soil structure, plant growth and microbial community and consequently the soil quality (Bandopadhyay *et al.*, 2018; Serrano-Ruiz *et al.*, 2020).

# **CHAPTER 8**

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## **CHAPTER 9**

**Annex** 

### 9.1 Supporting information

### 9.1.1 Supporting information to Chapter 3

#### Raster sampling

In May 2016, a raster sampling was conducted to identify potential gradients and inhomogeneities of selected soil properties that may interfere with our experiment design. The field size was 370 m (north-south direction) to 40 m and was always treated in north-south direction by the farmer. We used a rectangular sampling grid with 50 m distances in north-south direction and 10 m distances in east-west direction. Additionally, at three places in the field (north, middle and south) we took soil samples at 50 cm distances in east-west direction. We took soil samples from the 0-30 cm soil layer (plough layer) and the 30-60 cm soil layer (untreated subsoil) with a boring rod. We analyzed the soil samples on the following soil properties: pH in 0.01 M CaCl<sub>2</sub> (DIN EN 15933:2012-11), electrical conductivity (DIN CEN/TS 15937:2013-08), dissolved organic matter (according to DIN EN 1484:1997-05 with TOC analyzer (multiNC 2011S, Analytik Jena AG, Jena, Germany)), cations (with ICP-OES analysis (Agilent 720 ICP-OES spectrometer, Agilent Technologies Deutschland GmbH & Co. KG, Waldbronn, Germany)) and anions (with IC analysis (DX-500 Ion Chromatography System, Dionex Softron GmbH, Germering, Germany)).

**Table S1** Soil pH in the 0-30 cm soil layer

	Soil pH / a.u.								
Field width									
Field length	10 m	20 m	30 m	Mean	SD				
335 m	8.1	8.2	8.2	8.2	0.1				
285 m	8.1	8.3	8.2	8.2	0.1				
235 m	8.1	8.0	8.1	8.1	0.1				
185 m	8.1	8.1	8.3	8.2	0.1				
135 m	8.1	8.2	8.1	8.1	0.1				
85 m	8.3	8.2	8.2	8.2	0.1				
35 m	8.4	8.3	8.4	8.4	0.1				
Mean	8.2	8.2	8.2						
SD	0.1	0.1	0.1						

**Table S2** Soil pH in the 0-30 cm soil layer (50 cm distance)

	Soil pH / a.u.									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	8.3	8.3	8.3	8.1	8.2	8.2	0.1			
	Field width									
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	8.2	8.2	8.1	8.3	8.3	8.2	0.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	8.3	7.9	8.0	8.1	8.2	8.1	0.2			

**Table S3** Soil pH in the 30-60 cm soil layer

	Soil pH / a.u.								
Field width									
Field length	10 m	20 m	30 m	Mean	SD				
335 m	8.2	8.2	8.1	8.2	0.1				
285 m	8.1	8.3	8.2	8.2	0.1				
235 m	8.1	8.2	8.2	8.2	0.1				
185 m	8.1	8.2	8.0	8.1	0.1				
135 m	8.2	7.9	7.9	8.0	0.2				
85 m	8.1	8.2	8.2	8.2	0.1				
35 m	8.3	8.4	8.4	8.4	0.1				
Mean	8.2	8.2	8.1						
SD	0.1	0.2	0.2						

**Table S4** Soil pH in the 30-60 cm soil layer (50 cm distance)

	Soil pH / a.u.									
			Field width							
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	8.4	8.4	8.3	8.2	8.2	8.3	0.1			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	8.2	8.2	8.2	8.3	8.4	8.3	0.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	8.1	8.1	8.2	8.2	8.1	8.1	0.1			

**Table S5** Electrical conductivity in the 0-30 cm soil layer

	Electrical conductivity / μS cm <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	95.7	126.0	125.8	115.8	17.4				
285 m	113.4	126.7	125.8	122.0	7.4				
235 m	114.7	93.2	76.8	94.9	19.0				
185 m	109.1	133.3	96.5	113.0	18.7				
135 m	112.8	122.4	114.0	116.4	5.2				
85 m	113.5	132.0	128.4	124.6	9.8				
35 m	117.8	138.5	141.1	132.5	12.8				
Mean	111.0	124.6	115.5						
SD	7.2	14.8	22.0						

**Table S6** Electrical conductivity in the 0-30 cm soil layer (50 cm distance)

	Electrical conductivity / μS cm <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	125.0	118.7	119.4	126.6	125.8	123.1	3.7			
	Field width									
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	118.1	111.6	133.3	126.8	130.9	124.1	9.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	113.5	121.6	125.8	121.9	114.3	119.4	5.3			

**Table S7** Electrical conductivity in the 30-60 cm soil layer

	Electrical conductivity / μS cm <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	125.3	126.4	118.5	123.4	4.3				
285 m	126.2	123.3	115.9	121.8	5.3				
235 m	111.0	91.0	94.5	98.8	10.7				
185 m	123.2	149.2	102.4	124.9	23.4				
135 m	134.1	130.5	119.1	127.9	7.8				
85 m	139.0	159.3	150.3	149.5	10.2				
35 m	129.6	141.1	144.0	138.2	7.6				
Mean	126.9	131.5	120.7						
SD	8.9	22.0	20.3						

**Table S8** Electrical conductivity in the 30-60 cm soil layer (50 cm distance)

	Electrical conductivity / μS cm <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	116.0	122.9	131.7	128.3	115.9	123.0	7.1			
	Field width									
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	146.1	136.0	149.2	133.3	120.8	137.1	11.3			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	139.0	145.7	143.1	149.4	143.3	144.1	3.8			

**Table S9** Ca<sup>2+</sup> content in the 0-30 cm soil layer

Ca <sup>2+</sup> content / mg kg <sup>-1</sup>								
		Field width						
Field length	10 m	20 m	30 m	Mean	SD			
335 m	84.9	101.2	114.1	100.0	14.7			
285 m	99.0	104.2	105.7	103.0	3.6			
235 m	81.8	82.6	70.9	<b>78.4</b>	6.5			
185 m	92.4	110.7	88.0	97.0	12.1			
135 m	96.9	106.1	102.6	101.9	4.6			
85 m	96.8	113.0	109.0	106.3	8.4			
35 m	100.9	121.2	127.3	116.5	13.8			
Mean	93.2	105.6	102.5					
SD	7.3	12.1	18.3					

**Table S10** Ca<sup>2+</sup> content in the 0-30 cm soil layer (50 cm distance)

			•							
	Ca <sup>2+</sup> content / mg kg <sup>-1</sup>									
			Field width							
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	103.8	103.6	103.8	106.5	105.7	104.7	1.3			
Field width										
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	103.3	99.3	110.7	107.2	112.5	106.6	5.4			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	96.8	94.8	106.5	106.7	104.8	101.9	5.7			

**Table S11** Ca<sup>2+</sup> content in the 30-60 cm soil layer

	Ca <sup>2+</sup> content / mg kg <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	97.0	117.0	109.0	107.7	10.0				
285 m	112.3	106.4	109.4	109.4	2.9				
235 m	97.6	84.2	86.4	89.4	7.2				
185 m	100.9	118.6	97.3	105.6	11.4				
135 m	101.1	103.0	96.0	100.0	3.6				
85 m	104.1	112.8	105.0	107.3	4.8				
35 m	114.5	127.6	124.4	122.2	6.8				
Mean	103.9	110.0	103.9						
SD	6.9	13.9	12.2						

**Table S12** Ca<sup>2+</sup> content in the 30-60 cm soil layer (50 cm distance)

	Ca <sup>2+</sup> content / mg kg <sup>-1</sup>										
	Field width										
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD				
285 m	117.1	111.2	109.7	102.5	109.4	110.0	5.2				
	Field width										
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m						
185 m	112.8	119.1	118.6	113.6	108.5	114.5	4.4				
			Field width								
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m						
85 m	104.1	102.6	106.7	109.0	106.9	105.8	2.5				

Table S13  $Mg^{2+}$  content in the 0-30 cm soil layer

Mg <sup>2+</sup> content / mg kg <sup>-1</sup>								
		Field width						
Field length	10 m	20 m	30 m	Mean	SD			
335 m	8.6	5.8	7.9	7.4	1.4			
285 m	7.9	6.8	7.3	7.3	0.5			
235 m	8.5	8.7	8.9	8.7	0.2			
185 m	7.8	8.1	9.3	8.4	0.8			
135 m	8.9	8.9	9.4	9.1	0.3			
85 m	7.9	8.3	8.4	8.2	0.3			
35 m	6.9	7.8	7.6	7.4	0.5			
Mean	8.1	7.8	8.4					
SD	0.7	1.1	0.8					

**Table S14**  $Mg^{2+}$  content in the 0-30 cm soil layer (50 cm distance)

	Mg <sup>2+</sup> content / mg kg <sup>-1</sup>									
Field width										
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	7.1	7.2	7.4	7.4	7.3	7.3	0.1			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	7.5	7.5	8.1	7.8	8.0	7.8	0.3			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	7.9	7.5	8.5	8.1	7.9	8.0	0.4			

Table S15  $Mg^{2+}$  content in the 30-60 cm soil layer

Mg <sup>2+</sup> content / mg kg <sup>-1</sup>								
		Field width						
Field length	10 m	20 m	30 m	Mean	SD			
335 m	7.8	6.0	6.9	6.9	0.9			
285 m	7.8	6.4	6.1	6.8	0.9			
235 m	9.9	9.0	10.3	9.7	0.6			
185 m	7.1	8.4	10.5	8.6	1.7			
135 m	8.1	8.0	8.3	8.1	0.1			
85 m	7.7	7.0	6.8	7.2	0.5			
35 m	7.3	7.2	6.5	7.0	0.4			
Mean	7.9	7.4	7.9					
SD	0.9	1.1	1.8					

**Table S16**  $Mg^{2+}$  content in the 30-60 cm soil layer (50 cm distance)

	Mg <sup>2+</sup> content / mg kg <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	7.1	6.7	6.3	5.3	6.1	6.3	0.7			
	Field width									
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	7.8	8.7	8.4	8.4	8.3	8.3	0.3			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	7.7	7.1	7.0	7.2	7.0	7.2	0.3			

Table S17  $Na^+$  content in the 0-30 cm soil layer

	Na <sup>+</sup> content / mg kg <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	6.8	8.3	5.4	6.8	1.4				
285 m	7.2	12.9	8.2	9.5	3.1				
235 m	13.0	9.7	9.9	10.9	1.8				
185 m	10.8	11.3	9.1	10.4	1.2				
135 m	14.6	9.8	10.5	11.6	2.6				
85 m	7.8	9.5	10.8	9.4	1.5				
35 m	7.7	8.4	8.3	8.1	0.4				
Mean	9.7	10.0	8.9						
SD	3.1	1.6	1.8						

**Table S18** Na<sup>+</sup> content in the 0-30 cm soil layer (50 cm distance)

	Na <sup>+</sup> content / mg kg <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	11.2	7.1	6.2	6.4	8.2	<b>7.8</b>	2.1			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	9.9	8.6	11.3	14.1	10.0	10.8	2.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	7.8	8.7	13.0	11.5	9.7	10.1	2.1			

Table S19  $\mathrm{Na^{\scriptscriptstyle +}}$  content in the 30-60 cm soil layer

	Na <sup>+</sup> content / mg kg <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	9.1	9.3	7.0	8.5	1.3				
285 m	10.3	10.7	5.7	8.9	2.8				
235 m	14.4	12.7	13.0	13.4	0.9				
185 m	10.2	15.9	10.8	12.3	3.1				
135 m	17.1	12.0	11.5	13.5	3.1				
85 m	12.6	10.4	9.6	10.8	1.5				
35 m	10.4	8.5	7.6	8.8	1.4				
Mean	12.0	11.4	9.3						
SD	2.9	2.5	2.6						

**Table S20** Na<sup>+</sup> content in the 30-60 cm soil layer (50 cm distance)

					•		
		N	la+ content / n	ng kg <sup>-1</sup>			
			Field width				
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD
285 m	7.6	6.1	6.9	6.1	5.7	6.5	0.8
			Field width				
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m		
185 m	12.5	14.4	15.9	15.6	11.7	14.0	1.9
			Field width				
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m		
85 m	12.6	12.7	12.7	14.5	11.6	12.8	1.0

Table S21  $K^+$  content in the 0-30 cm soil layer

	K <sup>+</sup> content / mg kg <sup>-1</sup>								
Field width									
Field length	10 m	20 m	30 m	Mean	SD				
335 m	13.2	7.6	8.4	9.8	3.0				
285 m	15.8	13.9	10.2	13.3	2.8				
235 m	17.2	10.5	5.7	11.1	<b>5.7</b>				
185 m	17.8	15.8	7.2	13.6	<b>5.7</b>				
135 m	14.0	13.9	9.8	12.6	2.4				
85 m	12.5	14.0	10.5	12.3	1.8				
35 m	11.3	9.4	8.3	9.7	1.5				
Mean	14.5	12.2	8.6						
SD	2.4	3.0	1.7						

**Table S22** K<sup>+</sup> content in the 0-30 cm soil layer (50 cm distance)

	K <sup>+</sup> content / mg kg <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	11.6	7.3	6.8	7.1	10.2	8.6	2.2			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	10.7	12.7	15.8	9.7	10.2	11.8	2.5			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	12.5	13.7	18.4	16.0	14.1	14.9	2.3			

Table S23  $K^+$  content in the 30-60 cm soil layer

K <sup>+</sup> content / mg kg <sup>-1</sup>									
Field width									
Field length	10 m	20 m	30 m	Mean	SD				
335 m	7.4	4.9	5.3	5.9	1.3				
285 m	7.3	4.3	2.6	4.8	2.4				
235 m	7.9	10.5	6.1	8.2	2.2				
185 m	4.2	13.7	5.0	7.6	5.3				
135 m	5.3	7.6	4.0	5.6	1.8				
85 m	6.4	5.4	3.8	5.2	1.3				
35 m	6.7	4.2	3.9	4.9	1.5				
Mean	6.5	7.2	4.4						
SD	1.3	3.6	1.2						

**Table S24** K<sup>+</sup> content in the 30-60 cm soil layer (50 cm distance)

	K <sup>+</sup> content / mg kg <sup>-1</sup>									
	Field width									
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	3.8	3.4	4.0	3.1	2.6	3.4	0.6			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	6.6	6.5	13.7	8.6	7.0	8.5	3.0			
			Field width							
-	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	6.4	6.0	6.9	8.8	8.1	7.2	1.2			

**Table S25** NO<sub>3</sub><sup>-</sup> content in the 0-30 cm soil layer

NO <sub>3</sub> content / mg kg <sup>-1</sup>							
		Field width					
Field length	10 m	20 m	30 m	Mean	SD		
335 m	26.0	25.2	22.2	24.4	2.0		
285 m	27.6	27.2	28.2	27.6	0.5		
235 m	58.8	30.5	30.9	40.1	16.2		
185 m	33.3	37.3	37.0	35.9	2.2		
135 m	46.0	37.3	38.0	40.4	4.8		
85 m	30.3	33.6	44.3	36.1	7.3		
35 m	28.6	30.8	32.2	30.5	1.8		
Mean	35.8	31.7	33.2				
SD	12.1	4.7	7.2				

**Table S26** NO<sub>3</sub>-in the 0-30 cm soil layer (50 cm distance)

NO <sub>3</sub> - content / mg kg <sup>-1</sup>								
Field width								
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD	
285 m	18.2	25.2	26.6	29.9	28.2	25.6	4.5	
			Field width					
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m			
185 m	37.4	35.8	37.3	30.8	31.8	34.6	3.1	
			Field width					
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m			
85 m	30.3	38.5	38.7	32.2	30.5	34.0	4.2	

**Table S27** NO<sub>3</sub><sup>-</sup> content in the 30-60 cm soil layer

NO <sub>3</sub> - content / mg kg <sup>-1</sup>							
		Field width					
Field length	10 m	20 m	30 m	Mean	SD		
335 m	22.6	17.1	16.7	18.8	3.3		
285 m	17.2	12.5	12.6	14.1	2.7		
235 m	23.4	21.0	25.8	23.4	2.4		
185 m	18.1	25.6	23.3	22.3	3.8		
135 m	27.7	34.4	21.1	27.7	6.6		
85 m	23.0	23.2	32.2	26.1	5.3		
35 m	18.8	27.2	18.7	21.6	4.9		
Mean	21.5	23.0	21.5				
SD	3.7	7.1	6.4				

**Table S28** NO<sub>3</sub><sup>-</sup> content in the 30-60 cm soil layer (50 cm distance)

NO <sub>3</sub> - content / mg kg <sup>-1</sup>								
Field width								
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD	
285 m	8.5	18.8	15.6	20.3	12.6	15.2	4.8	
			Field width					
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m			
185 m	38.6	26.8	25.6	27.1	29.4	29.5	5.3	
			Field width					
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m			
85 m	23.0	22.8	18.9	24.5	28.4	23.5	3.4	

**Table S29**  $PO_4^{3-}$  content in the 0-30 cm soil layer

PO <sub>4</sub> <sup>3-</sup> content / mg kg <sup>-1</sup>							
		Field width					
Field length	10 m	20 m	30 m	Mean	SD		
335 m	3.6	2.2	0.9	2.2	1.3		
285 m	3.3	3.1	1.8	2.7	0.8		
235 m	6.4	6.8	3.2	5.5	2.0		
185 m	3.2	4.1	2.4	3.2	0.9		
135 m	5.9	3.6	1.8	3.8	2.1		
85 m	3.7	6.0	2.1	3.9	2.0		
35 m	3.3	2.4	0.7	2.1	1.3		
Mean	4.2	4.0	1.8				
SD	1.4	1.7	0.9				

**Table S30** PO<sub>4</sub><sup>3-</sup> content in the 0-30 cm soil layer (50 cm distance)

PO <sub>4</sub> <sup>3-</sup> content / mg kg <sup>-1</sup>								
Field width								
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD	
285 m	1.5	0.9	1.0	1.3	1.8	1.3	0.4	
Field width								
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m			
185 m	3.6	4.4	4.1	2.9	4.1	3.8	0.6	
Field width								
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m			
85 m	3.7	4.6	4.9	3.8	3.4	4.1	0.6	

**Table S31** PO<sub>4</sub><sup>3-</sup> content in the 30-60 cm soil layer

	PO <sub>4</sub> <sup>3-</sup> content / mg kg <sup>-1</sup>									
		Field width								
Field length	10 m	20 m	30 m	Mean	SD					
335 m	1.2	0.4	0.2	0.6	0.5					
285 m	0.8	0.0	0.0	0.3	0.4					
235 m	3.4	5.6	3.0	4.0	1.4					
185 m	0.9	2.7	0.9	1.5	1.0					
135 m	1.2	2.3	0.3	1.3	1.0					
85 m	0.5	1.2	0.3	0.7	0.5					
35 m	2.1	1.4	0.0	1.2	1.1					
Mean	1.5	1.9	0.7							
SD	1.0	1.9	1.1							

**Table S32** PO<sub>4</sub><sup>3-</sup> content in the 30-60 cm soil layer (50 cm distance)

		PC	O <sub>4</sub> <sup>3-</sup> content / 1	mg kg <sup>-1</sup>			
			Field width				
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD
285 m	0.0	0.0	0.2	0.5	0.0	0.1	0.2
			Field width				
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m		
185 m	3.1	1.1	2.7	3.1	2.3	2.5	0.8
			Field width				
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m		
85 m	0.5	0.8	1.2	1.9	2.0	1.3	0.7

**Table S33** SO<sub>4</sub><sup>2-</sup> content in the 0-30 cm soil layer

	SO <sub>4</sub> <sup>2-</sup> content / mg kg <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	0.3	0.6	0.4	0.4	0.1				
285 m	0.5	0.5	0.6	0.5	0.1				
235 m	1.0	0.4	0.5	0.6	0.3				
185 m	0.4	0.9	0.5	0.6	0.3				
135 m	0.5	0.5	0.6	0.5	0.1				
85 m	0.5	1.6	0.9	1.0	0.6				
35 m	0.4	0.7	0.4	0.5	0.1				
Mean	0.5	0.7	0.5						
SD	0.2	0.4	0.2						

**Table S34** SO<sub>4</sub><sup>2-</sup> content in the 0-30 cm soil layer (50 cm distance)

	SO <sub>4</sub> <sup>2-</sup> content / mg kg <sup>-1</sup>									
			Field width							
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	1.1	0.4	0.4	0.6	0.6	0.6	0.3			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	0.7	0.5	0.9	0.7	0.7	0.7	0.2			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	0.5	0.5	0.6	0.6	0.5	0.5	0.1			

**Table S35**  $SO_4^{2-}$  content in the 30-60 cm soil layer

		SO <sub>4</sub> <sup>2</sup> - conter	nt / mg kg <sup>-1</sup>							
	Field width									
Field length	10 m	20 m	30 m	Mean	SD					
335 m	0.3	0.4	0.5	0.4	0.1					
285 m	0.4	0.5	0.4	0.5	0.1					
235 m	0.6	0.5	0.4	0.5	0.1					
185 m	0.5	0.8	0.6	0.6	0.2					
135 m	0.6	0.8	0.8	0.7	0.1					
85 m	0.5	0.7	1.0	0.7	0.2					
35 m	3.2	1.8	0.4	1.8	1.4					
Mean	0.9	0.8	0.6							
SD	1.0	0.4	0.2							

**Table S36** SO<sub>4</sub><sup>2-</sup> content in the 30-60 cm soil layer (50 cm distance)

	SO <sub>4</sub> <sup>2-</sup> content / mg kg <sup>-1</sup>									
			Field width							
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	0.4	0.4	0.3	0.5	0.4	0.4	0.1			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	0.8	0.6	0.8	0.7	0.7	0.7	0.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	0.5	0.6	0.7	0.7	0.5	0.6	0.1			

**Table S37** DOC in the 0-30 cm soil layer

	DOC content / mg kg <sup>-1</sup>								
		Field width							
Field length	10 m	20 m	30 m	Mean	SD				
335 m	54.2	124.1	51.2	76.5	41.2				
285 m	71.8	61.2	61.4	64.8	6.1				
235 m	87.2	81.2	51.7	73.4	19.0				
185 m	67.1	59.3	179.6	102.0	67.3				
135 m	71.2	67.1	70.3	69.5	2.2				
85 m	73.6	60.5	129.2	87.8	36.5				
35 m	46.4	73.2	73.0	64.2	15.4				
Mean	67.4	75.2	88.1						
SD	13.4	23.0	48.3						

**Table S38** DOC content in the 0-30 cm soil layer (50 cm distance)

		De	OC content / 1	mg kg <sup>-1</sup>			
			Field width				
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD
285 m	68.9	51.7	50.0	65.0	61.4	<b>59.4</b>	8.3
			Field width				
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m		
185 m	49.7	98.7	59.3	49.7	83.2	68.1	21.9
			Field width				
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m		
85 m	73.6	68.1	72.3	81.4	69.0	72.9	5.3

 $\textbf{Table S39} \ \mathsf{DOC} \ \mathsf{content} \ \mathsf{in} \ \mathsf{the} \ \mathsf{30\text{-}60} \ \mathsf{cm} \ \mathsf{soil} \ \mathsf{layer}$ 

		DOC conter	nt / mg kg <sup>-1</sup>		
		Field width			
Field length	10 m	20 m	30 m	Mean	SD
335 m	*	*	*		
285 m	53.6	40.0	27.3	40.3	13.1
235 m	54.5	74.9	62.7	64.0	10.2
185 m	31.1	88.7	57.7	59.2	28.8
135 m	51.3	39.2	33.8	41.4	9.0
85 m	40.3	19.5	34.0	31.3	10.7
35 m	89.0	26.5	34.6	50.0	34.0
Mean	53.3	48.1	41.7		
SD	19.7	27.5	14.7		

<sup>\*</sup> Wrong filter used

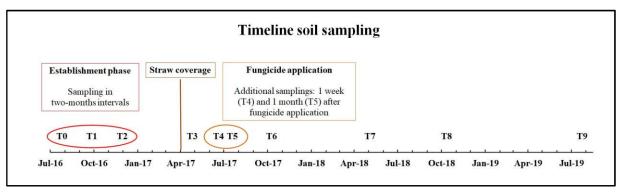
Table S40 DOC content in the 30-60 cm soil layer (50 cm distance)

	DOC content / mg kg <sup>-1</sup>									
			Field width							
Field length	28.0 m	28.5 m	29.0 m	29.5 m	30.0 m	Mean	SD			
285 m	57.6	30.8	69.2	80.7	27.3	53.1	23.5			
			Field width							
	19.0 m	19.5 m	20.0 m	20.5 m	21.0 m					
185 m	32.7	61.7	88.7	79.8	46.1	61.8	23.1			
			Field width							
	10.0 m	10.5 m	11.0 m	11.5 m	12.0 m					
85 m	40.3	19.5	55.0	63.1	50.8	45.7	16.8			

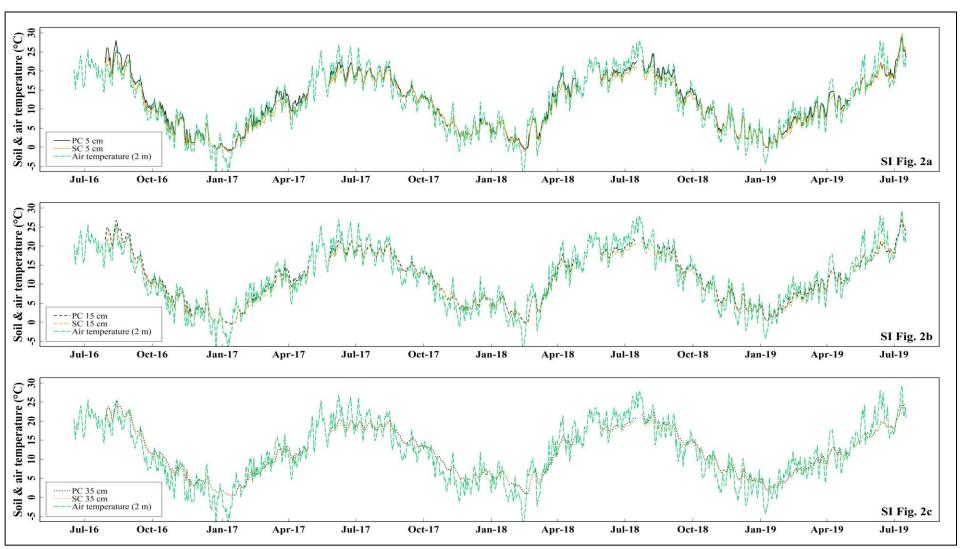
**Table S41** Monthly mean, maximum and minimum soil temperature measured at 5, 15 and 35 cm soil depth by field measuring station under plastic coverage (PC) and no-coverage treatment (NC) during the sampling period. Values are given as mean with standard deviation. (Values from July to 11<sup>th</sup> August are missing due to malfunction of the field measuring station).

			PC			NC		ΔT PC-NC
Date	Soil depth	Mean	Max.	Min.	Mean	Max.	Min.	of Mean
	•	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature
		/ °C	/ °C	/ °C	/ °C	/ °C	/ °C	/ °C
Aug. 16								
	5 cm	24.2±4.0	34.2	16.0	22.2±3.0	29.2	15.8	2.0
	15 cm	$23.7 \pm 2.4$	28.9	18.2	$22.4 \pm 2.1$	26.7	17.7	1.4
	35 cm	$22.9 \pm 1.6$	25.6	19.9	$22.3 \pm 1.4$	24.7	19.3	0.7
Sep.16								
-	5 cm	$20.2 \pm 3.5$	29.2	13.5	$18.7 \pm 3.2$	25.9	11.9	1.5
	15 cm	$20.2\pm2.9$	26.4	15.2	19.1±2.7	24.4	13.8	1.1
	35 cm	$20.4 \pm 2.4$	24.4	16.7	19.6±2.3	23.0	16.0	0.7
Oct. 16								
	5 cm	$11.3 \pm 2.0$	17.0	7.5	$10.4 \pm 2.0$	16.8	6.7	0.9
	15 cm	11.6±1.7	17.2	8.8	$11.0 \pm 1.8$	16.7	8.1	0.6
	35 cm	$12.5 \pm 1.7$	17.7	10.4	$12.0 \pm 1.7$	17.3	9.8	0.4
Nov. 16								
	5 cm	$6.3 \pm 2.0$	11.2	0.7	$5.7 \pm 2.2$	9.7	0.4	0.7
	15 cm	6.7±1.7	10.3	2.1	6.2±1.8	9.5	1.6	0.5
	35 cm	7.7±1.3	10.8	4.1	7.3±1.4	10.3	3.6	0.4

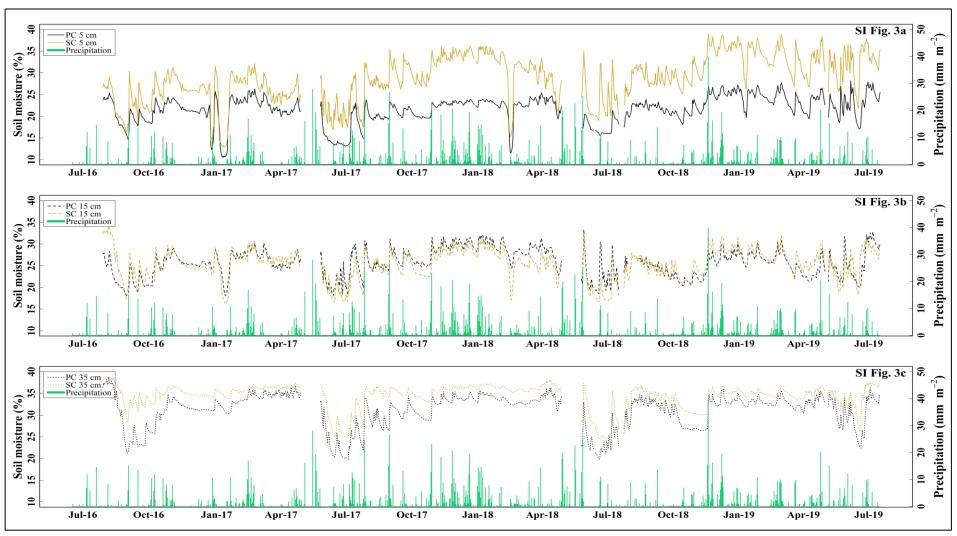
# 9.1.2 Supporting information to Chapter 5



SI Figure 1 Soil sampling scheme of the three-year field experiment

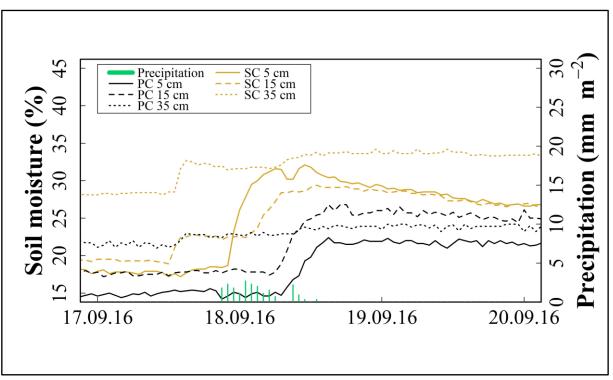


**SI Figure 2** Soil and air temperature **Fig. 2a-c** Daily mean soil temperature in strawberry cultivation, measured at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC) and daily mean air temperature measured 2 m above ground. The data exhibit data gaps from 01.07.2016–11.08.2016, 13.05.2017–08.06.2017 and 13.05.2018–08.06.2018 due to technical malfunction of the measuring station.

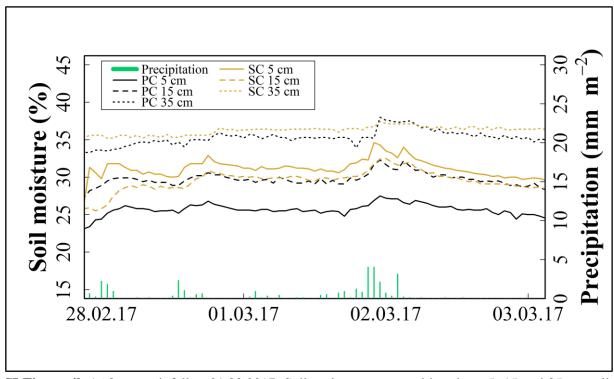


**SI Figure 3** Soil moisture and precipitation **Fig. 3a-c** Daily mean soil moisture in strawberry cultivation, measured at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC) and daily precipitation. The data exhibit data gaps from 01.07.2016–11.08.2016, 13.05.2017–08.06.2017 and 13.05.2018–08.06.2018 due to technical malfunction of the measuring station.

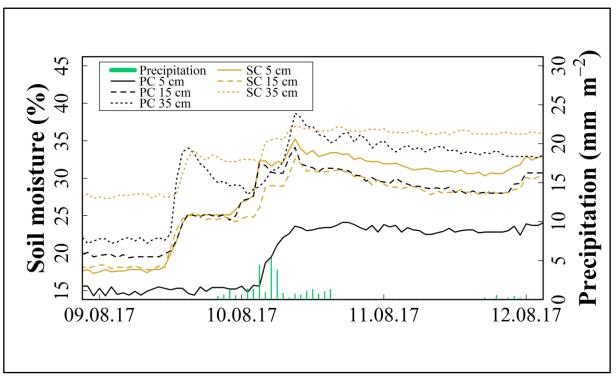
In order to estimate water flows and changes in soil moisture after rainfall under plastic coverage and straw coverage, the 20 largest daily rainfall events during the three-year sampling were graphically presented in chronological order



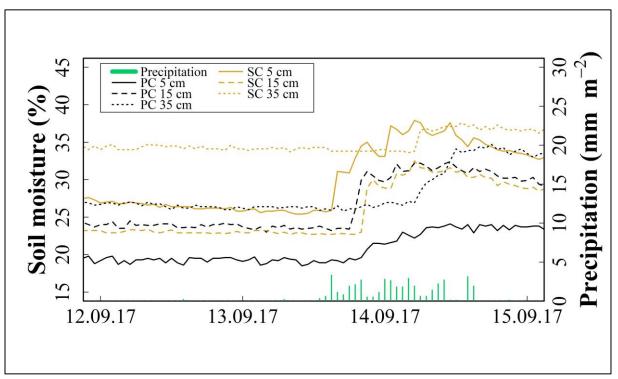
**SI Figure 4a** 15.1 mm rainfall at 18.09.2016. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



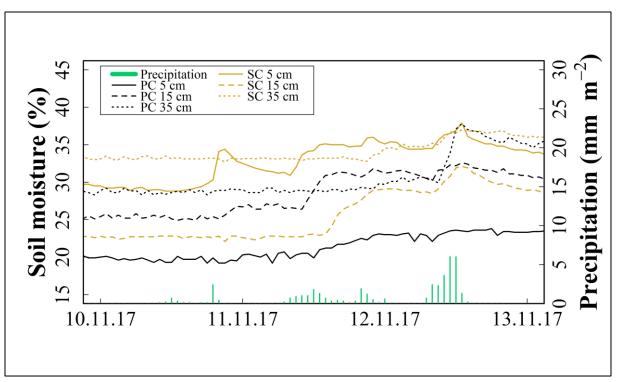
**SI Figure 4b** 16.8 mm rainfall at 01.03.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



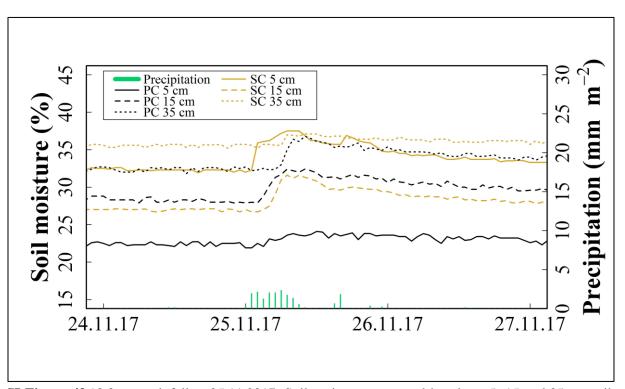
**SI Figure 4c** 24.8 mm rainfall at 10.08.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



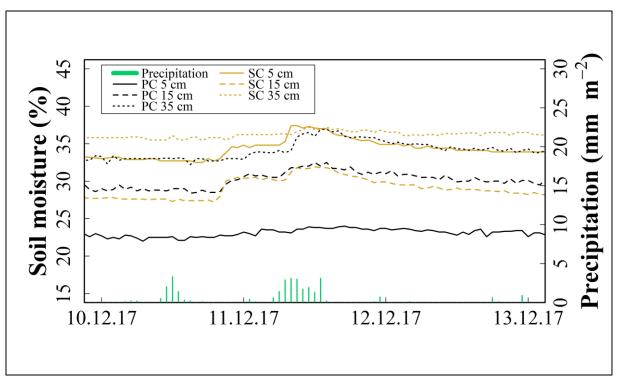
**SI Figure 4d** 15.1 and 26.6 mm rainfall at 13/14.09.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



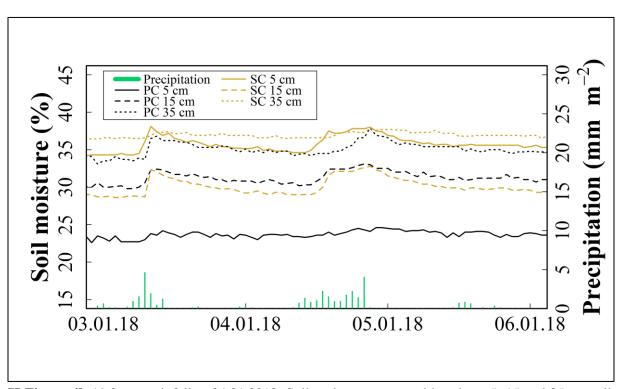
**SI Figure 4e** 23.0 mm rainfall at 12.11.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



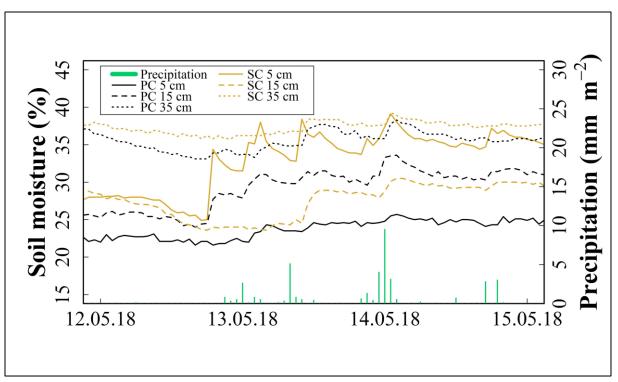
**SI Figure 4f** 18.3 mm rainfall at 25.11.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



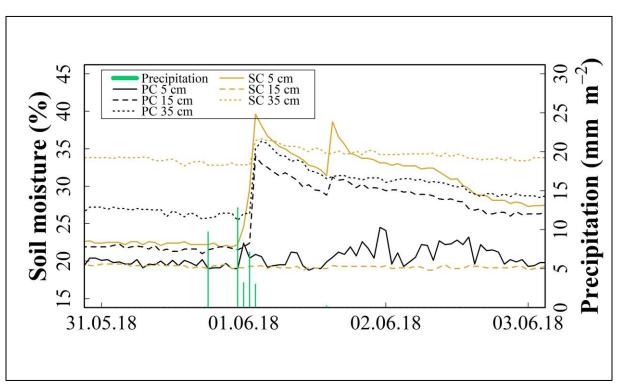
**SI Figure 4g** 20.5 mm rainfall at 11.12.2017. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



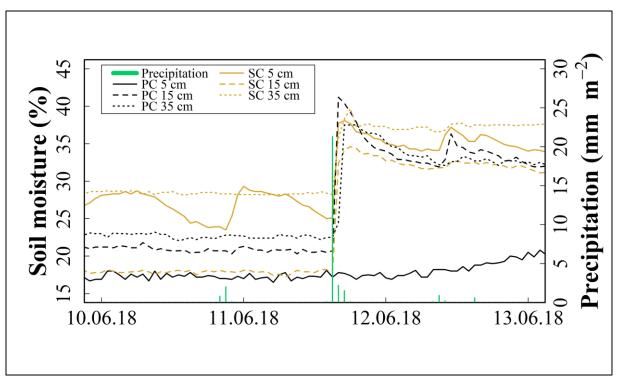
**SI Figure 4h** 19.2 mm rainfall at 04.01.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



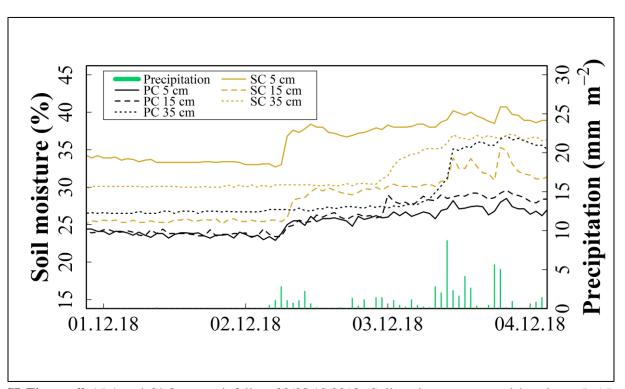
**SI Figure 4i** 17.5 and 19.9 mm rainfall at 13/14.05.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



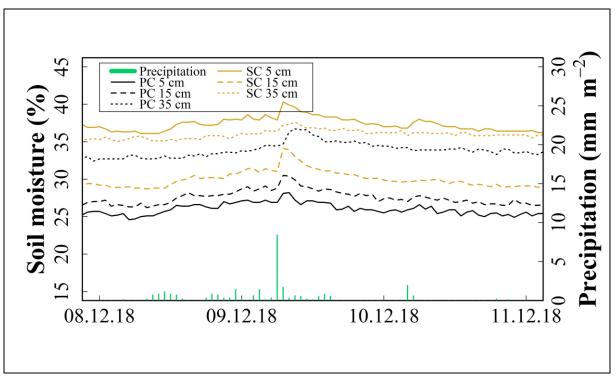
**SI Figure 4j** 22.5 mm rainfall at 31.05.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



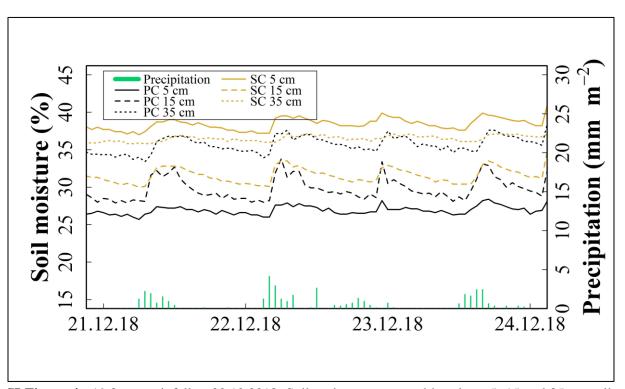
**SI Figure 4k** 25.2 mm rainfall at 11.06.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



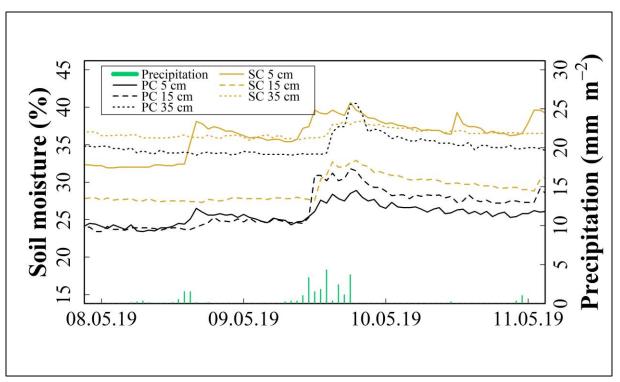
**SI Figure 4l** 15.4 and 39.8 mm rainfall at 02/03.12.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



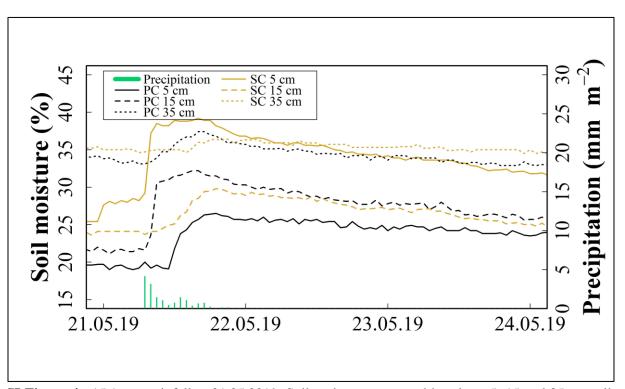
**SI Figure 4m** 16.2 mm rainfall at 09.12.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



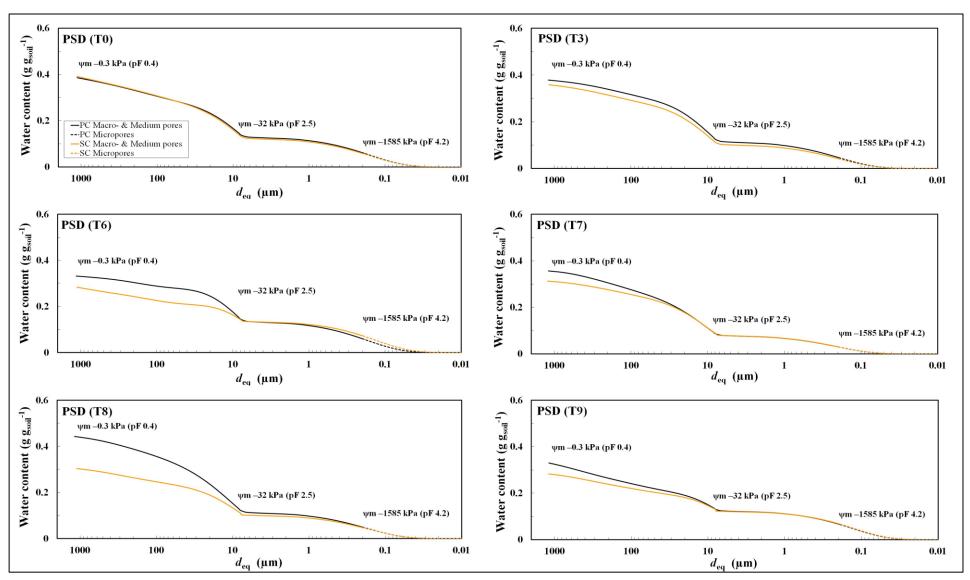
**SI Figure 4n** 19.3 mm rainfall at 22.12.2018. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



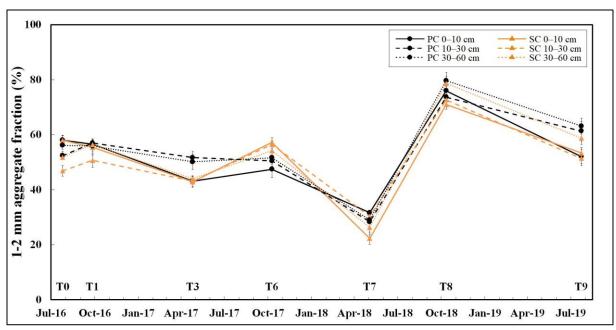
**SI Figure 4o** 20.2 mm rainfall at 09.05.2019. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



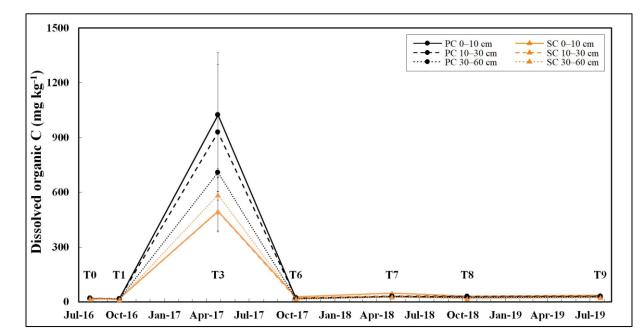
**SI Figure 4p** 15.1 mm rainfall at 21.05.2019. Soil moisture measured hourly at 5, 15 and 35 cm soil depth under plastic coverage (PC) and straw coverage (SC)



**SI Figure 5** Pore size distribution (PSD) measured in 0–5 cm soil layer under plastic coverage (PC) and straw coverage (SC) at six dates within the three-year field experiment, shown as mean (n=3)

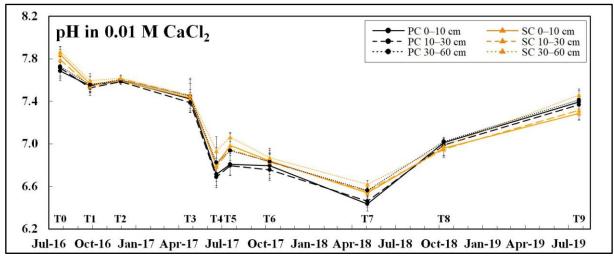


**SI Figure 6** Percentage of 1–2 mm aggregates in soil, determined gravimetrically in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at seven dates within the three-year field experiment, respectively, shown as mean with standard error (n=5)

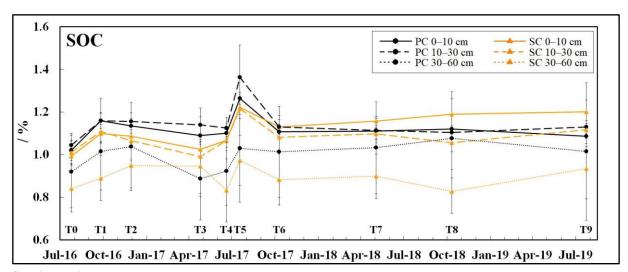


**SI Figure 7** Dissolved organic carbon (DOC) determined in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at seven dates within the three-year field experiment, respectively, shown as mean with standard error (n=5)

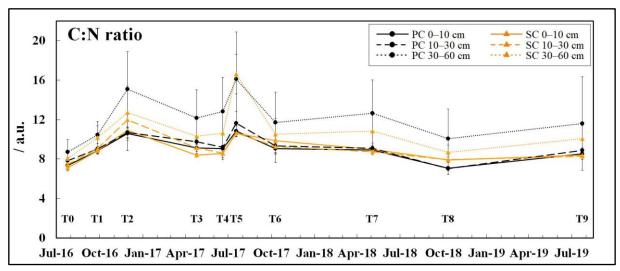
## 9.1.3 Supporting information to Chapter 6



**SI Figure 1** Soil pH (in 0.01 M CaCl<sub>2</sub>) determined in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates during the three-year field study, shown as mean with standard deviation (n=5) (Data summarized from Meyer et al. (2020, 2021a, b))



**SI Figure 2** Soil organic carbon (SOC) determined in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates during the three-year field study, shown as mean with standard deviation (n=5) (Data summarized from Meyer et al. (2020, 2021a, b))



**SI Figure 3** Carbon to nitrogen ratio (C:N ratio) determined in the 0–10, 10–30 and 30–60 cm soil layer under plastic coverage (PC) and straw coverage (SC) at ten dates during the three-year field study, shown as mean with standard deviation (n=5) (Data summarized from Meyer et al. (2020, 2021a, b))

**SI Table 1** The temperature range of the 100 largest and lowest soil temperatures measured under plastic and straw coverage at 5 cm soil depth during the three-year sampling period

Sampling year	Soil layer	Plastic cov	erage	Straw co	verage
		Maximum temperature	Minimum temperature	Maximum temperature	Minimum temperature
	/ cm	/ °C	/ °C	/ °C	/ °C
2016	0–10 cm	28.3 - 34.2	0.1 - 0.9	25.1 - 29.2	0.2 - 0.6
	10-30 cm	25.9 - 28.9	1.0 - 1.7	24.2 - 26.7	0.9 - 2.2
	30–60 cm	24.2 - 25.6	2.3 - 3.1	23.5 - 24.4	1.4 - 2.9
2017	0–10 cm	22.6 - 24.7	-1.7 – -0.7	21.3 - 22.4	-2.2 – -1.1
	10-30 cm	21.3 - 22.4	-0.50.3	20.5 - 21.3	-0.90.5
	30–60 cm	20.3 - 20.7	0.5 - 0.6	19.7 - 20.1	0.2 - 0.4
2018	0–10 cm	24.2 - 29.5	-0.80.2	21.7 - 23.9	-1.60.5
	10-30 cm	21.9 - 22.8	-0.2 - 0.0	21.3 - 22.4	-0.40.2
	30–60 cm	21.7 - 23.0	0.9 - 1.1	20.5 - 21.1	0.7 - 0.9
2019	0–10 cm	27.7 – 34.6	-0.2 - 0.0	21.7 – 25.9	-0.20.1
	10-30 cm	25.4 - 30.1	0.4 - 0.6	20.9 - 22.4	0.3 - 0.4
	30–60 cm	23.7 - 25.6	1.7 - 2.1	19.5 - 20.3	1.6 - 1.9

### 9.2 List of abbreviations

**SOM** Soil organic matter DOC Dissolved organic carbon PC Plastic-covered ridge-furrow system with subsurface drip irrigation SC Straw-covered ridge-furrow system with subsurface drip irrigation NC Uncovered ridge-furrow system with subsurface drip irrigation DON Deoxynivalenol NIV Nivalenol **ZEN** Zearalenone **WUE** Water use efficiency **NUE** Nutrient use efficiency 9.3 List of tables **Table 1** Ecosystem services and functions provided by soils (summarized from Costanza et al.,

# 9.4 List of figures

1997; Dominati et al., 2010; Powlson et al., 2011; Pereira et al., 2018) ......5

### 9.5 List of attached files on CD-ROM

- Dissertation thesis (PDF and Word format)
- Published articles (PDF format)

## 9.6 Curriculum Vitae

# Maximilian Meyer



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**Education** 

2007 Higher education entrance qualification

Pamina Gymnasium Herxheim

2007 – 2008 **Civilian service** 

Südpfalzwerkstatt, Offenbach

2008 – 2015 Study of Environmental Sciences (Final grade: 1.2)

University Koblenz-Landau, Campus Landau

Main subject: Geoecology
Minor subjects: Ecotoxicology

Biodiversity & Sustainability

Title Diploma Thesis (Grade: 1.0):

Determination of quantitative pore size distribution in soil

samples with <sup>1</sup>H-NMR Relaxometry

### **Employment history**

06.2008 - 07.2008	Vacation worker Daimler Chrysler, Wörth
03.2013 - 08.2013	Internship AgroScience GmbH, Neustadt-Mußbach Department of Environmental chemistry
07.2013 – 10.2013	Working student Dienstleistungszentrum Ländlicher Raum, Neustadt
06.2015 - 09.2015	Scholarship holder Publication scholarship of the research focus AufLand, financed by the Ministry of Education, Science, Further Education and Culture
Since 10.2015	Scientific assistant University Koblenz-Landau, Campus Landau, Institute for Environmental Sciences, Department of Environmental- and Soil Chemistry

### 9.7 Publications

### **Peer-reviewed articles:**

- 1. Buchmann, Christian; Meyer, Maximilian; Schaumann, Gabriele Ellen (2015): *Characterization of wet aggregate stability of soils by 1H-NMR relaxometry*. Magnetic Resonance in Chemistry, 53(9): 694-703.
- 2. Muñoz, Katherine; Buchmann, Christian; Meyer, Maximilian; Schmidt-Heydt, Markus; Steinmetz, Zacharias; Diehl, Dörte; Thiele-Bruhn, Sören; Schaumann, Gabriele Ellen (2017): Physicochemical and microbial soil quality indicators as affected by the agricultural management system in strawberry cultivation using straw or black polyethylene mulching. Applied Soil Ecology, 113: 36-44.
- 3. Meyer, Maximilian; Buchmann, Christian; Schaumann, Gabriele Ellen (2018): *Determination of quantitative pore-size distribution of soil with <sup>1</sup>H-NMR relaxometry*. European Journal of Soil Science, 69(3): 393-406
- 4. Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2020): *Analysis of biogeochemical processes in plastic-covered soil during establishment period in strawberry cultivation.* SN Applied Sciences. 2(10): 1-16
- 5. Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Agricultural mulching and fungicides Impacts on fungal biomass, mycotoxin occurrence and soil organic matter decomposition. Environmental Science and Pollution Research. 28(27):36535-36550

- 6. Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine (2021): Multiannual soil mulching in agriculture Analysis of biogeochemical soil processes under plastic and straw mulches in a three-year field study in strawberry cultivation. Journal of Soils and Sediments. 21:3733-3752
- 7. Muñoz, Katherine; Thiele-Bruhn, Sören; Kenngott, Kilian; Meyer, Maximilian; Diehl, Dörte; Steinmetz, Zacharias; Schaumann, Gabriele Ellen (2022): *Effects of Plastic versus Straw Mulching Systems on Soil Microbial Community Structure and Enzymes in Strawberry Cultivation*. Soil Systems 6, 21.
- 8. Meyer, Maximilian; Schaumann, Gabriele Ellen; Muñoz, Katherine (2022): *How does multiannual plastic mulching in strawberry cultivation influence soil fungi and mycotoxin occurrence in soil?* Mycotoxin Research. 38(2):93-105

# **Oral and poster presentations:**

- 1. Meyer, Maximilian; Buchmann, Christian; Schaumann, Gabriele Ellen: *Determination* of quantitative pore size distributions of soils with <sup>1</sup>H-NMR Relaxometry (oral presentation). DBG Jahrestagung, München, September 2015
- 2. Meyer, Maximilian; Buchmann, Christian; Schaumann, Gabriele Ellen: *Determination of quantitative pore size distributions of soils with <sup>1</sup>H-NMR Relaxometry* (Poster). Young Academics Conference Land-Water-Interactions, Klingenmünster, November 2015
- 3. Meyer, Maximilian; Diehl, Dörte; Thiele-Bruhn, Sören; Schaumann, Gabriele Ellen; Muñoz, Katherine: *Short-term soil response under plastic mulching in strawberry cultivation* (Poster). DBG Jahrestagung, Göttingen, September 2017
- 4. Meyer, Maximilian; Diehl, Dörte; Thiele-Bruhn, Sören; Schaumann, Gabriele Ellen; Muñoz, Katherine: *Short-term effects of plastic mulching on soil quality and mycotoxin occurence in strawberry cultivation* (Poster). SETAC GLB Jahrestagung, Neustadt, November 2017
- 5. Schaefer, Miriam; Heidbreder, Lea Marie; Meyer, Maximilian; Steinmetz, Zacharias; Milde, Jutta: *Project PLAST Dealing with environmental risks of plastic usage and consumption: An interdisciplinary contribution towards ecological transformation* (Poster). Symposium für Plastikmüll, Hamburg, Juni 2018
- 6. Meyer, Maximilian; Schaumann, Gabriele Ellen; Muñoz, Katherine: *Mycotoxin occurrence under short-term plastic mulching in strawberry cultivation* (Poster). Mycotoxin Workshop, München, Juni 2018
- 7. Meyer, Maximilian; Giradi, Johanna; Muñoz, Katherine: *Time-dependent response of microbial biomass and soil fungi to fungicide application in plastic and straw mulched soil* (Poster). SETAC GLB Jahrestagung, Münster, September 2018

- 8. Steinmetz, Zacharias; Meyer, Maximilian; Schaefer, Miriam; Heidbreder, Lea Marie; Milde, Jutta; Muñoz, Katherine; Schaumann, Gabriele Ellen: SOILPLAST Nachhaltiger Erhalt der Ressource Boden: Chancen und Risiken der intensiven Verwendung von Plastikfolien in der Landwirtschaft (Poster). SETAC GLB Jahrestagung, Münster, September 2018
- 9. Schaefer, Miriam; Heidbreder, Lea Marie; Werling, Kristen; Meyer, Maximilian; Steinmetz, Zacharias; Barkela, Berend; Muñoz, Katherine: *Project PLAST An interdisciplinary approach for the analysis of environmental risks due to plastic usage* (Poster). SETAC GLB Jahrestagung, Landau, September 2019
- 10. Meyer, Maximilian; Diehl, Dörte; Schaumann, Gabriele Ellen; Muñoz, Katherine: *Agricultural mulching and fungicides Impacts on fungal biomass and mycotoxin occurrence* (oral presentation). Mycotoxin Workshop, Münster (online), Juni 2021