

# Cover cropping in organic reduced tillage systems – soil fertility effects and measurement methodologies

Inaugural dissertation of the Faculty of Science  
University of Bern

Presented by  
Simon Oberholzer

from St.Gallen Tablat

Supervisor of the Doctoral Thesis:

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PD Dr. Markus Steffens

Institute of Geography, University of Bern, Switzerland



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## Summary

Sustainable agriculture strongly depends on fertile soils. Yet keeping soils in crop production while maintaining their fertility is highly challenging and most long-term studies indicate a decrease in soil fertility on arable land under continuous cropping. During the growing period of the cash crop, only limited options to improve soil fertility exist because there are normally very specific cultivation requirements. Therefore, the break periods between main crops have a high potential for soil fertility improvement because no harvest output is expected. Many studies have shown that long periods of bare soil should be avoided and during long fallow times the soil should be covered. For that purpose, different options of cover cropping have been developed. They differ in terms of plant species, frost tolerance, biomass input and termination methods. So far, in the scientific literature cover crops were mainly compared to bare soil treatments but different cover cropping strategies are only rarely compared, hence limiting knowledge on their performance relative to one another. In organic reduced tillage systems cover cropping has a high priority and since neither herbicides nor intense tillage can be used, frost-tolerant cover crops can only be terminated with shallow tillage methods that brings the cover crop biomass into the very topsoil layer. The challenge thereby is that within a reasonable time (around 2 weeks) the mixture of cover crop pieces and soil must result in proper seedbed for the next crop. One way to make this process easier is to reduce the amount of cover crop biomass by mowing and removing the aboveground biomass. Another approach is to use a microbial inoculant called “Effective Microorganisms” that is promised to facilitate the decomposition process and make seedbed preparation easier.

In the context of Switzerland, crop rotations are very diverse and accordingly also the fallow periods differ widely in length and seasonal growing condition. Thus, it is difficult to evaluate different cover cropping strategies in long-term experiments because the same fallow period only occurs once in several years. On the other hand, short-term experiments face the challenge that most soil properties show a high variability in space and changes are normally rather small which makes it very difficult to statistically detect management effects. The statistical power could be improved by increasing the sample size but due to the high costs of conventional soil analyses, the number of soil samples is normally limited. Infrared spectroscopy is a method that provides fast and cheap soil analyses and can therefore potentially be very useful in short-term soil experiments because the number of samples can be increased at little additional costs. Yet, spectral soil data need to be calibrated with measurements from conventional lab data and still little is known about the performance of spectral models at the local scale.

The goal of this thesis is to increase scientific knowledge about cover cropping effects on soil properties in organic reduced tillage systems. Thereby I formulated three major objectives: 1)

to compare the effects of two frequently used cover cropping strategies on soil fertility parameters, 2) to evaluate the suitability of infrared spectroscopy in soil sampling projects of local extent and 3) to investigate the effects of Effective Microorganisms (EM) on cover crop decomposition.

I thus investigated the effects of two cover cropping methods on soil properties at six fields in eastern Switzerland (Paper 1). A sampling design in very high temporal and spatial resolution was implemented and the high number of soil samples ( $n = 2574$ ) was analyzed in a combined approach of conventional soil analysis and soil spectroscopy in the visible and near-infrared range (vis-NIR). Thereby the reasons for the varying performance of spectral models between different fields were analyzed and summarized in Paper 2. A very similar spectral approach was used in a soil survey in northern Spain (Paper 5) and results are presented to complement the insights from Paper 2. The effects of EM on cover crop decomposition were tested in a lab incubation study and published in paper 3 and 4.

Regarding objective 1, the two cover cropping strategies that either maximized plant biomass input or soil cover were evaluated in the long fallow period between wheat harvest (End of July) and sowing of a next spring crop (Paper 1). In the double cover cropping (DCC) strategy two cover crops were sown subsequently and shallowly (3 cm) incorporated into the soil with the idea that the biomass input provides an energy source for the soil microorganisms. In the permanent soil cover (PSC) strategy, the soil was covered for the whole period with one cover crop, that was mowed, and the plant biomass was removed. In contrast to DCC, the PSC strategy had no aboveground plant biomass input into the soil but also no tillage throughout the period. The analysis of the two cover cropping strategies in high spatial and temporal resolution showed that the effects of differences between different sampling times were far more pronounced than differences between treatments. Nevertheless, in both treatments the increase in soil organic carbon was highest in 5-10 cm soil depth and significantly higher in the PSC compared to the DCC approach. The plant biomass input in the DCC treatment led to higher microbial biomass and mineral N compared to the PSC treatment. I conclude that the aboveground biomass input in the DCC strategy was beneficial for biological activity but the better soil cover and probably higher root biomass input in the PSC strategy was slightly more beneficial for soil organic carbon.

Addressing objective 2, the spectral models showed over all a good performance, but the model performance was lower on the two fields with high carbonate content (Paper 2). The prediction accuracy for fields with high carbonate content could not be improved when data of all fields were combined to build general models. I therefore conclude that especially in soils with low carbonate contents, soil spectroscopy is very suitable, and the prediction errors can be expected to be comparable to the lab measurement error. The application of the same

spectral approach at a study site in Spain showed clearly that much more detailed information can be obtained when conventionally analyzed samples are accompanied by additional spectral measurements (Paper 5).

Concerning objective 3, EM application did not show significant effects on cover crop decomposition dynamics, under the spring-like conditions mimicked in the incubation study (12° C) (Paper 3 and 4). Thanks to a sterilized control, I could distinguish effects caused by living microorganisms and effects caused from substrate (energy and nutrients) addition. Seven days after the start of the incubation, microbial taxa from EM solution could only be found when EM were applied in 100 times higher amounts than recommended in agricultural praxis.

This thesis shows that a high-resolution sampling design and the use of spectral methods allowed to evaluate different cover cropping strategies in a short-term experiment. I therefore consider the methodological approach to be very useful for evaluating innovative soil management strategies as soonest possible after their invention. This thesis is one piece of knowledge that aims to support decision making about cover cropping in organic reduced tillage systems and provides results about effects on soil fertility properties. More research is needed to evaluate effects of cover cropping on other agronomic variables like yields, weed pressure and profitability.

**Keywords:** Cover crop, soil spectroscopy, regenerative agriculture, effective microorganisms, temperate climates, soil fertility, soil organic matter, land abandonment

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on weather and management schemes and thanks to the student assistants, Evi Rothenbühler, Michael Müller, Aline Wicki, Cristina Joss and my brothers Basil und Linus Oberholzer, I could manage to take all the soil samples at the right time. I would also like to thank the lab technicians Daniela Fischer, Patrick Neuhaus, Maarika Bischoff and René Nussbaumer for their uncomplicated support in the lab. Furthermore, I would also like to thank the students I co-supervised in their Master theses: Maja Schneider, Nadine Harder and Christa Hermann. It was a pleasure to work with you and I wish you all the best for the future. Dr. Karl Herweg was not directly involved in my PhD thesis, but I thank him a lot for introducing me into transdisciplinary teaching and I could benefit a lot from the field course we did together for many years. Besides work also some fun is necessary to keep joy and motivation for a project. Therefore, I thank my PhD colleagues in the office Ademola Adenle, Pamela Tabi, Chima Iheaturu, Georges Agonvonon, Frank Mintah, Samuel Hepner and Phydias Agossou for the interesting discussions during lunch breaks about virtually everything under the sun that not only helped to get a better focus on the research topics but also took my horizon beyond the “Ivory tower” of academia. Finally, I would like to thank my wife for her patience during all the stages I had to go through.

22.10.2024, Simon Oberholzer

## **Preface**

This thesis was conducted in the group land systems and sustainable land management (LS-SLM) at the Institute of Geography at University of Bern in the context of its “sustainable soil management” project. The content of this PhD thesis was elaborated in a participatory approach with Swiss farmers who want to improve their management system and keep testing new methods. As an output I wrote three research papers and one policy brief as a first author and contributed to one paper as a co-author.

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## Abbreviations

Cmic:	Microbial carbon
DCC:	Double cover cropping
EM:	Effective microorganisms
Nmic:	Microbial nitrogen
Nmin :	Mineral nitrogen
POXC:	Permanganate oxidizable carbon
PSC:	Permanent soil cover
SOC:	Soil organic carbon
vis–NIR:	Visual and near-infrared



## Part I: Background and overview

### 1. Introduction

#### 1.1. Role of cover crops in soil fertility management

The concept of soil fertility captures the ability of a soil to sustain plant growth by providing essential plant nutrients and the physical, biological and chemical conditions enabling it to serve as habitat for plant growth (FAO, 2022). Soil fertility is a relatively narrow term that focuses on the suitability of soils for agricultural production (Patzel et al., 2000). It has been expanded to the terms of soil quality or soil health to account for further ecosystem services like water quality, biodiversity conservation, and climate regulation (Bünemann et al., 2018). Since in this thesis the focus lies on the question how different cover cropping management influences the soil conditions for the next crop, I consider soil fertility as the most adequate and most intuitive term. Soil fertility is a term that does not have a direct corresponding quantitative measurement and besides the application of visual methods in the field (Johannes et al., 2017) and some attempts to combine different measurements to a soil fertility index (Munnaf and Mouazen, 2021), most studies use measurements of soil organic matter stocks as a proxy for soil fertility (Tittonell et al., 2008). Soil organic matter improves the functioning of the soil in many different aspects through improved soil structure (aggregation, aeration, water retention) and improved elemental cycles (nutrient mineralization, carbon sequestration, compound retention) or just to serve as a biological habitat by itself (Hoffland et al., 2020). It is difficult to draw conclusion about soil fertility from soil organic matter stocks at two completely distinct locations but changes in soil organic matter stocks over time at one location have strong effects on soil fertility and are therefore very suitable as a proxy measurement.

In many world regions, agricultural management leads to decreasing soil organic matter stocks thereby threatening soil fertility which either directly decreases yields or makes them more dependent on external inputs (Lal, 2015). To reverse this global trend, many management strategies have been suggested, sometimes called soil-improving cropping systems (Rietra et al., 2022), sustainable land management practices (Ruiz et al., 2020) or regenerative agriculture (Rhodes, 2017), but always aiming at a high organic matter input (cover crops, organic fertilizers and amendments), high soil cover (mulch, cover crops), high plant species diversity in crop rotation and reduced tillage strategies (Liu et al., 2006).

Especially, in humid climates, where water is normally not a limiting factor, cover cropping is considered to be a major element to increase soil organic matter stocks and its turnover, which improves soil fertility (Scavo et al., 2022). The benefit of cover cropping is mainly to avoid bare soil periods that naturally do not exist in temperate climates and are normally

characterized by erosion, increasing nutrient losses, decreasing soil organic matter stocks and low microbial activity (Daryanto et al., 2018; Thorup-Kristensen et al., 2003).

The use of cover crops does not only bridge the time between two main crops to avoid bare soil periods but also offers the opportunity to push the agroecosystem into a more biodiverse and resilient direction. Main crops normally must fulfill clear requirements in terms of product quality and efficient machinery use at harvesting which limits the flexibility in the cultivation. Cover crops, on the other hand, can be handled more flexible and allow a direct focus on the improvement of soil conditions.

The major cover crop effect is its considerable organic matter input having the potential to increase soil organic matter stocks which is highly beneficial for soil fertility and relevant for carbon sequestration for climate change mitigation. Additionally, cover crops allow to increase the biodiversity in the agroecosystem with benefits for soil microbiology (McDaniel et al., 2014; Tiemann, 2015) whereby higher carbon uptake and microbial biomass has been measured in cover crop mixtures than in single species cover crops (Gentsch et al., 2020). Besides total microbial biomass, cover cropping increases also the functional diversity in the soil microbiome (Kim et al., 2020) which might then affect the resilience of an agroecosystem. Cover crops have furthermore a crucial role in nutrient cycling, especially nitrogen (N) retention (Thorup-Kristensen et al., 2003; Zhou et al., 2020), which reduces nutrient losses and potentially increases fertilizer efficiency. Especially, N losses from farmyard manure can be reduced if the manure is applied on growing plants whereby cover crops are very suitable because crop damage due to machine use is almost irrelevant (Cambardella et al., 2010; Everett et al., 2019).

However, in the agricultural practice, cover cropping must be well coordinated with the rotation of the main crops which makes the optimal cover cropping strategy strongly dependent on environmental conditions and the agricultural policy framework and therefore site-specific.

## **1.2. Agricultural policy frameworks regarding cover cropping and crop rotations in Switzerland**

Both the public and organized civil society and private actors influence soil management strategies in Switzerland. In the public domain, Swiss farmers must fulfill the proof of ecological performance (Ökologischer Leistungsnachweis) which also contains regulations about the bare soil periods (Swiss Ordinance 910.13, 2013) to receive direct payments. If a crop is harvested before August 31, a next crop or cover crop must be sown in the same year

(Swiss Ordinance 910.13: Article 17) with the effect that after cereal harvest and a next spring crop a cover crop or temporary ley must be sown. Additionally, there is an incentive payment per hectare if the bare soil period between crops or cover crops is shorter than seven weeks (Swiss Ordinance 910.13: Article 71).

In organic farming, additional requirements must be fulfilled. According to the regulations of BioSuisse (2022), a national membership organization of Swiss organic farmers, 20 % of the arable land must be covered with temporary grassland. Cover crops can also be counted (up to 10 %) according to their area and period if they stand longer than five months. Additionally, only a maximum of 50 % of the arable land is allowed to be bare over winter (15 Nov. – 15 Feb.; BioSuisse, 2022). Especially organic farms with little or no livestock are interested in cover cropping because they do not need grassland but must fulfill the label requirements.

These regulations show that in Switzerland cover cropping is widespread and that there are strong incentives to avoid periods of bare soil.

However, the question of cover cropping is strongly related to crop rotation because the main crops determine the agronomic requirements and periods for cover cropping. Switzerland is one of the few countries that has strict regulations on crop rotations. On a farm with more than three hectares arable land, at least four different crops must be grown per year, and for each crop, a clear pausing length in the crop rotation is specified (Swiss Ordinance 910.13 (2013), Article 16). These regulations along with the dynamic market situation (Bundesamt für Landwirtschaft, 2022) lead to highly diverse crop rotations that are constantly adjusted. Hence, they influence the durations of cover cropping and its contributions to soil organic matter.

### **1.3. Short-term versus long-term changes in soil organic matter**

There are two different views on soil organic matter. From a perspective of climate change mitigation mainly long-term increases in soil organic matter stocks are of interest because of their potential for carbon sequestration (Blanco-Canqui, 2022; Chahal et al., 2020). Short-term increases in soil organic matter stocks can be seen as short-lived carbon sinks and can accordingly also be quantified in carbon sequestration calculations (Leifeld, 2023) but their effect is of much lower importance compared to the long-term changes. From a soil fertility perspective long-term increases in soil organic matter are also very desired because of their positive effects on soil structure, and storage capacity, but also short-term changes are of interest, because the dynamic nature of soil organic matter is very beneficial for crop production (Janzen, 2006). The highest value of soil organic matter lies in its decay (Janzen, 2006) because nutrients are mineralized and become available for the crop (Hacker et al.,

2015). A sustainable cropping system is therefore mainly interested in a high turn-over of soil organic matter and needs periods of high organic matter formation (or inputs) that can then serve as a source for organic matter mineralization. A high increase in soil organic matter stocks in one year and an equal decrease in the next year is irrelevant from a climate perspective, but relevant in crop production because it might have been very beneficial for soil microbiology or nutrient supply. Cover crops are seen as a major element in a crop rotation to introduce periods of soil organic matter formation (McClelland et al., 2021), but the immediate effects of different cover cropping strategies on soil fertility parameters remain understudied. Given the diverse and flexible crop rotations in Switzerland, it is very difficult to evaluate a specific cover cropping approach in the long-term because it is probably applied only once in several years.

From a farmer's perspective, any measure without direct monetary benefit must pay off in a relevant time scale (Perez et al., 2007), which is probably mainly the performance of the crop in the following year or in a maximum of a few years. Long-term field trials are of very high scientific value but they cannot cover the whole range of management options in agricultural praxis as Chenu et al. (2019) argued. A participatory approach is thus needed that evaluates the short-term effects of different management (i.e. cover cropping) options on soil organic matter and soil fertility.

#### **1.4. Challenges in measuring changes in soil organic matter related soil properties**

Soil organic matter has three characteristics that make the detection of change a major challenge. According to Hoffmann et al. (2017), these are: a) its small scale spatial heterogeneity, b) its pronounced short-term temporal dynamics and c) the rather small magnitude of changes compared to the total stocks. In agricultural research the main strategy to deal with these challenges is to design long-term field trials, with the disadvantage that the short-term effects remain concealed and changes in soil organic matter are completely attributed to the long-term treatment effects (Schrumpp et al., 2011). Recently, Mayer et al. (2022) showed with data from a long-term field trial that the short-term dynamics of the occluded particulate organic matter, as part of total soil organic matter, can be substantial within days to weeks. Similar studies of total soil organic carbon (SOC) showed a very high temporal variability attributable to the combination of management activity (i.e. tillage, fertilization) and seasonal patterns (i.e. temperature, moisture; Wuest (2014)). The seasonal pattern during the growing season can be quite distinct from year to year and especially in diverse crop rotations, the management is highly variable making soil organic matter dynamics

on crop land very complex and unpredictable (Wuest, 2014). Thereby, the unpredictable speed of decomposition processes of crop residues, plant roots, cover crop biomass or organic fertilizers, plays a major role. A crucial factor is the time point when these organic substances reach a size of < 2 mm because only then they become part of the soil fine earth that is normally analyzed (Blume et al., 2016) and classified as soil organic matter. From a soil fertility perspective, the whole continuum of the decomposition processes from plant residues (organic matter > 2mm) as well as decomposition processes within the soil organic matter (organic matter < 2mm) are of relevance because all product of the decomposition processes interact with the soil mineral phase and the plant roots. This contentious nature of soil organic matter (Lehmann and Kleber, 2015) where organic matter is gradually more and more decomposed makes it recommendable to not only measure total soil organic matter but also associated soil properties like microbial parameters and nutrients as well as different fraction of soil organic matter (as e.g. particulate organic matter, mineral associated matter or permanganate oxidizable carbon). To tackle the high spatial and temporal resolution of soil organic matter related soil properties, the ideal solution would be to have a high spatial and temporal resolution in soil analysis which is constrained by the analysis costs per soil sample. Currently mostly applied in soil science is dry combustion, where the ground soil is burnt at 1150°C and produced CO<sub>2</sub> is quantified (Harris et al., 2014). In acidic soils the measured total carbon in a soil sample equals SOC but in soils containing carbonate a second measurement is necessary to determine the carbonate content, which makes the analytical costs per sample relatively high. Therefore, complementary measurement methods such as visible and near-infrared (vis–NIR) spectroscopy have the potential to make the analysis cheaper.

## **1.5. Soil visible and near infrared spectroscopy for cost-efficient soil sample analysis**

Diffuse reflectance spectroscopy has gained increasing attention in soil science because of its potential to provide fast, non-destructive, and cost-effective measurements (Nocita et al., 2015). Most spectrometers work either in the visible and near infrared range (vis–NIR, 350–2500 nm) or in the midinfrared (MIR, 2500–25'000 nm) range (Soriano-Disla et al., 2014). Comparisons between MIR and vis–NIR spectroscopy normally attribute a higher measurement accuracy to MIR spectrometers while vis–NIR spectroscopy is much cheaper and requires less soil sample preparation (no grinding) (Breure et al., 2022; Clairotte et al., 2016; Knox et al., 2015).

In general, spectral data need to be calibrated with reference values from standard laboratory measurements whereby different model approaches that can deal with multicollinearity in

the predictor variables (the reflectance value at each wavelength) such as partial least square regression or machine learning tools can be used (Viscarra Rossel et al., 2022). The vision is that one day the spectral analysis could potentially replace expensive lab measurements. In other disciplines this vision has become reality and spectral measurements are standard to measure for example the quality of agricultural products (cereals, forage,...), while in soil science, spectroscopy is still in a research status (Bellon-Maurel et al., 2010). The reason therefore is that biological products are clearly constrained by the plants genome which makes all grains or fruits of one plant species highly comparable, while soils normally show a high heterogeneity resulting in often highly skewed distribution of soil properties (Bellon-Maurel et al., 2010; Brown et al., 2006). The application of spectral models to soil samples from areas that have not been part of the calibration dataset is very challenging and one of the big topics in soil spectroscopy research (McBride, 2022; Ng et al., 2022; Tziolas et al., 2019).

However, even though the first vision of replacing standard lab measurements seems unlikely, soil spectroscopy has a high potential in combination with conventional lab measurements (Viscarra Rossel et al., 2022). Especially, regionally and locally calibrated spectral models predicting soil properties related to soil organic matter have shown good performance (Angelopoulou et al., 2020; Breure et al., 2022). Therefore, soil spectroscopy has a high potential to increase the number of samples at little additional costs which is crucial to tackle the high spatial and temporal variability of soil organic matter. However, in conventional lab methods, the measurement error can be very well estimated beforehand, which is not the case for spectral models. Each local spectral model has its own performance and the prediction error (corresponding to measurement error in conventional methods) cannot be estimated beforehand. Therefore, the application of soil spectroscopy bears the risk that the model performance might not match the measurement accuracy that is required by the research question.

## **1.6. Cover crops in organic reduced tillage systems: decomposition is crucial**

In reduced tillage systems cover crops decompose on the soil surface either as mulch or slightly mixed with topsoil. The optimal decomposition dynamics depend on the management objective and the following crop and influences the cover crop selection: A slow decomposition is desired if the cover crop mulch should suppress weeds (Brito et al., 2019), a decomposition in balance with main crop growths is desired if the cover crop mainly serves as nutrient source (Dhakal et al., 2020) and a fast decomposition is desired if the following crop

has strict requirements about seedbed preparation and mulch sowing is not possible. The decomposition of cover crops depends highly on diurnal variations of moisture and temperature (Thapa et al., 2021) and therefore the cover crop decomposition in reduced tillage systems can be slower than desired (Varela et al., 2017), with negative consequences for seedbed preparation for the next crop. In Switzerland, weather in spring can be cold and wet which prevents a fast cover crop decomposition and the moist and potentially slimy cover crop material on the soil surface can cause seedbed preparation and sowing problems (Thapa et al., 2021).

### **1.7. A microbial inoculant to accelerate the decomposition of cover crops**

With the promise to enhance cover crop decomposition and boost soil microbiology, a microbial inoculation product based on the effective microorganism (EM) technology (Higa, 1991) was introduced into the Swiss market (EM Schweiz, 2023). Many Swiss farmers practicing the shallow incorporation of cover crops apply EM. The general idea of microbial inoculants, often summarized under the term plant growth promoting rhizobacteria, is to influence the plant-microbial associations in the soil thereby aiming at better crop performance (Backer et al., 2018; Gouda et al., 2018). Effective microorganisms are advertised to have a wide application range that goes far beyond agriculture which makes their scientific evaluation very difficult. The existing peer-reviewed literature about the application of EM in agriculture is highly controversial whereby beneficial effects of EM were most times observed in tropical or subtropical regions in Asia (Hu, 2018; Khaliq et al., 2006), while studies in Europe did not detect statistically significant effects of EM (Mayer et al., 2010; Pranagal et al., 2020; Schenck zu Schweinsberg-Mickan and Müller, 2009). So far, the main conclusion about EM application is, that it has to be applied in combination with organic matter, which is the recommendation of the developer (Higa, 2003) as well as the conclusion of research studies that found significant effects (Hu and Qi, 2013; Javaid, 2011; Olle, 2021; Van Fan et al., 2018). The combined application of EM with cover crop termination fulfills this criterium but the promised effects of faster decomposition, higher nutrient efficiency and formation of soil organic matter (EM Schweiz, 2023) has not yet been evaluated.

### **1.8. Summarizing the identified scientific gaps**

Much empirical research has been conducted to compare cover cropping and bare soil treatments and results have been summarized in meta-analyses (Jian et al., 2020; McClelland et al., 2021). These two meta-analyses summarize various data from long-term cover cropping

field trials, but 68 % respectively 54 % of the considered field experiments were in monoculture system and most of the rest in systems with two or three crops (Jian et al., 2020; McClelland et al., 2021). The evaluation of cover cropping in diverse (more than three crops) crop rotation is highly understudied because time windows for cover cropping keep changing and do not occur every year. In Switzerland, diverse crop rotations are general praxis and due to the federal regulations (see section 1.2) bare soil treatments in long fallow periods are not of practical relevance. Therefore, there is a clear need to evaluate different cover cropping strategies in agricultural systems with diverse crop rotations. Since the same cover cropping time window only occurs once in several years, long-term field trials would only be of limited use, respectively their cost-benefit ratio would be questionable. Yet, measurement of short-term changes in soil properties related to organic matter is challenging (see section 1.3) and requires a high spatial and temporal resolution of soil sampling and therefore a large sample size. Soil vis–NIR spectroscopy has the potential to analyze large number of samples but its suitability in field experiments is understudied. Local spectral models at field or on-farm level have been developed (e.g. Kuang and Mouazen (2011) or Singh et al. (2022)) but their accuracies are highly varying for unknown reasons. This uncertainty hampers the application of vis–NIR spectroscopy at the local scale and therefore there is a strong need to analyze the factors that influence the performance of local spectral models.

Furthermore, the rate of cover crop decomposition is crucial in organic reduced tillage systems and so far, it is not clear if the application of EM can alter the cover crop decomposition process. There is no systematic analysis of the effects of cover crop incorporation with EM application regarding soil microbial activity and community composition as well as released elements during the cover crop decomposition.

## **1.9. Research objectives**

The goal of this thesis is to enhance knowledge on cover crop management, elucidate short-term changes in soil fertility and elaborate suitable methods for such assessments. Its objectives can be divided into three major topics that have been published as individual papers: 1) analyze the effects of different cover cropping strategies on soil fertility parameters, 2) to explore the suitability of vis–NIR spectroscopy for soil sampling projects of local extent, 3) to examine the effects of EM on cover crop decomposition. These topics were structured by the following research questions:



- 1) Effect of different cover cropping strategies on soil fertility (Paper 1):
  - A) Do cover cropping strategies that differ in terms of species composition, growing period and aboveground biomass input show different effects on soil C and N fractions?
  - B) Which of the analyzed soil properties and which soil depth segment is most sensitive to implemented cover cropping strategies?
- 2) Suitability of vis–NIR spectroscopy for soil sampling projects of local extent (Paper 2 and 5):
  - A) To what extent do the prediction errors of local spectral models differ from the lab measurement error?
  - B) Can spectral data of several target sites be combined to a general model without substantial decrease in model performance?
  - C) How do field and soil characteristics (e.g., field size, soil texture, carbonate content, correlations of soil properties) of the target site relate to the performance of spectral models?
  - D) Does an increased sample size analyzed with vis–NIR spectroscopy in a local experiment improve the statistical power compared to a lower sample size with conventional analysis?
- 3) Effect of EM on cover crop decomposition: (Paper 3 and 4):
  - A) Do EM alter the dynamics of cover crop decomposition?
  - B) Do EM alter the concentration of water-soluble ions or elements?
  - C) Can microbial taxa from the EM solution establish themselves in the soil?

## **2. Methodology**

### **2.1. Initiating a participatory on-farm experiment**

The starting point for this thesis was a privately organized soil course (Bodenkurs im Grünen, Konzepte der regenerativen Landwirtschaft), held by the practitioners Dietmar Näser (Grüne Brücke, 2022) and Friedrich Wenz (Humusfarming, 2022), that I participated with around 20 farmers in 2017. This course is not offered anymore but follow-up programs still exist, and their content can be checked on the websites of Grüne Brücke and Humusfarming. A big topic of the course was cover cropping with shallow incorporation and EM application, which was assured to show beneficial effects on soil fertility parameters (Näser, 2021). Together with five interested farmers, we decided to scientifically evaluate two contrasting cover cropping approaches in short-term field experiments on their farms (Objective 1, Paper 1). The experiment took place on six fields in eastern Switzerland that were a maximum of 12.8 km apart from one another (Figure 1). The field experiment in this PhD theses took place from

end of July (cereal harvest) to end of April or beginning of May (sowing of spring crop) and in this long fallow period a bare soil treatment is not an option and was therefore also not considered in the study design.

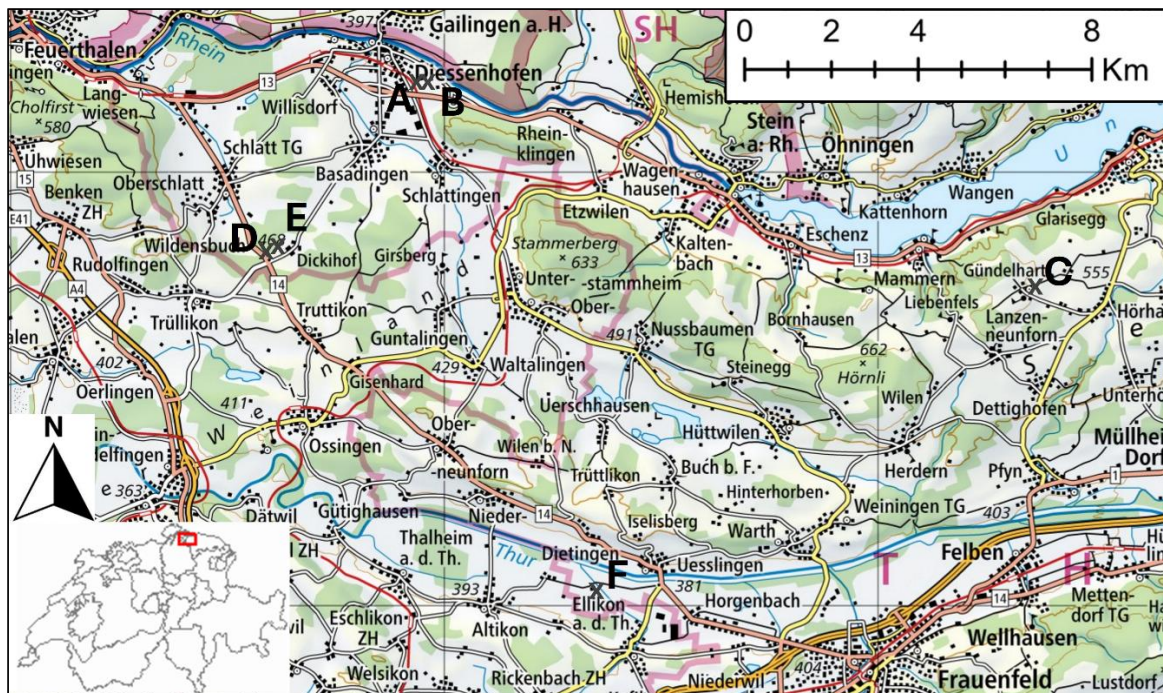


Figure 1: Location of the six fields (A-F) in the canton of Thurgau in eastern Switzerland.

## 2.2. Application of vis–NIR spectroscopy

The need for more studies to contribute to the emerging evidence about the potential of vis–NIR spectroscopy to complement laboratory soil analysis and thus enable a cost-effective analysis of larger soil samples was another motivation (Metzger et al., 2024). The availability of portable vis–NIR spectrometers allows to collect data through direct in situ measurements. However, I took soil samples in the field and conducted the spectroscopy measurements on dried and sieved samples in the lab. There are three reasons why I did not conduct in situ measurements on the field. First, I would not have had enough time in the field to do proper measurements and the time window was limited due to the cover cropping management schedule. Second, the highly variable water content under field conditions at different time points would probably have resulted in lower model performance compared to lab measurements (Hutengs et al., 2019). Thirdly, it is a big advantage to have all soil samples available in the lab, because the spectra can be analyzed and a representative subset for wet chemistry analysis can be chosen. With field measurements, the reference samples must be determined beforehand without knowing if they are representative for the whole dataset. The suitability of vis–NIR spectroscopy application in field experiments (objective 2) was evaluated in Paper 2 and Paper 5.

## 2.3. Effects of EM tested in a lab incubation experiment

The scientific literature about the effects of EM on organic matter decomposition (see section 1.7) indicated that a potential effect of EM on cover crop decomposition might, if at all, only be minor. Therefore, I did not include EM as an experimental factor in the field trial but ran a lab incubation experiment with different EM levels and a sterilized control (Objective 3, Paper 3 and 4). The lab experiment allowed to keep the environmental conditions stable and work with a high temporal resolution.

## 2.4. Overview of research papers

This thesis contains three first author papers, one policy brief and one co-authored paper. Figure 2 shows which of the collected datasets were used in which paper and how these different papers contribute to the three objectives of this thesis. Table 1 provides an overview about the different research outputs and in which journal they were published or have been accepted.

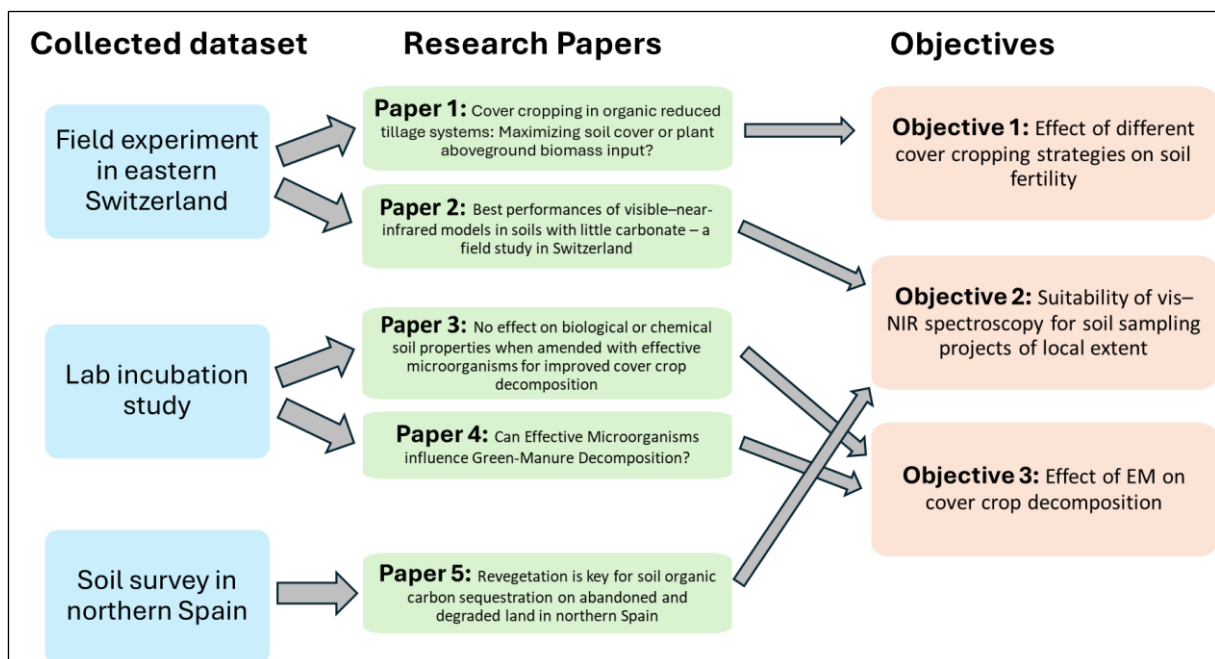


Figure 2: Overview of the connections between collected datasets, research papers and objectives of this PhD thesis.

Table 1: Overview of research outputs

No.	Objective	Authors	Title	Journal	Status
1	1	Simon Oberholzer, Klaus A. Jarosch, Markus Steffens, Nadine Harder and Chinwe Ifejika Speranza	Cover cropping in organic reduced tillage systems: Maximizing soil cover or plant aboveground biomass input?	European Journal of Soil Science	Accepted on October 25, 2024
2	2	Simon Oberholzer, Laura Summerauer, Markus Steffens and Chinwe Ifejika Speranza	Best performances of visible–near-infrared models in soils with little carbonate – a field study in Switzerland	Soil	Published on April 10, 2024
3	3	Simon Oberholzer, Christa Herrmann, Natacha Bodenhausen, Hans-Martin Krause, Adrien Mestrot, Chinwe Ifejika Speranza and Klaus A. Jarosch	No effect on biological or chemical soil properties when amended with effective microorganisms for improved cover crop decomposition	Applied Soil Ecology	Published on March 2, 2024
4	3	Simon Oberholzer, Christa Herrmann, Natacha Bodenhausen, Hans-Martin Krause, Adrien Mestrot, Chinwe Ifejika Speranza and Klaus A. Jarosch	Can Effective Microorganisms influence Green-Manure Decomposition?	Policy Brief in Agrarforschung Schweiz	Published on April 6, 2024
5	2	Maja V. Schneider, Simon Oberholzer and Chinwe Ifejika Speranza	Revegetation is key for soil organic carbon sequestration on abandoned and degraded land in northern Spain	Geoderma Regional	Published on July 11, 2024

### 3. Key insights and discussion

#### **Paper 1: Cover cropping in organic reduced tillage systems: Maximizing soil cover or plant aboveground biomass input?**

- Comparison of permanent soil cover (PSC) that maximizes soil cover and minimizes tillage and double cover cropping (DCC) that maximizes cover crop aboveground biomass input.
- Measurement of C and N fractions in high spatio-temporal resolution in a field experiment.
- Aboveground biomass input led generally to higher microbial biomass, mineral N and POXC (0-5 cm) in the DCC treatment.
- The PSC treatment showed significantly higher increase in SOC in 5-10 cm compared to the DCC treatment which was probably caused by the higher belowground C inputs.

*Box 1: Short summary of the Cover crop paper (Paper 1, objective 1)*

The evaluation of the two cover cropping strategies revealed that the temporal variability of soil properties was very high, which posed a challenge to the identification of management effects. As the pronounced short-term variability was expected beforehand, I chose a methodological approach that allowed to collect soil samples in high temporal and spatial resolution. Despite the high measurement resolution using vis-NIR spectroscopy, only small differences for the soil properties SOC, total N and POXC between the treatments could be detected. For the whole analyzed soil depth (0-20 cm) we did not find differences between treatments. Only when sub-setting per depth segment, we found significantly higher increases for SOC in 5-10 cm in the PSC treatment but significantly higher increases for POXC in 0-5 cm in the DCC treatment. The cover crop in the PSC system had much more time to develop its root system and I hypothesize that therefore the belowground C inputs were probably higher in the PSC treatment, and which would explain the significantly higher SOC in 5-10 cm soil depth compared to the DCC treatment. The significantly higher POXC in 0-5 cm in the DCC treatment was most probable caused by the aboveground biomass input that took place in that soil depth. The spectral data were accompanied by conventional lab measurements with a lower spatial sampling resolution. Also, for these soil properties (Cmic, Nmic and Nmin) we found a pronounced variability over time, but they were, as expected more sensitive to treatment effects than SOC, total N and POXC. At the end of the field trial, I measured generally higher Cmic, Nmic and Nmin in the DCC treatment compared to the PSC treatment. I concluded therefore that the aboveground biomass input in the DCC strategy was beneficial for soil microbiology and nitrogen availability, but the constant soil cover and minimum tillage of PSC tended to be more beneficial for SOC.

## **Paper 2: Best performances of visible–near-infrared models in soils with little carbonate – a field study in Switzerland**

- Out of 30 local spectral models, 24 showed an accurate performance ( $RPD > 2$ ) and six models a low model performance ( $RPD < 2$ ).
- The low performing models were from the two fields with highest carbonate content.
- Analysis of variable importance in projection (VIP), and correlations between spectral variables and target soil properties, confirmed that high carbonate content masked absorption features for SOC.
- When combining data of different fields to build one general model, fields with high carbonate content showed a strong decrease in model performance compared to the local model.

*Box 2: Short summary of the spectroscopy paper (Paper 2, objective 2)*

The spectroscopy paper (see Box 2) showed that most local models had an accurate performance and the highest prediction error for SOC ( $2.43 \pm 0.55 \text{ g kg}^{-1}$ ) was not much higher than the lab measurement error of the conventional lab analysis using a CNS analyzer ( $1.01 \pm 0.40 \text{ g kg}^{-1}$ ). Additionally, all local models showed a very low bias (see Table 2 in Paper 2), which means that the spectral models did not systematically over- or underestimate the measured values. However, soils with high carbonate content (Fields A and F) showed in general a lower model performance than the other four fields (A, B, C, D) and the utilization of vis–NIR spectroscopy on fields with high carbonate content remains challenging and should be addressed by further research. A very similar methodological approach as in Paper 2 was applied in Paper 5 where in an area of 300 ha soil samples were taken in high spatial resolution to investigate the effect of contrasting land management (tilled fields, fallow fields and forest). In this study, the sampling design could also be statistically evaluated by only using the soil samples where wet chemistry data were available and without using any spectral data. The comparison between evaluation with wet chemistry data only (57 samples) and with spectral data (487 samples), showed that the number of statistically significant relations between treatments could be drastically increased (see Figure 8 in Paper 5). We conclude therefore that the application of soil spectroscopy in soil survey projects of local extent allows to increase the number of samples at very little additional costs which can provide more detailed information than when less samples with only wet chemistry data are used.

**Paper 3: No effect on biological or chemical soil properties when amended with effective microorganisms for improved cover crop decomposition**

- EM at recommended dose did not change the soil respiration, nor microbial C and N.
- EM at 100 times of recommended dose showed effects due to added substrate and not due to the added living microorganisms.
- Seven days after the start of the incubation, EM taxa were only detected in the samples that were treated with a 100 times higher EM dose than recommended.
- EM did not coherently change the concentration of water-soluble ions and elements.

*Box 3: Short summary of the EM paper (Paper 3, objective 3)*

Effective microorganisms did not change soil respiration nor any of the measured biological or biochemical soil property (see Box 3). I therefore conclude that the decomposition was not influenced by the addition of EM in the study setting (laboratory experiment). With the specific experimental set up, it cannot be excluded that under certain environmental conditions EM might influence cover crop decomposition but since a general effect was lacking, I expect such a potentially occurring effect to be minor.

#### **4. Synthesis and outlook**

Different cover cropping options have different short-term effects as could be shown by Paper 1. Though, a cover cropping strategy must be integrated into a farming system and therefore one cover cropping approach cannot be qualified as superior *per se* compared to another one. Taking the main results of the two compared approaches in Paper 1, we found that PSC had a stronger effect on SOC whereas the DCC approach had a stronger effect on soil microorganisms. For crop rotations that include crops requiring a lot of “soil movement” like ridge crops (potatoes, carrots, etc.) or multiple sets during the vegetation season (vegetables) the effects of the PSC approach would probably not be sustainable because it would be counteracted very soon by the following crop. Since in these crop rotations (independent of the farming system) a lot of physical soil management takes place, the recovery of soil structure (aggregates) is probably most important which can only be achieved through a high microbial activity. Therefore, in such systems I would rather recommend cover cropping options with biomass input that foster soil microbiology like the DCC approach. On the other hand, in reduced tillage systems with little soil movement and crop rotations with mainly threshing crops (cereals, rapeseed, etc.), low tillage intensity and high soil cover strategy like in the PSC approach is suitable because soil organic matter mineralization is probably relatively low and soil structure relatively good and therefore plant biomass input is not a priority. However, there are many more cover cropping options than the ones tested in Paper 1 but only limited or no knowledge about them is available and more research needs to be

done. The main question thereby should not be “what is the best cover cropping strategy?” but rather “Which cover cropping strategy can best compensate the negative impacts of a specific crop rotation on soil fertility?”.

For this type of research at the local extent, soil vis–NIR spectroscopy can be very helpful to cope with a high number of samples. However, as also shown by this study, soil organic matter related parameters (SOC, total N, POXC) can be well predicted with vis–NIR spectroscopy but additional measurements like nutrients or microbial properties might also be necessary for the evaluation of cover cropping strategies but are normally less accurately predicted by vis–NIR spectroscopy. These latter properties are quite sensitive to management and even with a small sample size, effects can be identified, hence no added value or additional insights from spectral assessment. The performance of vis–NIR spectroscopy was lower on soils with low carbonate contents and therefore more research is needed to improve the prediction accuracy for these soils. This issue is probably even more important for larger scale spectral libraries with highly varying carbonate contents. I can imagine three possible ways to address this problem but all of them must be first evaluated. First, samples could be pre-treated with acid to remove carbonates before conducting the measurements. A comparison in model performance with acid-treated samples and untreated samples could be a first step in that direction. Second, there might be a possibility to “correct” a reflectance spectrum for its carbonate content as it was e.g. successfully done for the water content by Ji et al. (2015). However, since the important areas for organic carbon and carbonate are overlapping, such correction approach might be challenging. Thirdly, there is still the option to rely on MIR spectroscopy in soils with high carbonate where the prediction of carbonate is much better.

Even though measuring immediate effects of land management on soil properties is very challenging, a big motivation to improve the methodology is the farmer’s perspective. Independent of the management approach that is evaluated, it is more motivating and relevant for farmers, if immediate effects can be measured and communicated. Due to the high sample number, soil vis–NIR spectroscopy might be one piece to improve the methodology to tackle the challenge of short-term effects on soil fertility. If short-term effects can be made better visible, it is easier to set up research projects with a high participation of people working in the agricultural praxis. The participation of practitioners in research projects has been identified as a crucial factor to improve soil fertility (Cheik and Jouquet, 2020) and could potentially be fostered by the implementation of local soil spectroscopy projects. Like farmers, also other stakeholders like the agricultural ministry, food retailers and label organization have an interest in fast evaluation of new measures for sustainable soil management and might therefore be more interested to participate in research projects.



Besides its unquestionable positive effects on soil fertility, cover crops also bring challenges for soil management in the agricultural praxis. Plant or litter material on the soil surface may hinder efficient machine operation. In Paper 3 we did not find any effect of EM on cover crop decomposition and therefore conclude that the decomposition process is probably mainly governed by temperature, soil moisture and the microbial community present in the soil. Especially, the cover crop moisture content (which is determined by prevailing weather conditions) has been shown to be the most crucial factor that determines cover crop decomposition (Thapa et al., 2021). It may be that other microbial inoculants or EM under different experimental conditions might show an effect on cover crop decomposition but, this effect is probably very small. Therefore, I conclude that the method to incorporate cover crops should be chosen according to the crop rotation planning and the prevailing environmental conditions.

## Part II: Research papers

### Paper 1: Cover crops

**Authors:** Simon Oberholzer, Klaus A. Jarosch, Nadine Harder, Markus Steffens and Chinwe Ifejika Speranza

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<https://doi.org/10.1111/ejss.70012>

## RESEARCH ARTICLE

# Cover cropping in organic reduced tillage systems: Maximizing soil cover or plant above ground biomass input?

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**Abstract**

Cover crops are grown between two main crops to reduce periods of bare fallow. In highly diverse crop rotations, the lengths of break periods between two main crops vary highly over time and consequently the cover cropping management differs from year to year. Long-term field trials are thus of limited use because the same cover cropping approach only appears once in several years. This increases the need to better determine the immediate effects of different cover cropping strategies on soil properties. This study evaluated two cover cropping strategies and monitored the temporal development of several soil properties on six fields in Eastern Switzerland in the 9 months period between harvest of winter wheat and sowing of spring crops. The two tested strategies were (a) double cover cropping (DCC) where two cover crops mixtures were grown subsequently and shallowly (3 cm) incorporated into the topsoil and (b) permanent soil cover (PSC) with one grass-clover mixture, which was harvested and thus not incorporated into the soil. Soil samples at three different soil depths (0–5, 5–10 and 10–20 cm) were sampled four times in high spatial resolution and analysed using a combined approach of visible near infrared spectroscopy and conventional lab methods. Differences between the sampling times and field sites were stronger than effects of different treatments. For soil organic carbon (SOC), no significant difference was measured between treatments in 0–20 cm soil depth. Only when analysed per depth segment, the PSC treatment showed significantly higher SOC increase in 5–10 cm soil depth than the DCC treatment. This could be due to the longer soil cover and thereby associated longer root growth period in the PSC treatment, leading to higher below ground C inputs than in the DCC treatment. On the other hand, the DCC treatment showed generally higher increases in permanganate oxidizable carbon stocks (0–5 cm), microbial C (0–10 cm), microbial N (0–10 cm) and mineral N (0–10 cm) than the PSC treatment. We conclude that maximizing

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cover crop above ground biomass input by planting two cover crops (DCC) benefitted soil microorganisms on most fields but was less beneficial on SOC than permanent soil cover (PSC) in 5–10 cm soil depth.

#### KEYWORDS

microbial biomass, regenerative agriculture, shallow incorporation, soil fertility, soil organic matter, soil spectroscopy, temperate climate

## 1 | INTRODUCTION

Soil fertility is crucial for sustainable crop production but is decreasing in arable soils across the world (Lal, 2015). Depletion in soil organic carbon (SOC) is an important driver of this process which has also been observed in Europe (Gubler et al., 2019). The beneficial effects of soil organic matter (SOM) lie in its dynamic nature where short-term formation and mineralization of organic matter influence nutrient availability and crop performance (Hacker et al., 2015; Janzen, 2006). Cover crops are an important element to promote SOM formation in a crop rotation (Jian et al., 2020; Kaye & Quemada, 2017; McClelland et al., 2021; Poepflau & Don, 2015), but region-specific limitations hamper their adoption in Europe (Heller et al., 2024). Cover crops, also referred to as catch crops or intercrops, are sown in the period between two main crops to avoid periods with bare soil. Additionally, cover crops can also be undersown in a main crop to increase the species richness on the field. The major goal of cover cropping is to improve nutrient cycling, avoid nutrient losses, increase SOC stocks, enhance microbial activity, increase soil cover and reduce erosion (Daryanto et al., 2018; Thorup-Kristensen et al., 2003).

While the overall benefits of cover crops are well documented, very little information is available on the effects of different cover cropping strategies on soil properties. Cover cropping strategies differ in terms of species diversity, incorporation method, biomass input and the frequency they are applied in a crop rotation. All these factors are relevant for both the decomposition and the accumulation of organic matter in soil. For example, SOM formation is more efficient when above ground residues were mixed with topsoil than just put on the soil surface (Mitchell et al., 2016, 2018; Sokol et al., 2019).

Several parameters have been suggested to evaluate the performance of cover crops. Since total SOC is a slowly reacting C pool, the analysis of labile C fractions to evaluate the effect of different agricultural management techniques has been recommended (Bongiorno et al., 2019; Wang et al., 2014). Among them, permanganate oxidizable carbon (POXC), also referred as active C, has been shown to be influenced by cover cropping (Jagadamma et al., 2019;

#### Highlights

- Monitoring of two cover cropping strategies in high spatial and temporal resolution
- Permanent soil cover (PSC) strategy increased soil organic carbon in 5–10 cm depth
- Double cover cropping (DCC) increased soil microbial biomass on most fields
- Above ground biomass input in DCC strategy increased mineral N on most fields

Lucas & Weil, 2021). Another fast reacting and management sensitive C pool is soil microbial biomass carbon (Cmic), of which some studies have measured an increase due to cover cropping (Kim et al., 2020). This effect was more pronounced with species mixtures than with single species cover crops (Gentsch et al., 2020). Other studies showed that POXC and Cmic correlate with SOC and therefore suggested them as indicators for SOC development (Bongiorno et al., 2019; Lange, 2015). Besides soil C fractions, cover crops also influence the soil nitrogen (N) cycle, whereby some N fractions are more sensitive to cover cropping than others (Mohammed et al., 2020; Wang et al., 2007). Similar to SOC, total soil N is a slowly reacting N pool and cover crop research focuses mainly on the labile N pools such as mineral N (Nmin) and microbial N (Nmic). Cover crops use Nmin for their growth and can thereby prevent the leaching of some Nmin into deeper soil layers or into ground water (Tonitto et al., 2006). On the other hand, cover crops enhance the uptake of Nmin into the microbial biomass (immobilization) because microbial growth benefits from cover crop's labile C inputs (in't Zandt et al., 2018).

Two main mechanisms explain the beneficial effects of cover crops on soil C and N fractions. First, cover crops increase the organic matter input into the soil. Second, cover crops are used to suppress weed growth, which reduces the need for mechanical weed control and thereby prevents SOC mineralization (Singh et al., 2023). Traditionally, organic farming systems mainly rely on cover crops for increasing organic matter inputs whereas

conservation agriculture systems see the main benefit in the reduction of soil tillage. In both systems, cover crops are well established (Büchi et al., 2017; Hubbard, 2013; Welch et al., 2016). However, the combination of conservation tillage with organic farming remains challenging mainly because of increased weed pressure and reduced yields (Leifeld et al., 2009; Zikeli & Gruber, 2017). In conservation tillage systems, cover crops are often killed with herbicides, roller crimper or by frost periods whereas in organic systems cover crops are normally incorporated by inversion tillage (Alonso-Ayuso et al., 2020; Wayman et al., 2015).

New cover cropping approaches try to combine methods from both organic farming and reduced tillage by shallowly (3 cm) incorporating cover crop mixtures with a rotary tiller. The resulting plant-soil mixture serves as an energy source for the soil microbiome. Labile C inputs enhancing the soil microbiology are a key element for the stabilization of SOM (Cotrufo et al., 2013). Thereby, the microbial by-products and the microbial necromass can play a major role in SOM formation (Kallenbach, 2016; Miltner et al., 2012; Vidal et al., 2021). This shallow incorporation of cover crop mixtures is often used in 'regenerative agriculture' that has gained popularity in agricultural practice in recent years (Giller et al., 2021; Rhodes, 2017), yet, is still not clearly defined.

Most research on the effects of cover crops focuses on the comparison between a cover crop treatment and a bare soil control. However, in Switzerland long-term bare soil periods are not allowed (Swiss Ordinance 910.13, 2013) and cover cropping is widely applied (Heller et al., 2024). Also other European countries try to foster the adoption of cover crops (Kathage et al., 2022). The question on the type of cover cropping strategy and their effects on soil properties will thus become in future more important than whether or not to implement cover crops at all. In Swiss organic reduced tillage systems, two different types of cover cropping are commonly applied in the up to 9 months period between cereal harvest (end of July) and sowing of a next spring crop (April–May). The so-called 'double cover cropping' (DCC) aims to maximize fresh organic matter into the soil by sowing, growing and shallowly incorporating a summer cover crop mixture and a winter cover crop mixture subsequently. The DCC approach is expected to show beneficial effects on soil fertility parameters because it has a high above ground biomass input into the soil that is decomposing in interaction with the soil mineral phase. However, the double shallow incorporation requires shallow but intensive tillage that might increase SOM mineralization in the topsoil. Alternatively, the 'permanent soil cover' (PSC) aims for maximized soil cover and reduced soil tillage. This is achieved by a temporary ley where the above ground biomass can be harvested and used as forage. The same effect

can also be achieved by undersowing a cover crop with grasses and clover in the cereal stand and use it as a temporary ley after the cereal harvest. In contrast to DCC, the PSC approach does not have any above ground biomass input into the soil but also no disturbance.

Given the increasing implementation of cover cropping, it becomes more and more relevant to evaluate the effects of these different strategies as management options on soil fertility. We thus monitored the immediate effects of the DCC and the PSC approach on soil C and N fractions at three different soil depths (0–5, 5–10 and 10–20 cm) over a period of 9 months in six fields in Switzerland. In highly diversified crop rotations, a long fallow period that is suitable for either the DCC or PSC cover cropping approach appears only once within several years. For this reason, the effects of these cover cropping approaches cannot be evaluated in experiments that span over several cropping seasons, as their immediate effects would be covered by any other crop or management effect. We thus took soil samples in high spatial and temporal resolution using a combination of near infrared spectroscopy and conventional lab methods to enable detection of small changes in the analysed parameters. This was done to achieve a better understanding of the effects of either maximizing cover crop biomass input (DCC) or soil cover (PSC) on soil fertility using cover crops. We formulated three hypotheses:

1. Given the short time period of the experiment, SOC and total N will not significantly differ between the two treatments.
2. The DCC treatment with above ground biomass input will show higher labile C and N (POXC and N<sub>min</sub>), compared to the PSC treatment with no such above ground biomass input.
3. The DCC treatment with above ground biomass input will promote the soil microbial biomass (C<sub>mic</sub> and N<sub>mic</sub>), compared to the PSC treatment with no such above ground biomass input.

## 2 | METHODS

### 2.1 | Study sites and experimental set-up

The trial was conducted on six agricultural fields in the canton of Thurgau, Switzerland (Table 1). All fields were at maximum 12 km apart from each other. In 2019, the mean temperature in the region was 10.8°C and total annual precipitation summed up to 815 mm, which was a bit warmer and drier than the long-term average (1991–2020) of 8.7°C and 853 mm. The trial comprised the period of 9 months between cereal harvest at the end of July and sowing of a cash crop in late spring (Figure 1). Before the onset of the

TABLE 1 Description of the trial sites.

Field	Elevation (m a. s. l.)	Trial area (ha)	Soil class (world reference base)	Soil texture (% of sand/silt/clay)	pH (CaCl <sub>2</sub> )	Crop rotation (4 years before trial)	Last ploughing (year)	Fertilization
A	420	0.84	Eutric Cambisol	50/29/21 Sandy loam	7.18	2015: Temporary ley 2016: Celeriac 2017: Rye 2018: Potato 2019: Winter wheat	2012	None
B	420	0.67	Eutric Cambisol	44/35/20 Sandy loam	6.56	2015: Potato 2016: Dwarf beans 2017: Temporary ley 2018: Corn 2019: Rye	2016	Processed organic fertilizer (Bio-Enne, Timac Agro, Switzerland) N: 72 kg ha <sup>-1</sup> C: 210 kg ha <sup>-1</sup> Applied: 27.04.2020
C	600	0.44	Eutric Cambisol	27/35/38 Clay loam	7.19	2015: Sugar beet 2016: Winter wheat 2017: Temporary ley 2018: Temporary ley 2019: Winter wheat	2015	None
D	460	0.64	Eutric Cambisol	28/44/28 Clay loam	6.88	2015: Oat 2016: Spelt 2017: Field beans 2018: Red clover 2019: Winter wheat	2018	Chicken manure N: 112 kg ha <sup>-1</sup> C: 740 kg ha <sup>-1</sup> Applied: 01.04.2020
E	460	1.05	Eutric Cambisol	30/48/23 Sandy loam	6.6	2015: Spelt 2016: Dwarf bean and peas 2017: Winter wheat 2018: Linen 2019: Winter wheat	2018	None
F	380	0.3	Eutric Cambisol	39/43/18 Sandy loam	7.49	2015: Winter wheat 2016: Sugar beet 2017: Corn 2018: Potato 2019: Winter wheat	2017	None

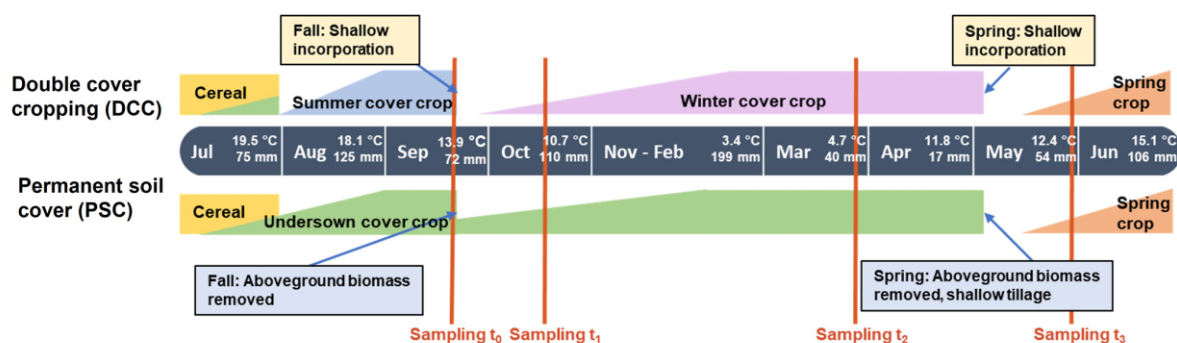


FIGURE 1 Timeline for the two treatments double cover cropping (DCC) and permanent soil cover (PSC). For every month the average temperature and total precipitation are indicated.

trial, every field was planted with winter cereal and an undersown cover crop called GreenCarbonFix that was purchased at Camena Samen (Germany) and contained six species: 55% perennial ryegrass (*Lolium perenne* L.), 25% crimson clover (*Trifolium incarnatum* L.), 5% white clover (*Trifolium repens* L.), 5% hop clover (*Medicago lupulina* L.), 5% bird's-foot trefoil (*Lotus corniculatus* L.) and 5% camelina (*Camelina sativa* L.). After the cereal harvest in July each field was divided into a PSC plot in the middle and two DCC plots on both sides. Plot sizes were between 1000 and 3500 m<sup>2</sup>. Each plot comprised 13 GPS-referenced sampling points (circles with a radius of 1 m) that were homogeneously distributed across the plot in an unaligned design (Webster & Lark, 2013). The results of the two DCC plots (26 subplots) were combined and referred here as DCC plot. The unequal sample number for each treatment was accounted for in all statistical analyses (see Section 2.7). The management was conducted by the farmers and therefore we used a strip design and not a randomized block design which would have made the machine handling very complicated. In the DCC plots, two commercial cover crop mixtures were sown subsequently (Figure 1). The summer cover crop mixture (Dominanzgemenge; Camena Samen) was sown after cereal harvest (end of July) and comprised 12 species: 20% buck wheat (*Fagopyrum esculentum* MOENCH), 20% flax (*Linum usitatissimum* L.), 20% serradella (*Ornithopus sativus* BROT.), 8% corn (*Zea mays* L.), 7% sunflower (*Helianthus annuus* L.), 5% bristle oat (*Avena strigosa* SCHREB.), 5% camolina (*Camelina sativa* L.), 4% winter oilseed rape (*Brassica napus* L.), 4% white mustard (*Sinapis alba* L.), 3% deeptill radish (*Raphanus sativus* var. *oleiformis*), 2% sudan grass (*Sorghum sudanense* STEUD.), 2% lacy phacelia (*Phacelia tanacetifolia* BENTH.). After the shallow incorporation of the fall cover crop in September, a frost tolerant winter cover crop mixture (Wintergrün, Camena Samen) was sown that contained five species: 62% winter rye (*Secale cereale* L.), 26% Hungarian vetch (*Vicia*

*pannonica* CRANTZ.), 10% crimson clover (*Trifolium incarnatum* L.), 1% winter turnip rape (*Brassica rapa* L.). The winter cover crop was shallowly incorporated at the end of April or beginning of May. The shallow incorporation was done each time with a rotary tiller with right-angled knives that cut the plants 3 cm below the soil surface. The result was a plant soil mixture on the surface that was left on the soil for 10 days. After that the soil surface was again treated with a rotary tiller and the winter cover crop, respectively the spring cash crop, was sown. In the PSC plot the GreenCarbonFix mixture undersown in the cereal was kept and further on managed equal to a temporary ley. In fall, when the cover crop in the DCC plot was incorporated, the PSC plot was mowed and the above ground biomass was removed from the field. In spring, when the winter cover crop on the DCC plot was incorporated, the PSC plot was mowed again, and the stubbles were incorporated the same way as in the DCC plot using a rotary tiller. The exact dates and management details of the four sampling times are provided in Table S.1 and an overview about the used cover crop mixtures is provided in Table S.2. For the fields E and F, soil sampling had to be reduced to three time points because of management issues with the seedbed preparation in these two fields. Consequently, those two fields were ploughed in spring and therefore the soil sampling before the incorporation of spring cover crop ( $t_2$ ) was the last sampling time on these two fields. All cover crops were grown without any fertilizer and under organic farming conditions. Yet, on fields B and D for the spring cash crop, an organic fertilizer was applied after cover crop incorporation between  $t_2$  and  $t_3$ . Same amounts of fertilizers were applied on both treatment plots. The C and N inputs from fertilization can be seen in Table 1. Right before the cover crops were incorporated with a rotary tiller (DCC in fall, DCC and PSC in

spring) 100 L ha<sup>-1</sup> commercially purchased effective microorganisms (EM; Rottelenker, EM Schweiz AG, Switzerland) were sprayed on the cover crops. The objective of this measure is to improve the decomposition process and reduce C and N losses (Oberholzer, Herrmann, et al., 2024). This practice is commonly used by farmers in the region when they shallowly incorporate a cover crop and was therefore part of both cover cropping systems.

## 2.2 | Plant biomass sampling

In the DCC plots cover crop biomass was cut right before cover crop incorporation in a square of 50 × 50 cm with seven replications per field and subsequently dried at 65°C for 48 h to determine the dry weight. The sampling replication with the median weight was ground and analysed for C and N content by dry combustion (vario MICRO tube, Elementar, Germany), separately for each field. The concentrations of plant C and N were multiplied by the dry matter weight to obtain the cover crop C and N input.

## 2.3 | Soil sampling and sample treatment

Soil sampling was done before incorporation of the fall cover crop ( $t_0$ , September), about 4 weeks after the shallow incorporation ( $t_1$ , October), in early spring ( $t_2$ , March) and about 4 weeks after the incorporation of the spring cover crop ( $t_3$ , May; Table S.1 in the Supplementary Material). At every sampling time, three batches of soil samples were obtained for different analyses. Batch one to determine SOC, POXC and total N was sampled by taking five samples per sampling point using an auger (0–20 cm, 2 cm diameter) and subsequently separated per depth segment of 0–5, 5–10 and 10–20 cm. The GPS reference for each sampling point was done using a dGPS device (Geo7X, Trimble, USA) with an approximate measurement accuracy of 10 cm, allowing for point specific monitoring of soil properties over time. In total, six fields with each three plots (two DCC, one PSC), each with 13 sampling points, were sampled in three depths at four (field A, B, C and D) respectively three (field E and F) sampling times which resulted in a total number of 2574 soil samples. These samples were dried for 72 h or constant weight at 40°C and sieved to 2 mm. Batch two to determine Cmic, Nmic and Nmin was obtained by randomly sampling 15 subsamples in 0–10 cm depth in four replicates per plot and sampling time ( $n = 264$ ). Samples were stored at 4°C and sieved to 2 mm before the analysis of Cmic and Nmic.

Thereof a part of sieved soil was frozen at –20°C for the analysis of Nmin. Batch three to determine soil bulk density and soil water content was obtained by sampling three undisturbed soil cores per plot and sampling time from 0 to 20 cm with 5 cm diameter that were taken with an impact probe (HumaxTube®, Switzerland). These cores were cut into 5 cm segments, weighed and dried at 105°C for at least 48 h to assess soil bulk density and water content for each 5 cm layer ( $n = 792$ ).

## 2.4 | Spectral measurement and modelling

All 2574 samples of batch one were measured with a vis-NIR spectrometer (350–2500 nm, ASD FieldSpec 4 Hi-Res, Malvern Panalytical, USA) in five replicates using a contact probe in a dark room. We treated the samples from each field as one individual dataset ( $n = 468$  for fields A, B, C and D and  $n = 351$  for fields E and F) for the spectral modelling. For every field 15% of the samples were selected as reference samples for wet chemistry analysis based on a Kennard-Stones algorithm that uses the principal component scores to select a representative subset of a given dataset (Wadoux, 2021). Therein, sampling times and soil depth were similarly represented. For each parameter (SOC, POXC and total N) and for every field a spectral model was calibrated with the reference samples to predict the values for the other samples. For every spectral model we selected the optimal preprocessing technique and applied a partial least square regression (PLSR; Wold et al., 1983). A five times repeated fivefold cross-validation approach was used to calibrate for a spectral model for each field and soil property. We evaluated the model performance using the three model performance parameters, coefficient of determination ( $R^2$ ), root mean standard error (RMSE) and the ratio of performance to deviation (RPD) which is the ratio of standard deviation of the measured reference values to RMSE. According to Chang et al. (2001) and Zhang et al. (2018) we considered an RPD above 3 as excellent, above 2 as accurate, above 1.4 as approximate and below 1.4 as poor model performance. The RMSE has always the unit of the measured parameter and therefore does not allow a generalized evaluation scheme. The executed preprocessing steps and the accuracy of the final chosen model for SOC, POXC and total N can be found in Table S.3 in the Supplementary Material. Spectral models for SOC and POXC on fields A and F showed an approximative performance while all other models showed an accurate or even excellent performance. The slightly lower model performance of fields A and F can probably be explained by their higher carbonate content (see Oberholzer, Summerauer, et al. (2024)). The RMSE



ranged between 1.07 and 2.43 g kg<sup>-1</sup> for SOC, 0.03 and 0.05 g kg<sup>-1</sup> for POXC and between 0.09 and 0.14 for total N. These achieved RMSE from spectral models were relatively close to the lab measurement errors that were 1.01 ± 0.40 g kg<sup>-1</sup> for SOC, 0.02 ± 0.01 g kg<sup>-1</sup> for POXC and 0.07 ± 0.02 g kg<sup>-1</sup> for total N (Oberholzer, Summerauer, et al., 2024).

## 2.5 | Chemical soil analyses

For the reference samples of batch one ( $n = 386 \triangleq 15\%$  of all samples) concentrations of total C and total N were determined by dry combustion (vario MICRO tube, Elementar, Germany). Inorganic C was determined through dissolution of carbonate in 10% HCl-solution and measurement of the volume of the evolved CO<sub>2</sub>, and SOC as the difference between total C and inorganic C. POXC was measured based on the protocol of Weil et al. (2003) with the modifications of Lucas and Weil (2012), where 2.5 g instead of 5 g soil were used to make sure that enough reactant (0.2 M KMnO<sub>4</sub>) is available (Culman et al., 2012; Lucas & Weil, 2012).

Cmic and Nmic were measured based on the protocol of Vance et al. (1987) with some adaptations: We weighed moist soil equal to 10 g dry matter and used 40 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub>. After the extraction, dissolved C and N were measured with a TOC-analyser (DIMATOC<sup>®</sup> 2100, DIMATEC Analysetechnik GmbH, Germany). We did not use any conversion factor and report Cmic and Nmic as chloroform labile C and N. For the measurement of Nmin as the sum of nitrate and ammonium, 4 g of soil were extracted with 40 mL 1 M KCl. Nitrate was determined by using vanadium (III) as a reductant according to the Protocol of Garcia-Robledo et al. (2014). Nitrate content in the solution was colorimetrically determined by measuring the absorbance at 540 nm with a Spectrophotometer (UV-1800, Shimadzu Corporation, Japan). Ammonium was determined as described in Rhine et al. (1998) with salicylate as a reactant. The ammonium absorbance was measured at 650 nm with the same spectrophotometer.

## 2.6 | Calculation of soil organic carbon, permanganate oxidizable carbon and total N stocks

Due to seasonal and management induced changes in soil bulk density over the nine-month period of the trial, we used an equivalent soil mass (ESM) approach to calculate stocks and stock changes of SOC, POXC and total N. The concept of ESM was introduced by Ellert and Bettany (1995) and evaluated by Lee et al. (2009). When the soil

bulk density varies over time and between treatments, stocks of a fixed depth (FD) contain different soil masses, which makes a comparison between them uneven. The ESM approach uses a reference soil mass that is used for a correction to obtain stocks of same soil masses among all treatments and sampling times. We used here the minimum ESM approach (Lee et al., 2009) and used the sampling time with the lowest bulk density to set the minimum reference soil mass. The SOC, POXC and total N stocks of the other sampling times were accordingly adjusted to an equivalent soil mass. For every soil layer (i) the FD stock was calculated as:

$$C_{i, fixed} = conc_i * M_i$$

where  $conc_i$  is the concentration and  $M_i$  the dry soil mass of the corresponding layer. Then for every soil layer the surplus soil mass ( $M_{i, add}$ ) was calculated:

$$M_{i, add} = M_i - M_{i, equiv}$$

where  $M_{i, equiv}$  is the equivalent or reference soil mass of the corresponding layer. The stocks of the first soil layer (0–5 cm) were obtained by subtracting the surplus soil mass times the concentration in 0–5 cm:

$$C_{0-5} = C_{0-5, fixed} - M_{0-5, add} * conc_{0-5}$$

For the 5–10 cm layer ESM stocks were obtained by adding the surplus stock of the 0–5 cm layer and deducting the surplus soil masses from 0–5 and 5–10 cm times the concentration of the 5–10 cm layer:

$$C_{5-10} = C_{5-10, fixed} + M_{0-5, add} * conc_{0-5} - (M_{0-5, add} + M_{5-10, add}) * conc_{5-10}$$

Accordingly, the calculation was also done for the 10–20 cm layer. At the end there remains a soil mass that is unaccounted and must be dropped to obtain an ESM.

We calculated stocks based on the ESM approach for SOC, POXC and total N. We did not calculate stocks for Cmic, Nmin and Nmic because we measured them only in one depth (0–10 cm).

## 2.7 | Data evaluation and statistics

### 2.7.1 | Field specific evaluation

The objective of this study was to assess the influence of two cover cropping strategies on soil parameters. We thus do not focus much on absolute values but rather on the changes of these values over time. For SOC, POXC and

total N, we subtracted the measured values of sampling  $t_0$  from the values of sampling  $t_1$ ,  $t_2$  and  $t_3$  to obtain a stock change for every GPS-referenced sampling point and sampling time  $t_1$ ,  $t_2$  and  $t_3$ . Since samples for Cmic, Nmin and Nmic were not GPS referenced, we subtracted the mean of sampling  $t_0$  from concentration values of samplings  $t_1$ ,  $t_2$  and  $t_3$ . These changes relative to sampling  $t_0$  showed for all data a normal distribution or could be transformed to fulfil the requirement of normality with  $\log(x)$ ,  $\sqrt{x}$  or  $1/x$ . Since we had an unequal sample size, we used the Levene's-test to check for equal variances. To detect significant differences in changes between treatments a Welch two-sample  $t$ -test was applied for every field and sampling time separately.

To test the changes over time within one treatment we applied a multiple pairwise comparison using a paired  $t$ -test for the GPS referenced samples (SOC, POXC, total N). When the different sampling times of the samples of batch two (Cmic, Nmin and Nmic) were combined per field, the data often could not be transformed to a normal distribution. For these samples we therefore used the non-parametric Kruskal–Wallis test followed by a Dunn's post hoc test to detect significant changes over time within one treatment. For both, the multiple  $t$ -test as well as the Dunn's test, we used the Holm method to correct for multiple pairwise comparisons.

### 2.7.2 | Statistics across fields

To test the treatment influence in different soil depths, we took the changes in SOC, POXC and total N between  $t_0$  and  $t_3$  for field A, B, C and D and applied a general mixed model with treatment and soil depth as fixed factors and field as random factor.

We related the changes between  $t_0$  and  $t_1$  to the fall cover crop input and the changes between  $t_2$  and  $t_3$  to the spring cover crop input to analyse the relationship between C inputs and changes in SOC, POXC and Cmic as well as N inputs and changes in total N, Nmin and Nmic. For this analysis we only considered the data from the DCC plots since the PSC plots did not have any above ground input. For field C and D, we added the fertilizer C and N input to the spring cover crop input.

All analyses were performed in R version 4.0.3 (R Core Team, 2020). The spectral datasets were analysed using the R-package `simplerspec` (Baumann, 2019) in combination with the packages `prospectr` (Stevens & Ramirez-Lopez, 2020) and `caret` (Kuhn, 2020). In the figures and in the text means and standard errors are presented.

## 3 | RESULTS

### 3.1 | Cover crop performance

Cover crop above ground biomass showed large differences between the six fields in the DCC treatment. In Figures 2 and 3, fields are therefore ordered according to total cover crop biomass in the DCC treatment (fall and spring) whereby field A had the highest ( $716 \text{ g m}^{-2}$ ) and field F the lowest ( $102 \text{ g m}^{-2}$ ) cover crop biomass produced in the entire duration of the trial. The cover crop C content ranged between 38% and 42% and the N content between 1.8% and 3.1%. All figures that show changes in the selected parameters also indicate the cover crop C or N inputs for each field.

### 3.2 | Changes in soil C fractions (SOC, POXC, Cmic)

Soil organic carbon stocks ranged from  $4.2 \pm 0.1$  (Field E) to  $8.2 \pm 0.2 \text{ kg m}^{-2}$  (Field F) at  $t_0$  and on each field, the changes over time in 0–20 cm soil depth were quite similar between the DCC and PSC treatment (Figure 2a). The only significant difference between treatments was observed on field F for  $t_1$  where the PSC treatment showed significantly higher increases in SOC stocks than the DCC treatment. On every field we determined significant differences in SOC stocks over time in at least one treatment. At the end of the nine-month trial, the maximum increase in SOC stocks over time in 0–20 cm soil depth was measured on the PSC plot of field A with  $+0.46 \pm 0.06 \text{ kg m}^{-2}$ . The maximum decrease in SOC stocks was measured on the DCC plot on field E at  $t_1$  ( $-0.38 \pm 0.05 \text{ kg m}^{-2}$ ). In relative terms, SOC stocks changed between  $-8.5 \pm 1.1\%$  (DCC field E,  $t_1$ ) and  $+8.3 \pm 1.0\%$  (PSC field A,  $t_2$ ) over the monitoring period of 9 months in 0–20 cm soil depth.

At the start of the experiment ( $t_0$ ), POXC stocks ranged between  $181 \pm 8 \text{ g m}^{-2}$  (Field E) and  $225 \pm 8 \text{ g m}^{-2}$  (Field C) and only a few differences between PSC and DCC treatment were measured over time (Figure 2b). The DCC treatment exceeded the PSC treatment significantly on field C at  $t_2$  and on field D at  $t_1$  (Figure 2b). On the other hand, the PSC treatment on field F showed significantly higher changes in POXC stocks than the DCC treatment at  $t_1$ . On all fields, the POXC stocks in the DCC treatment were significantly higher at the last sampling time than at  $t_0$ . For the PSC treatment, only on field B, the POXC did not significantly increase during the trial while all other fields showed significantly higher POXC stocks at the last sampling time compared to  $t_0$ . The maximum significant increase in POXC stocks over time was  $+18.9 \pm 2.1 \text{ g m}^{-2}$  (PSC field F,  $t_2$ ), which corresponds to a relative increase of  $+10.4 \pm 1.2\%$ .

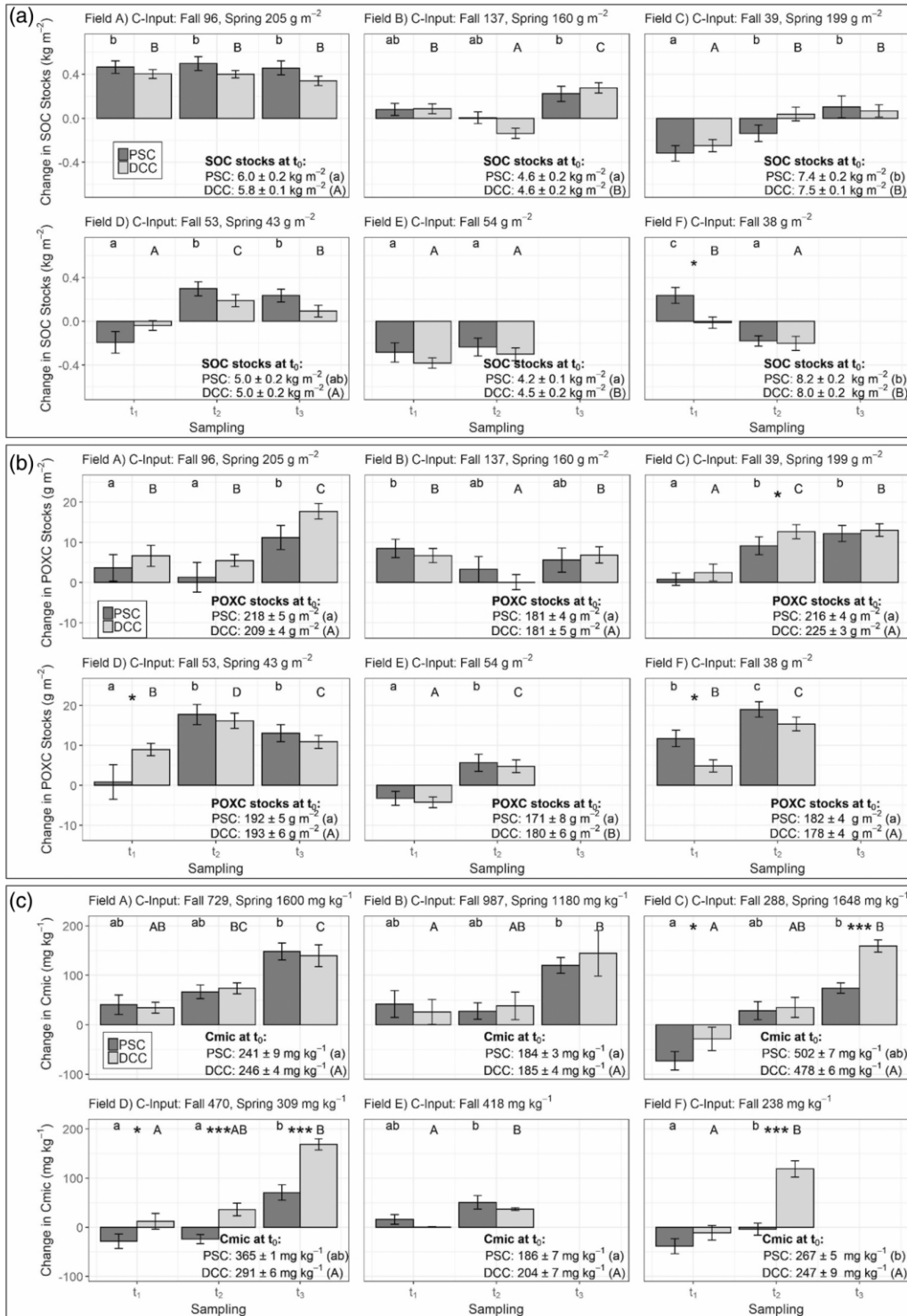


FIGURE 2 Legend on next page.

Microbial C ranged from  $184 \pm 3 \text{ mg kg}^{-1}$  (Field B) to  $502 \pm 7 \text{ mg kg}^{-1}$  at  $t_0$  and increased over time reaching highest values in all treatments at the last sampling time (Figure 2c). On fields C, D and F, microbial C was at least at one sampling time significantly higher in the DCC than in PSC treatment. At the end of the trial on field C, D and F the changes in  $C_{mic}$  in the DCC plots exceeded the PSC plots significantly by  $85 \pm 23$ ,  $97 \pm 27$  and  $123 \pm 29 \text{ mg kg}^{-1}$  which corresponds to a relative increase of  $+17.8 \pm 4.5$ ,  $+33.6 \pm 9.3$  and  $+49.6 \pm 11.5\%$  compared to  $t_0$ .

### 3.3 | Changes in soil N fractions (total N, $N_{min}$ and $N_{mic}$ )

Soil N stocks ranged from  $496 \pm 19 \text{ g m}^{-2}$  (Field E) to  $734 \pm 6 \text{ g m}^{-2}$  (Field C) at  $t_0$  and the development of total N stocks over time was very distinct on the different fields and did not show a clear pattern. Only at two time points significant differences in changes of total N stocks (0–20 cm) between the two treatments were observed ( $t_1$  on field D and F; Figure 3a). Compared to  $t_0$ , total N stocks varied between  $-7.6 \pm 1.1\%$  (PSC field E,  $t_2$ ) and  $+7.3 \pm 1.0\%$  (PSC field F,  $t_1$ ).

Mineral N ranged from  $55 \pm 7 \text{ mg kg}^{-1}$  to  $112 \pm 5 \text{ mg kg}^{-1}$  at  $t_0$  and showed on most fields a higher increase in the DCC treatment. On fields A, B, D and F we observed at least at one sampling time significantly higher  $N_{min}$  changes in the DCC treatment with highest differences in spring ( $t_3$ ) where the  $N_{min}$  changes in the DCC plots of fields A, B and D exceeded the changes in the PSC plots by  $+19 \pm 10$ ,  $+11 \pm 9$  and  $+18 \pm 6 \text{ mg kg}^{-1}$  (Figure 3b). On all fields  $N_{min}$  decreased from  $t_0$  to  $t_2$  after winter between  $-21 \pm 17\%$  (PSC plot field C) and  $-77 \pm 10\%$  (DCC plot field E) compared to  $t_0$ . The ratio between nitrate-N and ammonium-N did not show a treatment effect but varied substantially over time and was for all fields highest in fall at  $t_0$  or  $t_1$  (between 9 and 15) and lowest in spring at  $t_2$  or  $t_3$  (between 0.5 and 6; Figure S.1 in the Supplementary Material).

Similar to  $N_{min}$ ,  $N_{mic}$  showed similarly large differences between treatments at several time points on all fields except field E (Figure 3c). Highest differences were measured at  $t_3$  where changes in the DCC plots significantly exceeded the changes in the PSC plot on field B, C

and D by  $+25 \pm 15$ ,  $+29 \pm 7$  and  $+25 \pm 8 \text{ mg kg}^{-1}$ . This corresponds to percental increases of  $+63 \pm 29$ ,  $+102 \pm 12$  and  $+97 \pm 8\%$  in  $N_{mic}$  compared to  $t_0$  for fields B, C and D, respectively.

### 3.4 | Changes in SOC, POXC and total N in different soil depths

During the experimental period of 9 months, SOC, POXC and total N generally showed an increasing trend, yet depth-specific differences (Figure 4, fields A–D only). In particular, the 5–10 cm depth segment always showed the highest increases, compared to the 0–5 cm and the 10–20 cm depth segments (Figure 4). In 5–10 cm soil depth, the PSC treatment showed significantly higher increases in SOC (but not POXC or total N) than the DCC treatment ( $p = 0.026$ ). On the other hand, in depth 0–5 cm the DCC treatment showed significantly higher increases in POXC stocks ( $p = 0.037$ ) compared to the PSC treatment. The absolute stocks for SOC, POXC and total N per depth segment and sampling time can be seen in Table S4 in the Supplementary Material. The same analysis applied on concentrations instead of stocks obtained the same results (see Figure S.2 in the Supplementary Material).

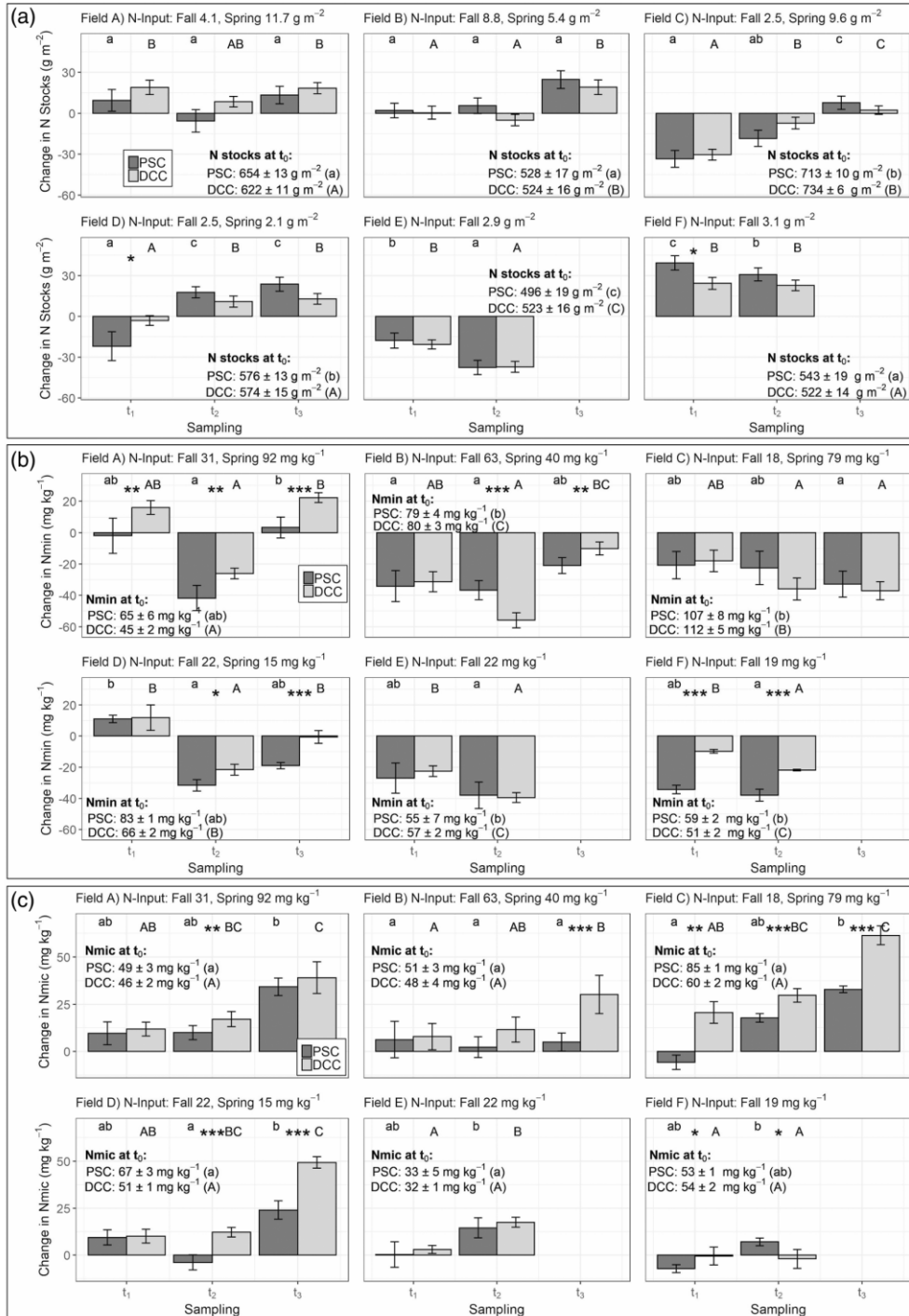
### 3.5 | Relationship between C and N input by double cover cropping and soil C and N fractions

There was a significant linear relationship between above ground cover crop plus fertilizer C or N input by the two cover crop incorporations in the DCC treatment and changes in  $C_{mic}$  or  $N_{mic}$  (Figure 5). Parameters SOC, POXC, total N and  $N_{min}$  did not show a significant relationship with above ground C or N inputs (data not shown).

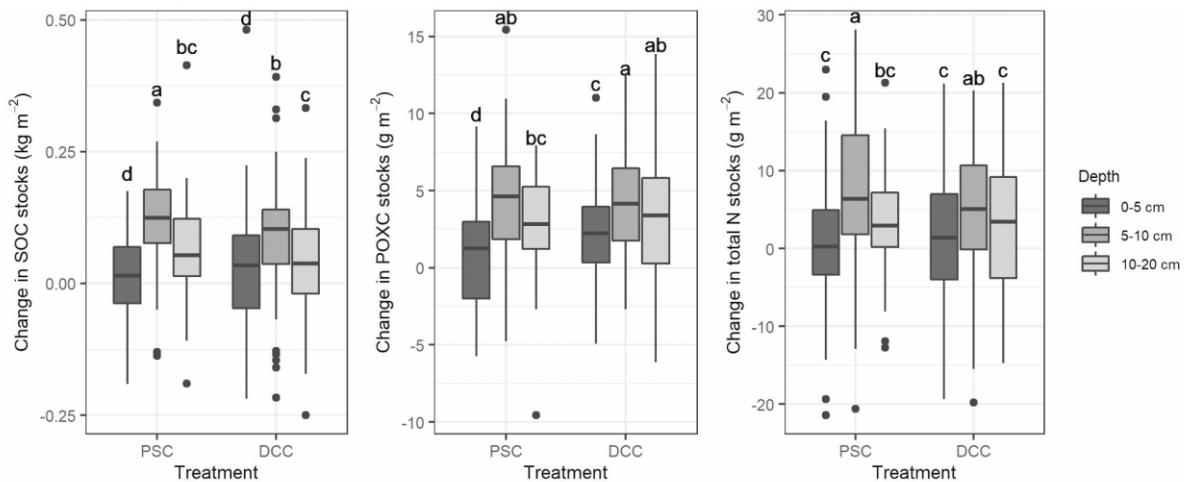
## 4 | DISCUSSION

Cover crop growth on the six fields showed a high variability and reflects the difficulties to predict nitrogen dynamics under organic farming conditions with no mineral N fertilization. All cover crops were grown without

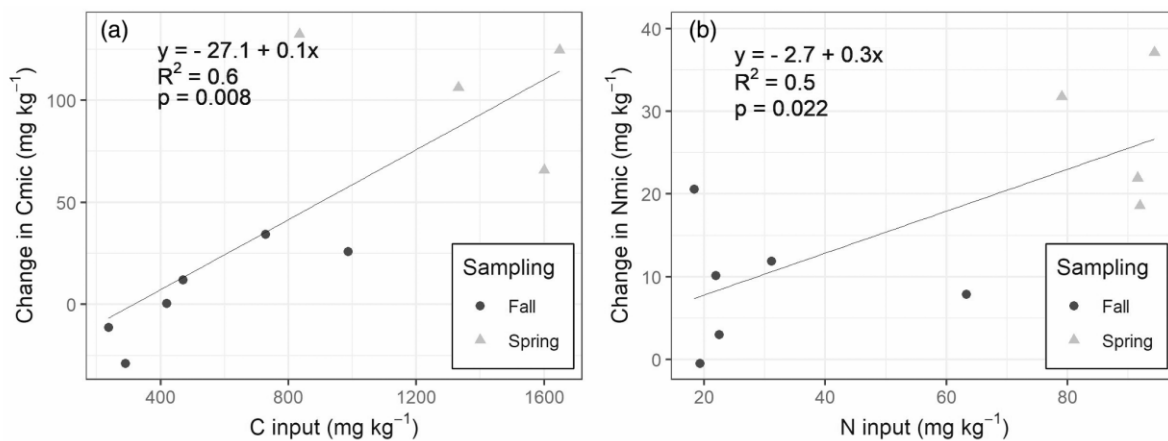
**FIGURE 2** Changes in soil organic C stocks (SOC, 0–20 cm, a), permanganate oxidizable C stocks (POXC, 0–20 cm, b) and microbial biomass C ( $C_{mic}$ , 0–10 cm, c) over time relative to sampling  $t_0$ , which is listed for each field in the subplots. For every field A–F, the above ground cover crop C input in the double cover cropping (DCC) treatment is given in the title. Within each field, significant differences between treatments were tested with a *t*-test and are indicated with the codes: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. Significant changes over time within each treatment are indicated with letters for both treatments separately and were tested with a paired *t*-test for SOC and POXC and with a Kruskal–Wallis-test for  $C_{mic}$ . Error bars represent standard errors.



**FIGURE 3** Changes in total N stocks (0–20 cm, a), mineral N (Nmin, 0–10 cm, b) and microbial biomass N (Nmic, 0–10 cm, c) over time relative to sampling t<sub>0</sub>, which is listed for each field in the subplots. For every field A–F, the above ground cover crop N input in the double cover cropping (DCC) plot is given in the title. Within each field, significant differences between treatments were tested with a *t*-test and are indicated with the codes: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. Significant changes over time within each treatment are indicated with letters for both treatments separately and were tested with a paired *t*-test for total N and with a Kruskal–Wallis-test for Nmin and Nmic. Error bars represent standard errors.



**FIGURE 4** Boxplot of changes in soil organic C (SOC) stocks, permanganate oxidizable C (POXC) stocks and total N stocks per depth and treatment between sampling  $t_0$  and  $t_3$  (only fields A–D). The values from segment 10–20 cm were divided by 2 to reach equal layer thickness. Different letters indicate significant differences between depth: treatment combinations according to the mixed linear model with fixed factors treatment and depth and random factor field.



**FIGURE 5** Correlation of organic C and N inputs (cover crop + fertilization) with changes in microbial biomass C (Cmic, a) and N (Nmic, b) in the double cover cropping treatment. Changes are calculated for the cover crop incorporation in fall (between sampling  $t_0$  and  $t_1$ ) and in spring (between sampling  $t_2$  and  $t_3$ ).

any starter fertilization which resulted in poor cover crop growth on fields E and F with lowest initial Nmin concentration (Figure 3c). Despite the variability, the following general trends were observed between and within the two cover cropping strategies.

#### 4.1 | High short-term temporal variability

We observed significant differences between the two cover cropping strategies in Cmic, Nmin and Nmic at multiple

time points, but only at very rare occasions in SOC, POXC and total N in 0–20 cm soil depth (Figures 2 and 3). Due to the high spatial and temporal variability, SOC, POXC and total N did not show consistent effects that could be attributed to cover crop management or above ground biomass input. However, we determined a few significant but non-consistent changes over time for SOC and total N. Changes in SOC stocks in 0–20 cm soil depth ranged between  $-0.38 \pm 0.05 \text{ kg m}^{-2}$  and  $+0.46 \pm 0.06 \text{ kg m}^{-2}$  in both treatments established during the 9 months cover cropping period. This latter number is much larger than the estimated annual C sequestration potential of cover

cropping of  $0.02$  to  $0.06 \text{ kg m}^{-2} \text{ yr}^{-1}$  in the latest literature (Jian et al., 2020; McClelland et al., 2021; Poeplau & Don, 2015). The difference may be explained by the fact that we looked here into the immediate changes in SOC that is induced by different cover cropping methods, while the cited meta-analyses only considered SOC changes from longer-term trials. Therefore, short-term changes should not be used to deduce long-term C-sequestration rates. Nevertheless, the relative changes in SOC stocks between  $t_0$  and other sampling times ranging between  $-8.5 \pm 1.1\%$  and  $8.3 \pm 1.0\%$  agree with a study reporting SOC to vary up to 15% around the annual mean (Wuest, 2014). The temporal variability of total N (between  $-7.6 \pm 1.1\%$  and  $+7.3 \pm 1.0\%$ ) was very similar to the one of SOC. This is plausible, because both parameters are strongly connected with soil organic matter dynamics. We can confirm our first hypothesis because for the full sampled soil depth (0–20 cm), we did not measure consistent differences between the two treatments. On the other hand, POXC showed a consistent and significant increase in most fields and time points and both treatments over time (Figure 2b). The maximum changes in POXC stocks between two sampling dates were  $+18.9 \pm 2.1 \text{ g m}^{-2}$  which corresponds to a concentration change of  $+72.7 \pm 8.1 \text{ mg kg}^{-1}$  (assumed bulk density =  $1.3 \text{ g cm}^{-3}$ ) were in the same range as the maximum changes of POXC concentrations in Lucas and Weil (2021) after a two-year cover cropping period. However, in a soil depth of 0–20 cm we did not observe consistent treatment effects on POXC, which does not confirm our second hypothesis of higher POXC stocks in the DCC treatment due to above ground biomass input.

#### 4.2 | Relating changes in soil C fractions with C input

The maximum above ground C input of both cover crops in the DCC treatment of around  $300 \text{ g m}^{-2}$  (field A) was in the same range as the maximum changes in SOC stocks and around 15 times higher than the maximal changes in POXC stocks (Figure 2a,b). We did not find any relationship between above ground biomass C input and SOC or POXC stock changes. This suggests that most C input by incorporated cover crops was quickly used by soil microorganisms as also indicated by the strong relationship between C inputs of cover crop biomass and changes in  $C_{mic}$  (Figure 5). The consistent increase in POXC stocks with cover cropping was also observed by Burke et al. (2019) and was probably related to active cover crop root growth and not to above ground biomass input.

POXC is often seen as a sensitive indicator for agricultural management and changes in POXC are sometimes considered as an indicator for changes in SOC

(Bongiorno et al., 2019; Jagadamma et al., 2019). We found significant linear relationships between SOC and POXC concentrations ( $0.23 \leq R^2 \leq 0.85$ ,  $p < 0.001$ ) on every field (Figure 6a) and for fields B, C, D and E also a significant positive relationship between changes in POXC and SOC stocks ( $0.11 \leq R^2 \leq 0.59$ ,  $p < 0.001$ ; Figure 6b). However, the relationship between changes in POXC and SOC stocks is relatively weak indicating that in the short-term these two C fractions can react quite independent from one another.

#### 4.3 | Differences between treatments in different soil depths

Taking the fields with four sampling times (A–D) together we found significantly higher changes in SOC, POXC in 5–10 cm soil depth compared to 0–5 cm soil depth in both treatments (Figure 4). We see three potential mechanisms that might explain why the highest increase in SOC was measured for both treatments in 5–10 cm and not in 0–5 cm depth. First, the 0–5 cm soil depth was also the layer that was intensively tilled in fall and spring for the DCC and only in spring for the PSC treatment. Despite the shallow tillage depth of around 3 cm, one must be aware that a rotary tiller is a very intensive tillage method since it cuts the cover crop plants below ground with a speed of around 500 revolutions per minute and therefore potentially broke soil aggregates (Li et al., 2023) which could have led to C loss due to increased microbial respiration. This intensive tillage in the 0–5 cm soil depth was likely a main driver why, despite higher organic matter input, lower accumulation rates of SOC and POXC were observed than in the below layer of 5–10 cm soil depth. A second explanation might be that the lower C saturation in the 5–10 cm layer fostered C accumulation more compared to the top 0–5 cm where SOC concentrations were already higher. Thirdly, and in relation to that, dissolved organic C (DOC) might have leached from the topsoil and absorbed in the 5–10 cm layer.

Looking at each depth segment separately, we also found significant treatment effects for SOC and POXC: in the PSC treatment, we found significant higher increases in SOC stocks in 5–10 cm depth but significantly lower increases in POXC in 0–5 cm depth compared to the DCC treatment. The higher increase of SOC in the PSC treatment in 5–10 cm soil depth can be explained by possibly higher below ground C inputs in the PSC compared to the DCC plot. Literature values demonstrated that the cover crop species in the PSC treatment (mainly perennial ryegrass and clovers) had higher root/shoot ratio than most other cover crop species that were present in the mixtures of the DCC treatment (Hu et al., 2018).

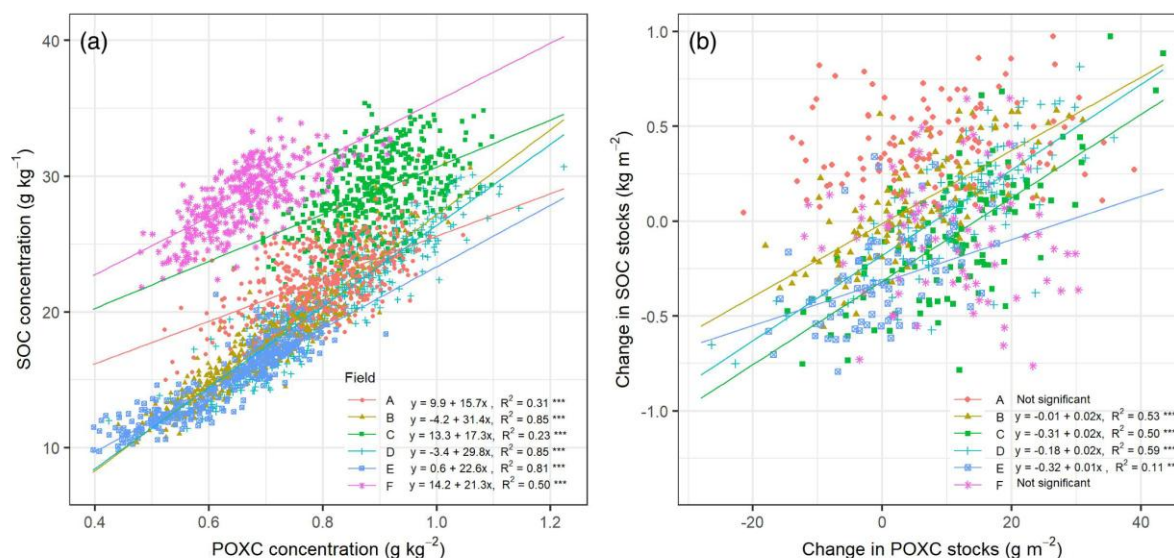


FIGURE 6 (a) Scatter plot of soil organic C (SOC, 0–20 cm) and permanganate oxidizable C (POXC, 0–20 cm) with a regression for each field. (b) Scatter plot of changes in SOC and POXC stocks with a regression for each field (if significant). Significant codes: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05.

Additionally, since there was only one cover crop mixture sown in the PSC treatment the root system had a longer time to develop than in the DCC treatment and root biomass is considered to be more important than above ground biomass for stabilizing soil organic matter (Balesdent et al., 2017; Ghafoor et al., 2017). The significantly higher increase in POXC in 0–5 cm depth in the DCC compared to the PSC treatment is probably related to the increase in microbial biomass. As can be seen in Figure 7, Cmic is stronger related to POXC than SOC and we think that the significant treatment difference in POXC in the topsoil (0–5 cm) is related to the stronger increase in microbial biomass and potentially microbial necromass in the DCC treatment.

#### 4.4 | Microbial C

In general, among the analysed C-fractions, Cmic was most sensitive to the two treatments (Figure 2c). On three fields (C–F) we found significantly higher changes in Cmic for the DCC than the PSC treatment, which confirms our third hypothesis that the above ground biomass input increases Cmic. Above ground plant C input was linearly correlated with Cmic (Figure 5a) suggesting that the incorporated plant material had a large effect on Cmic ( $R^2 = 0.6$ ). Besides cover crop C input, also tillage could have triggered soil microbial activity. In fall at  $t_1$  when the DCC plots were shallowly tilled and the PSC plot were not, we observed significantly higher increases in Cmic on fields C and D in the DCC treatment

compared to the PSC treatment. These differences between treatments on field C and D became even more pronounced in spring ( $t_3$ ) when both plots were tilled the same way. Increases in Cmic in spring might be explained by the combination of labile C-inputs and rising temperatures. Other studies also saw an increase in Cmic of 27% to 40% due to cover cropping which is in the range of our results (18% to 50%; Kim et al., 2020; Muhammad et al., 2021). Unlike the cited literature, we found these increases in microbial biomass in a single cover cropping period which suggests that bringing labile organic material directly into the biologically most active soil layer (shallow incorporation) led to an immediate response of microorganisms. However, on fields A and B, we did not see a significant difference in changes in Cmic between the DCC and the PSC treatment even though these fields had highest amount of above ground cover crop input. The beneficial effects of the DCC treatment on Cmic could in the long-term lead to an increase in SOC and POXC stocks since labile organic matter input leads to microbial products that form a big part of stable soil organic matter as it is proposed by the ‘microbial efficiency-matrix stabilization (MEMS) framework’ (Cotrufo et al., 2013; Robertson et al., 2019).

#### 4.5 | Mineral and microbial N

The two labile N fractions, Nmin and Nmic showed more pronounced treatment effects than Cmic (Figure 3). For both parameters we measured on four fields higher



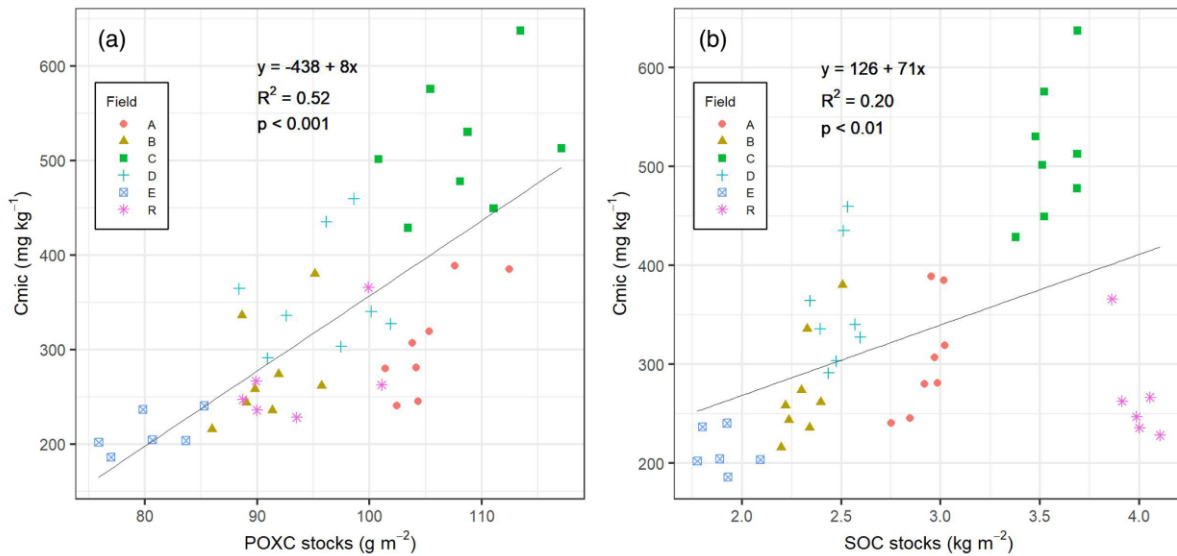


FIGURE 7 (a) Scatter plot of microbial biomass C ( $C_{mic}$ , 0–10 cm) and permanganate oxidizable C stocks (POXC, 0–10 cm, a) and soil organic carbon stocks (SOC, 0–10 cm, b) with a regression for all fields together.

increases in the DCC than in the PSC plot which confirms our third hypothesis. Despite their similar treatment effects,  $N_{min}$  and  $N_{mic}$  showed an opposite development over time.  $N_{min}$  decreased on most fields whereas  $N_{mic}$  increased on most fields. The decrease in  $N_{min}$  by 21% to 77% was in a similar range as in other cover crop studies (Kramberger et al., 2009; Mohammed et al., 2020; Zhou et al., 2020) and can be explained by four possible mechanisms: uptake through growing plants, leaching into deeper soil layer or ground water, microbial immobilization, and gaseous N losses. We found on all fields, irrespective of the treatment, lowest  $N_{min}$  values at sampling time  $t_3$  after winter and since plant growth and microbial activity is low during winter, it is very probable that despite the cover crops some N was lost through leaching. This assumption is supported by the decreased ratio between nitrate-N and ammonium-N after winter since mainly nitrate is susceptible to leaching (Figure S.1 in the Supplementary Material). The increase in  $N_{mic}$  can be explained by cover crop N input and immobilization of already present  $N_{min}$  in the soil. Though, we did not find a quantitative relationship between the decrease in  $N_{min}$  and the increase in  $N_{mic}$ . During cover crop biomass decomposition, gaseous N losses ( $N_2O$ ) may play a crucial role (Baggs et al., 2000; Carter et al., 2014), but are quantity-wise often in much lower ranges (Skinner et al., 2019). However, since we observed higher changes in  $N_{min}$  and  $N_{mic}$  in the DCC plot compared to the PSC plot, we assume that at least some of the above ground plant biomass N stayed within the plant-soil-microbial system.

Since  $N_{mic}$  is not available for plants, one cannot expect an immediate fertilization effect (Kramberger et al., 2009; Nevins et al., 2021), moreover the crop might not meet its N demand (Thorup-Kristensen et al., 2003). Research dealing with the benefits of cover cropping on N management mainly focuses on  $N_{min}$  (White et al., 2017), while the dynamics of immobilized N ( $N_{mic}$ ) in cover cropping systems is still understudied. Late incorporation time, as in this study, favours immobilization over mineralization of cover crop N input (Andersen & Jensen, 2001; Wyland et al., 1995) but we cannot make any assumption if and when this immobilized N may become plant available for the following crop.

## 5 | SUMMARY AND CONCLUSION

The widespread implementation of different cover cropping strategies requires information on their effects on soil organic matter dynamics for optimal management decisions. By assessing these dynamics in close spatial and temporal resolution for two cover cropping strategies during a nine-month period, we saw a high variability over time and between the six experimental sites. For SOC, total N and POXC we did not observe clear differences between strategies in 0–20 soil depth. When considering different soil depth segments, we observed that the strategy of PSC had significantly higher increases in SOC in 5–10 cm soil depth. The strategy of DCC showed instead significantly higher increases in POXC stocks in

0–5 cm soil depth. For the labile fractions N<sub>min</sub>, C<sub>mic</sub> and N<sub>mic</sub>, we observed generally higher increases in the DCC treatment, but these effects have not been observed consistently on all experimental fields.

We therefore conclude that the above ground biomass input in the DCC strategy was more beneficial for soil microbiology and N<sub>min</sub>, but the PSC strategy was more beneficial for short-term changes in SOC stocks. We hypothesize that the longer soil cover in the PSC treatment was accompanied by increased root growth and therefore higher below ground C inputs which seemed to be more important for SOC stocks than above ground biomass input. To find explanations for the effects of different cover cropping systems on SOC dynamics in more depth we therefore highly recommend to also measure below ground C inputs.

#### AUTHOR CONTRIBUTIONS

**Simon Oberholzer:** Writing – original draft; conceptualization; investigation; methodology; validation; visualization; writing – review and editing; software. **Klaus A. Jarosch:** Conceptualization; writing – review and editing; visualization. **Nadine Harder:** Investigation; methodology. **Markus Steffens:** Conceptualization; writing – review and editing; supervision. **Chinwe Ifejika Speranza:** Conceptualization; writing – review and editing; funding acquisition; supervision.

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#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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#### REFERENCES

- Alonso-Ayuso, M., Gabriel, J. L., Hontoria, C., Ibanez, M. A., & Quemada, M. (2020). The cover crop termination choice to designing sustainable cropping systems. *European Journal of Agronomy*, *114*, 126000. <https://doi.org/10.1016/j.eja.2020.126000>
- Andersen, M. K., & Jensen, L. S. (2001). Low soil temperature effects on short-term gross N mineralisation-immobilisation turnover after incorporation of a green manure. *Soil Biology and Biochemistry*, *33*(4–5), 511–521. [https://doi.org/10.1016/S0038-0717\(00\)00192-9](https://doi.org/10.1016/S0038-0717(00)00192-9)
- Baggs, E. M., Watson, C. A., & Rees, R. M. (2000). The fate of nitrogen from incorporated cover crop and green manure residues. *Nutrient Cycling in Agroecosystems*, *56*(2), 153–163. <https://doi.org/10.1023/A:1009825606341>
- Balesdent, J., Basile-Doelsch, I., Chadoeuf, J., Cornu, S., Fekiacova, Z., Fontaine, S., Guenet, B., & Hatté, C. (2017). Turnover of deep organic carbon in cultivated soils: An estimate from a review of isotope data. *Biotechnology, Agronomy, Society and Environment*, *21*(3), 1–10.
- Baumann, P. (2019). philipp-baumann/simplerspec: Beta release simplerspec 0.1.0 for zenodo, Zenodo [software]. <https://doi.org/10.5281/zenodo.3303637>
- Bongiorno, G., Bünemann, E. K., Oguejiofor, C. U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., & de Goede, R. (2019). Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecological Indicators*, *99*, 38–50. <https://doi.org/10.1016/j.ecolind.2018.12.008>
- Büchi, L., Wendling, M., Amosse, C., Jeangros, B., Sinaj, S., & Charles, R. (2017). Long and short term changes in crop yield and soil properties induced by the reduction of soil tillage in a long term experiment in Switzerland. *Soil and Tillage Research*, *174*, 120–129. <https://doi.org/10.1016/j.sti.2017.07.002>
- Burke, J. A., Lewis, K. L., Ritchie, G. L., Moore-Kucera, J., DeLaune, P. B., & Keeling, J. W. (2019). Temporal variability of soil carbon and nitrogen in cotton production on the Texas High Plains. *Agronomy Journal*, *111*(5), 2218–2225. <https://doi.org/10.2134/agronj2019.02.0066>
- Carter, M. S., Sorensen, P., Petersen, S. O., Ma, X. Z., & Ambus, P. (2014). Effects of green manure storage and incorporation methods on nitrogen release and N<sub>2</sub>O emissions after soil application. *Biology and Fertility of Soils*, *50*(8), 1233–1246. <https://doi.org/10.1007/s00374-014-0936-5>
- Chang, C. W., Laird, D. A., Mausbach, M. J., & Hurburgh, C. R. (2001). Near-infrared reflectance spectroscopy-principal components regression analyses of soil properties. *Soil Science Society of America Journal*, *65*(2), 480–490. <https://doi.org/10.2136/sssaj2001.652480x>
- Cotrufo, M. F., Wallenstein, M. D., Boot, C. M., Deneff, K., & Paul, E. (2013). The microbial efficiency-matrix stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? *Global Change Biology*, *19*(4), 988–995. <https://doi.org/10.1111/gcb.12113>

- Culman, S. W., Snapp, S. S., Freeman, M. A., Schipanski, M. E., Beniston, J., Lal, R., Drinkwater, L. E., Franzluebbers, A. J., Glover, J. D., Grandy, A. S., Lee, J., Six, J., Maul, J. E., Mirsky, S. B., Spargo, J. T., & Wander, M. M. (2012). Permanganate oxidizable carbon reflects a processed soil fraction that is sensitive to management. *Soil Science Society of America Journal*, 76(2), 494–504. <https://doi.org/10.2136/sssaj2011.0286>
- Daryanto, S., Fu, B. J., Wang, L. X., Jacinthe, P. A., & Zhao, W. W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth Science Reviews*, 185, 357–373. <https://doi.org/10.1016/j.earscirev.2018.06.013>
- Ellert, B. H., & Bettany, J. R. (1995). Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Canadian Journal of Soil Science*, 75(4), 529–538. <https://doi.org/10.4141/cjss95-075>
- García-Robledo, E., Corzo, A., & Papaspyrou, S. (2014). A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. *Marine Chemistry*, 162, 30–36. <https://doi.org/10.1016/j.marchem.2014.03.002>
- Gentsch, N., Boy, J., Batalla, J. D. K., Heuermann, D., von Wiren, N., Schwenecker, D., Feuerstein, U., Gross, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Cespedes, F. C., & Guggenberger, G. (2020). Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. *Biology and Fertility of Soils*, 56(7), 943–957. <https://doi.org/10.1007/s00374-020-01475-8>
- Ghafoor, A., Poeplau, C., & Katterer, T. (2017). Fate of straw- and root-derived carbon in a Swedish agricultural soil. *Biology and Fertility of Soils*, 53(2), 257–267. <https://doi.org/10.1007/s00374-016-1168-7>
- Giller, K. E., Hijbeek, R., Andersson, J. A., & Sumberg, J. (2021). Regenerative Agriculture: An agronomic perspective. *Outlook on Agriculture*, 50(1), 13–25. <https://doi.org/10.1177/0030727021998063>
- Gubler, A., Wächter, D., Schwab, P., Müller, M., & Keller, A. (2019). Twenty-five years of observations of soil organic carbon in Swiss croplands showing stability overall but with some divergent trends. *Environmental Monitoring and Assessment*, 191(5), 277. <https://doi.org/10.1007/s10661-019-7435-y>
- Hacker, N., Ebeling, A., Gessler, A., Gleixner, G., Mace, O. G., de Kroon, H., Lange, M., Mommer, L., Eisenhauer, N., Ravenek, J., Scheu, S., Weigelt, A., Wagg, C., Wilcke, W., & Oelmann, Y. (2015). Plant diversity shapes microbe-rhizosphere effects on P mobilisation from organic matter in soil. *Ecology Letters*, 18(12), 1356–1365. <https://doi.org/10.1111/ele.12530>
- Heller, O., Bene, C. D., Nino, P., Huyghebaert, B., Arlauskienė, A., Castanheira, N. L., Higgins, S., Horel, A., Kir, A., Kizekova, M., Lacoste, M., Munkholm, L. J., O'Sullivan, L., Radzikowski, P., Rodríguez-Cruz, M. S., Sandén, T., Sarunaite, L., Seidel, F., Spiegel, H., ... Vanwindekens, F. (2024). Towards enhanced adoption of soil-improving management practices in Europe. *European Journal of Soil Science*, 75(2), e13483. <https://doi.org/10.1111/ejss.13483>
- Hu, T., Sorensen, P., Wahlstrom, E. M., Chirinda, N., Sharif, B., Li, X. X., & Olesen, J. E. (2018). Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass. *Agriculture, Ecosystems & Environment*, 251, 141–148. <https://doi.org/10.1016/j.agee.2017.09.024>
- Hubbard, R. K. (2013). Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. *Soil and Tillage Research*, 126, 276–283.
- in't Zandt, D., Fritz, C., & Wichern, F. (2018). In the land of plenty: Catch crops trigger nitrogen uptake by soil microorganisms. *Plant and Soil*, 423(1–2), 549–562. <https://doi.org/10.1007/s11104-017-3540-2>
- Jagadamma, S., Essington, M. E., Xu, S. T., & Yin, X. H. (2019). Total and active soil organic carbon from long-term agricultural management practices in West Tennessee. *Agricultural and Environmental Letters*, 4(1), 5. <https://doi.org/10.2134/aerl2018.11.0062>
- Janzen, H. H. (2006). The soil carbon dilemma: Shall we hoard it or use it? *Soil Biology and Biochemistry*, 38(3), 419–424. <https://doi.org/10.1016/j.soilbio.2005.10.008>
- Jian, J. S., Du, X., Reiter, M. S., & Stewart, R. D. (2020). A meta-analysis of global cropland soil carbon changes due to cover cropping. *Soil Biology and Biochemistry*, 143, 107735. <https://doi.org/10.1016/j.soilbio.2020.107735>
- Kallenbach, C. M. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature Communications*, 7(1), 13630. <https://doi.org/10.1038/ncomms13630>
- Kathage, J., Smit, B., Janssens, B., Haagsma, W., & Adrados, J. L. (2022). How much is policy driving the adoption of cover crops? Evidence from Four EU Regions. *Land Use Policy*, 116, 106016. <https://doi.org/10.1016/j.landusepol.2022.106016>
- Kaye, J. P., & Quemada, M. (2017). Using cover crops to mitigate and adapt to climate change. A Review. *Agronomy for Sustainable Development*, 37(1), 1–17. <https://doi.org/10.1007/s13593-016-0410-x>
- Kim, N., Zabaloy, M. C., Guan, K. Y., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biol. The Biochemist*, 142, 107701. <https://doi.org/10.1016/j.soilbio.2019.107701>
- Kramberger, B., Gselman, A., Janzekovic, M., Kaligarić, M., & Bracko, B. (2009). Effects of cover crops on soil mineral nitrogen and on the yield and nitrogen content of maize. *European Journal of Agronomy*, 31(2), 103–109. <https://doi.org/10.1016/j.eja.2009.05.006>
- Kuhn, M. (2020). caret: Classification and Regression Training, R package. <https://CRAN.R-project.org/package=caret>
- Lal, R. (2015). Restoring soil quality to mitigate soil degradation. *Sustainability*, 7(5), 5875–5895. <https://doi.org/10.3390/su7055875>
- Lange, M. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, 6(1), 6707. <https://doi.org/10.1038/ncomms7707>
- Lee, J., Hopmans, J. W., Rolston, D. E., Baer, S. G., & Six, J. (2009). Determining soil carbon stock changes: Simple bulk density corrections fail. *Agriculture, Ecosystems & Environment*, 134(3–4), 251–256. <https://doi.org/10.1016/j.agee.2009.07.006>
- Leifeld, J., Reiser, R., & Oberholzer, H. R. (2009). Consequences of conventional versus organic farming on soil carbon: Results from a 27-year field experiment. *Agronomy Journal*, 101(5), 1204–1218. <https://doi.org/10.2134/agronj2009.0002>
- Li, Z. J., Liu, H. G., Yang, H. C., & Wang, T. A. (2023). Effects of deep vertical rotary tillage management methods on soil quality in saline cotton fields in southern Xinjiang. *Agriculture-Basel*, 13(10), 1864. <https://doi.org/10.3390/agriculture13101864>

- Lucas, S., & Weil, R. (2021). Can permanganate oxidizable carbon predict soil function responses to soil organic matter management? *Soil Science Society of America Journal*, 85, 1768–1784. <https://doi.org/10.1002/saj2.20282>
- Lucas, S. T., & Weil, R. R. (2012). Can a labile carbon test be used to predict crop responses to improve soil organic matter management? *Agronomy Journal*, 104(4), 1160–1170. <https://doi.org/10.2134/agronj2011.0415>
- McClelland, S. C., Paustian, K., & Schipanski, M. E. (2021). Management of cover crops in temperate climates influences soil organic carbon stocks: A meta-analysis. *Ecological Applications*, 31(3), e02278. <https://doi.org/10.1002/eap.2278>
- Miltner, A., Bombach, P., Schmidt-Brucken, B., & Kastner, M. (2012). SOM genesis: Microbial biomass as a significant source. *Biogeochemistry*, 111(1–3), 41–55. <https://doi.org/10.1007/s10533-011-9658-z>
- Mitchell, E., Scheer, C., Rowlings, D., Conant, R. T., Cotrufo, M. F., & Grace, P. (2018). Amount and incorporation of plant residue inputs modify residue stabilisation dynamics in soil organic matter fractions. *Agriculture, Ecosystems & Environment*, 256, 82–91. <https://doi.org/10.1016/j.agee.2017.12.006>
- Mitchell, E., Scheer, C., Rowlings, D. W., Conant, R. T., Cotrufo, M. F., van Delden, L., & Grace, P. R. (2016). The influence of above-ground residue input and incorporation on GHG fluxes and stable SOM formation in a sandy soil. *Soil Biology and Biochemistry*, 101, 104–113. <https://doi.org/10.1016/j.soilbio.2016.07.008>
- Mohammed, Y. A., Patel, S., Matthees, H. L., Lenssen, A. W., Johnson, B. L., Wells, M. S., Forcella, F., Berti, M. T., & Gesch, R. W. (2020). Soil nitrogen in response to interseeded cover crops in maize-soybean production systems. *Agronomy-Basel*, 10(9), 1439. <https://doi.org/10.3390/agronomy10091439>
- Muhammad, I., Wang, J., Sainju, U. M., Zhang, S. H., Zhao, F. Z., & Khan, A. (2021). Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma*, 5, 381. <https://doi.org/10.1016/j.geoderma.2020.114696>
- Nevins, C. J., Lacey, C., & Armstrong, S. (2021). Cover crop enzyme activities and resultant soil ammonium concentrations under different tillage systems. *European Journal of Agronomy*, 126, 126277. <https://doi.org/10.1016/j.eja.2021.126277>
- Oberholzer, S., Herrmann, C., Bodenhausen, N., Krause, H.-M., Mestrot, A., Ifejika Speranza, C., & Jarosch, K. A. (2024). No effect on biological or chemical soil properties when amended with effective microorganisms for improved cover crop decomposition. *Applied Soil Ecology*, 197, 105358. <https://doi.org/10.1016/j.apsoil.2024.105358>
- Oberholzer, S., Summerauer, L., Steffens, M., & Speranza, C. I. (2024). Best performances of visible-near-infrared models in soils with little carbonate – a field study in Switzerland. *The Soil*, 10(1), 231–249. <https://doi.org/10.5194/soil-10-231-2024>
- Poepflau, C., & Don, A. (2015). Carbon sequestration in agricultural soils via cultivation of cover crops – a meta-analysis. *Agriculture, Ecosystems & Environment*, 200, 33–41. <https://doi.org/10.1016/j.agee.2014.10.024>
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. URL <https://www.R-project.org/>
- Rhine, E. D., Sims, G. K., Mulvaney, R. L., & Pratt, E. J. (1998). Improving the Berthelot reaction for determining ammonium in soil extracts and water. *Soil Science Society of America Journal*, 62(2), 473–480. <https://doi.org/10.2136/sssaj1998.03615995006200020026x>
- Rhodes, C. J. (2017). The imperative for regenerative agriculture. *Science Progress*, 100(1), 80–129. <https://doi.org/10.3184/003685017x14876775256165>
- Robertson, A. D., Paustian, K., Ogle, S., Wallenstein, M. D., Lugato, E., & Cotrufo, M. F. (2019). Unifying soil organic matter formation and persistence frameworks: The MEMS model. *Biogeosciences*, 16(6), 1225–1248. <https://doi.org/10.5194/bg-16-1225-2019>
- Singh, G., Sharma, K. R., Bhatt, R., Singh, J., Wani, O. A., Dewidar, A. Z., & Mattar, M. A. (2023). Soil carbon and biochemical indicators of soil quality as affected by different conservation agricultural and weed management options. *Land*, 12(9), 1783. <https://doi.org/10.3390/land12091783>
- Skinner, C., Gattinger, A., Krauss, M., Krause, H. M., Mayer, J., van der Heijden, M. G. A., & Mader, P. (2019). The impact of long-term organic farming on soil-derived greenhouse gas emissions. *Scientific Reports*, 9, 1702. <https://doi.org/10.1038/s41598-018-38207-w>
- Sokol, N. W., Sanderman, J., & Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. *Global Change Biology*, 25(1), 12–24. <https://doi.org/10.1111/gcb.14482>
- Stevens, A. S., & Ramirez-Lopez, L. (2020). An introduction to the prospectr package, R package. <https://cran.r-project.org/web/packages/prospectr/vignettes/prospectr.html>
- Swiss Ordinance 910.13. (2013). Direktzahlungsverordnung, 17.
- Thorup-Kristensen, K., Magid, J., & Jensen, L. S. (2003). Catch crops and green manures as biological tools in nitrogen management in temperate zones, in Sparks, D. L., ed. *Advances in Agronomy*, 79, 227–302.
- Tonitto, C., David, M. B., & Drinkwater, L. E. (2006). Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. *Agriculture, Ecosystems & Environment*, 112(1), 58–72. <https://doi.org/10.1016/j.agee.2005.07.003>
- Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass-C. *Soil Biology and Biochemistry*, 19(6), 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6)
- Vidal, A., Kloffel, T., Guigue, J., Angst, G., Steffens, M., Hoeschen, C., & Mueller, C. W. (2021). Visualizing the transfer of organic matter from decaying plant residues to soil mineral surfaces controlled by microorganisms. *Soil Biology and Biochemistry*, 160, 108347. <https://doi.org/10.1016/j.soilbio.2021.108347>
- Wadoux, A. M. J. C. (2021). *Soil spectral inference with R: Analysing digital soil spectra using the R programming environment*. Springer.
- Wang, Q. R., Li, Y. C., & Klassen, W. (2007). Changes of soil microbial biomass carbon and nitrogen with cover crops and irrigation in a tomato field. *Journal of Plant Nutrition*, 30(4–6), 623–639. <https://doi.org/10.1080/01904160701209410>
- Wang, Q. Y., Wang, Y., Wang, Q. C., & Liu, J. S. (2014). Impacts of 9 years of a new conservation agricultural management on soil organic carbon fractions. *Soil & Tillage Research*, 143, 1–6. <https://doi.org/10.1016/j.still.2014.05.004>

- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D., & Bary, A. (2015). The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30(5), 450–460. <https://doi.org/10.1017/s1742170514000246>
- Webster, R., & Lark, R. M. (2013). *Field sampling for environmental science and management*. Routledge.
- Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., & Samson-Liebig, S. E. (2003). Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture*, 18(1), 3–17. <https://doi.org/10.1079/AJAA200228>
- Welch, R. Y., Behnke, G. D., Davis, A. S., Masiunas, J., & Villamil, M. B. (2016). Using cover crops in headlands of organic grain farms: Effects on soil properties, weeds and crop yields. *Agriculture, Ecosystems & Environment*, 216, 322–332. <https://doi.org/10.1016/j.agee.2015.10.014>
- White, C. M., DuPont, S. T., Hautau, M., Hartman, D., Finney, D. M., Bradley, B., LaChance, J. C., & Kaye, J. P. (2017). Managing the trade off between nitrogen supply and retention with cover crop mixtures. *Agriculture, Ecosystems & Environment*, 237, 121–133. <https://doi.org/10.1016/j.agee.2016.12.016>
- Wold, S., Martens, H., & Wold, H. (1983). The multivariate calibration problem in chemistry solved by the PLS method. In *Proceedings matrix pencils* (Vol. 1983, pp. 286–293). Springer. <https://doi.org/10.1007/BFb0062108>
- Wuest, S. (2014). Seasonal variation in soil organic carbon. *Soil Science Society of America Journal*, 78(4), 1442–1447. <https://doi.org/10.2136/sssaj2013.10.0447>
- Wyland, L. J., Jackson, L. E., & Schulbach, K. F. (1995). Soil-plant nitrogen dynamics following incorporation of a manure rye cover crop in a lettuce production system. *The Journal of Agricultural Science*, 124, 17–25. <https://doi.org/10.1017/s0021859600071203>
- Zhang, L., Yang, X. M., Drury, C., Chantigny, M., Gregorich, E., Miller, J., Bittman, S., Reynolds, W. D., & Yang, J. Y. (2018). Infrared spectroscopy estimation methods for water-dissolved carbon and amino sugars in diverse Canadian agricultural soils. *Canadian Journal of Soil Science*, 98(3), 484–499. <https://doi.org/10.1139/cjss-2018-0027>
- Zhou, Y. X., Roosendaal, L., & van Eerd, L. L. (2020). Increased nitrogen retention by cover crops: Implications of planting date on soil and plant nitrogen dynamics. *Renewable Agriculture and Food Systems*, 35(6), 720–729. <https://doi.org/10.1017/s1742170519000383>
- Zikeli, S., & Gruber, S. (2017). Reduced tillage and No-till in organic farming systems, Germany-status quo, potentials and challenges. *Agriculture-Basel*, 7(4), 35. <https://doi.org/10.3390/agriculture7040035>

### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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## **Paper 2: Soil spectroscopy**

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## Best performances of visible–near-infrared models in soils with little carbonate – a field study in Switzerland

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**Abstract.** Conventional laboratory analysis of soil properties is often expensive and requires much time if various soil properties are to be measured. Visual and near-infrared (vis–NIR) spectroscopy offers a complementary and cost-efficient way to gain a wide variety of soil information at high spatial and temporal resolutions. Yet, applying vis–NIR spectroscopy requires confidence in the prediction accuracy of the infrared models. In this study, we used soil data from six agricultural fields in eastern Switzerland and calibrated (i) field-specific (local) models and (ii) general models (combining all fields) for soil organic carbon (SOC), permanganate oxidizable carbon (POXC), total nitrogen (N), total carbon (C) and pH using partial least-squares regression. The 30 local models showed a ratio of performance to deviation (RPD) between 1.14 and 5.27, and the root mean square errors (RMSE) were between 1.07 and 2.43 g kg<sup>−1</sup> for SOC, between 0.03 and 0.07 g kg<sup>−1</sup> for POXC, between 0.09 and 0.14 g kg<sup>−1</sup> for total N, between 1.29 and 2.63 g kg<sup>−1</sup> for total C, and between 0.04 and 0.19 for pH. Two fields with high carbonate content and poor correlation between the target properties were responsible for six local models with a low performance (RPD < 2). Analysis of variable importance in projection, as well as of correlations between spectral variables and target soil properties, confirmed that high carbonate content masked absorption features for SOC. Field sites with low carbonate content can be combined with general models with only a limited loss in prediction accuracy compared to the field-specific models. On the other hand, for fields with high carbonate contents, the prediction accuracy substantially decreased in general models. Whether the combination of soils with high carbonate contents in one prediction model leads to satisfying prediction accuracies needs further investigation.

### 1 Introduction

The application of spectroscopy in the visible and near-infrared (vis–NIR) range is increasing in soil science and related disciplines, with the main objective being to gain information on the soil properties of more samples at lower costs than with conventional laboratory methods. With a larger sample size, the spatial or temporal resolution can be increased, which allows conclusions about the within-field or within-farm variability but might potentially also increase the statistical power in agricultural experiments (Greenberg et al., 2022). Despite its tendency to be less accurate compared to mid-infrared (MIR) spectroscopy, vis–NIR spectroscopy

is widely applied because of less sample preparation, lower costs and generally easier portability (Soriano-Disla et al., 2014).

On-site vis–NIR measurements are therefore feasible, but laboratory measurements with dried and sieved soil samples have so far shown higher accuracy (Allory et al., 2019; Hutengs et al., 2019). In particular, soil properties related to soil organic matter can be estimated appropriately by laboratory vis–NIR spectroscopy (Angelopoulou et al., 2020). In most cases, the focus is to provide soil information over large areas (e.g., soil maps) where a high sample number is present and only a moderate prediction accuracy is needed.

Hence, large-scale spectral libraries have been developed to further reduce the need for wet chemistry data. Due to the high complexity within spectral libraries, the application of a general model to a local context leads to high prediction errors. Recent research shows that the localization of these infrared models substantially improves the predictive performance in a local context, for example by spiking (Brown, 2007; Li et al., 2020; Ng et al., 2022; Seidel et al., 2019; Wetterlind and Stenberg, 2010; Zhao et al., 2021), memory-based learning (Ramirez-Lopez et al., 2013), resampling algorithms (Lobsey et al., 2017) or deep learning (Shen et al., 2022). However, for analyzing small-scale variability (field or farm level), a local model is often still the best choice because of its low prediction errors. Theoretically, developing local models is supported by the finding that, in the vis–NIR range, spectral features that influence specific soil properties vary strongly between different datasets, which makes highly heterogeneous large datasets prone to insufficient model performance (Angelopoulou et al., 2020; Grunwald et al., 2018). The development of local spectral models has the main purpose of coping with a large sample size at the local scale, but such local models have no utility beyond the analysis of the specific local dataset.

Spectral vis–NIR models developed from local datasets showed a very high variability in model performance, ranging from excellent models (Breure et al., 2022; Seidel et al., 2019) to those with relatively poor model performance (Carmargo et al., 2022; Kuang and Mouazen, 2011). The reasons for these different performances of local models are understudied and remain unclear. Among many different possible modeling approaches, including support vector machine regression, artificial neural networks, cubist and random forest, partial least-squares regression (PLSR) is the most frequently used model type to build spectral models with small datasets (Alomar et al., 2021; Zhao et al., 2021).

The number of samples is crucial for local models, often, only a limited number of samples with reference laboratory data are available. Kuang and Mouazen (2012) showed that local models improve with an increasing number of calibration samples and that a sample size of at least 50 provides accurate prediction models. Some studies thus combined multiple target sites and developed a general model by combining all the local datasets to reach a larger sample size and potentially better model performance (Kuang and Mouazen, 2011; Singh et al., 2022). In these studies, the general model showed an intermediate performance, and the general prediction error was between the best- and the poorest-performing local model. However, these studies only calculated the overall prediction error of the general model; therefore, it is not clear if the prediction for target sites with poorly performing local models could be improved by applying a general model.

For vis–NIR spectroscopy application at local scales, it is therefore very difficult to estimate the measurement accuracy for the predicted samples beforehand. This uncertainty

is probably the main reason that hampers the application of vis–NIR spectroscopy because researchers prefer to rely on conventional lab measurements with a smaller sample size (and smaller spatial resolution) where the measurement accuracy is known before sampling and measurements are conducted. Applying spectroscopy at the field or farm scale thus bears the risk that the measurement accuracy (RMSE) may be beyond the tolerable threshold, which might then bring a whole project into question. Thus, in this paper, we analyze the performance of field-specific (local) spectral models of a field experiment conducted in six fields in eastern Switzerland and that of a general model combining the data from all six fields to ascertain their influencing factors. We ask the following questions:

1. To what extent do the prediction errors of local spectral models differ from the lab measurement error?
2. Does a general model that includes several target sites improve the prediction on a target site with a poor local-model performance?
3. How do field and soil characteristics (e.g., field size, soil texture, carbonate content, correlations of soil properties) of the target site relate to the performance of spectral models?

By answering these questions, we want to provide insights into the estimations of prediction accuracies for vis–NIR studies at the local scale, with the objective of supporting decision-making during the development of a sampling design and the planning of laboratory reference measurements for subsequent calibration modeling.

## 2 Methods

### 2.1 Datasets from a cover-cropping experiment at six field sites

We used datasets from six fields (A, B, C, D, E, F) of a cover-cropping experiment in the Canton of Thurgau, eastern Switzerland (paper in preparation). The six fields were up to 13 km apart from one another, and the soil type for all of them was Eutric Cambisol that had developed on base moraine (Table 1). The aim of the study was to compare the influence of two different cover-cropping regimes on short-term soil organic matter cycling. Each field had 39 differential-GPS (dGPS)-referenced sampling points in an unaligned sampling design. At each dGPS-referenced point, soil was sampled three to four times at three depths (0–5, 5–10 and 10–20 cm) during one long cover-cropping period (August 2019 to May 2020). Fields A, B, C and D had four sampling times, resulting in 468 samples per field. Fields E and F had three sampling times, resulting in 351 samples per field. All samples were dried at 40 °C to a constant weight (around 72 h) and then gently crushed and sieved to 2 mm. For the total sample size of 2574 samples, soil properties were esti-



mated using vis–NIR soil spectroscopy, whereas 386 samples were analyzed conventionally by wet chemistry for subsequent calibration modeling. These 386 samples for laboratory analysis were selected for each field separately using the Kennard–Stones algorithm (Kennard and Stone, 1969) to ensure coverage of the whole spectral variability. Thereby, the Kennard–Stones algorithm was run with two to seven principal components, and the number of principal components was chosen such that it covered at least 99 % of the spectral variance and provided a reference sample selection that represented well the different sampling times, soil depths and spatial distributions. The laboratory analysis comprised soil organic C (SOC), total C, total N, permanganate oxidizable C (POXC) (also called active C) and pH.

## 2.2 Chemical soil analyses and its accuracy

Total C and N concentrations were measured on a ground aliquot by dry combustion (vario MICRO tube, Elementar, Germany). Inorganic C was analyzed for each sample in triplicates through the dissolution of carbonate in a Scheibler apparatus with 10 % HCl solution and the measurement of the evolved CO<sub>2</sub> volume. SOC was then calculated as the difference between total C and the mean of the three measurements for inorganic C. POXC was measured according to the Protocol of Weil et al. (2003), with the adaption of Lucas and Weil (2012). In brief, 2.0 mL of 0.2 M KMnO<sub>4</sub> was added to 2.5 g of soil, and after a reaction time of 10 min, the absorption of the liquid was measured at 550 nm with a spectrophotometer (UV-1800, Shimadzu Corporation, Japan). The measurement of pH was done in a 0.01 M CaCl<sub>2</sub> solution.

To estimate the lab measurement error, we took three samples per field (in total 18) where we conducted the measurements for total C, total N, POXC and pH in triplicates to calculate a standard deviation. We estimated the lab measurement error for SOC ( $\sigma_{\text{SOC}}$ ) according to Eq. (1):

$$\sigma_{\text{SOC}} = \sqrt{\sigma_{\text{Total C}}^2 + \sigma_{\text{Inorganic C}}^2}, \quad (1)$$

where  $\sigma_{\text{Total C}}$  is the standard deviation of the total C measurement, and  $\sigma_{\text{Inorganic C}}$  is the standard error of the inorganic C measurement because inorganic C measurements were done for all samples in triplicates. The measurement errors of all 18 triplicates were then averaged to obtain the overall lab measurement error for a soil property.

To characterize the spatial variability of soil texture in the field, we measured grain size for 20 samples per field (every second sampling point in 10–20 cm soil depth). Organic matter in the samples was oxidized with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and then grain size was measured with laser-diffraction analysis (LDA) after dispersion of the sample (22 mM sodium carbonate and 18 mM sodium hexaphosphate) using a Mastersizer 2000 (Malvern Panalytical, UK). Since the LDA underestimates the clay content compared to the standard grain size methods (Taubner et al., 2009),

we measured one composite sample per field with the improved integral suspension pressure method (ISP+; Durner and Iden, 2021) on a PARIO Plus Soil Particle Analyzer (METER Group, Germany and USA). We rescaled the mean sand, silt and clay content of the LDA data to the mean of the IPS+ method while keeping the coefficient of variation constant (see Table S3 in the Supplement).

## 2.3 Spectral measurement and pre-processing of spectra

All samples were measured with a vis–NIR spectrometer (ASD FieldSpec 4 Hi-Res, Malvern Panalytical, USA) with a sampling interval of 1.4 nm from 350 to 1000 nm and 1.1 nm from 1000 to 2500 nm. The device then provides a reflectance spectrum with a resolution of 1 nm and 2151 wavelengths. Measurements were done with a contact probe, containing an internal halogen bulb, which was in a fixed position, and soil samples, placed in a petri dish of 1.5 cm height and 3 cm diameter, were lifted with a laboratory scissor jack until coming into close contact with the probe to ensure a stable measurement position. For each sample, five petri dishes were filled to provide five replicate spectra per sample. Each of these five replicates consisted of 30 internal repetitive scans that were automatically averaged by the device's internal RS3 software. Between samples, the contact probe was carefully cleaned with water and ethanol. After the five replicates of a sample, the calibration of the spectrometer was checked with a 100 % reflectance white reference panel (Spectralon, 12 × 12 cm, Labsphere, USA). The infrared data of each sample were kept in two versions, once as reflectance spectra, as provided by the spectrometer, and once as absorbance spectra using the  $\log(1/\text{reflectance})$  transformation. Several pre-processing options and their combinations were tested on both the reflectance and the absorbance spectra: (a) resampling of the spectra in an interval from 1 to 6 nm, (b) cutting of the beginning (350–400 nm) or the end (2450–2500 nm) of the spectra, (c) first- or second-order derivative, (d) Savitzky–Golay (SG) smoothing in a third-order polynomial with window sizes ranging from 5 to 51, (e) gap segment derivative (GSD) with window widths between 5 and 51 and segment sizes between 1 and 21, (f) standard normal variate (SNV) combined with GSD, and (g) SG smoothing combined with multiplicative scatter correction (MSC). All applied pre-processing techniques are frequently used in soil spectroscopy and are well described in Ellinger et al. (2019). The pre-processing techniques from (a) to (g) led to around 100 meaningful combinations that were tested in model building, and the final pre-processing option was selected based on the smallest RMSE.

**Table 1.** Description of the datasets of the six different fields A to F. All fields were classified as Eutric Cambisol developed on base moraine. Soil texture was measured with the improved integral suspension pressure method (ISP+).

Field	Coordinates	Elevation (m above sea level)	Area (ha)	Mean soil texture (sand/silt/clay) (%)	Number of samples	
					Spectroscopy	Wet chemistry
A	47°40′58″ N, 08°45′54″ E	420	0.84	Sandy loam (50/29/21)	468	70
B	47°40′54″ N, 08°46′05″ E	420	0.67	Sandy loam (44/35/20)	468	70
C	47°38′01″ N, 08°57′02″ E	600	0.44	Sandy loam (27/35/38)	468	70
D	47°38′43″ N, 08°42′58″ E	460	0.64	Clay loam (28/44/28)	468	70
E	47°38′49″ N, 08°43′06″ E	460	1.05	Sandy loam (30/48/23)	351	53
F	47°34′22″ N, 08°48′52″ E	380	0.3	Sandy loam (39/43/18)	351	53

#### 2.4 Development and evaluation of field-specific local models

We used for all 30 local models (6 fields  $\times$  5 properties) a PLSR modeling approach (Wold et al., 1983). Model performance was assessed using the statistics of the hold-out folds of each five-times-repeated five-fold cross-validation because it was evaluated as a robust method for smaller datasets (Kuhn and Johnson, 2013; Molinaro et al., 2005). To avoid model overfitting, we set the maximum of latent variables in the PLSR model to 12. For each number of latent variables (1, 2, ..., 12) the dataset was randomly split five times into five folds, of which four were used for model training, and the remaining fold was held out and used for model validation. The RMSE (Eq. 2) of the hold-out samples was averaged among the five repeats, resulting in a cross-validated RMSE per number of latent variables. The final number of latent variables was then chosen according to the “1-standard-error rule”, which means that, instead of directly choosing the number of latent variables with the smallest mean RMSE, the most parsimonious (fewer latent variables) model within 1 standard error of the mean RMSE of the optimal model was selected (Hastie et al., 2017). The 1-standard-error rule was also applied during optimization of pre-processing to avoid model overfitting. The final model was trained using all training data with an optimized number of latent variables.

A proper validation of a spectral model is very crucial and is particularly important in this study where soil was repeatedly sampled at different depths at the same GPS point. To analyze the correlation among the samples and define a grouping factor for the cross-validation, we calculated the mean Euclidean distance between all samples and compared it with the mean distance (1) between samples at the same GPS point but different depths, (2) between samples at the same point and depth but different sampling times, and (3) between samples at the same point but different depth and sampling times (Fig. S1 in the Supplement). Thereby, we observed that the soil samples from the three different soil depths sampled at the same GPS point at the same sampling time had a substantially lower mean Euclidean distance

compared to the overall mean. Consequently, we grouped the samples from the same GPS point at the same sampling time and kept them in the same fold to avoid a too-optimistic model evaluation during cross-validation.

Since we used a cross-validation approach at the field scale, all models showed a very small bias (see Table 2). We therefore do not discuss the bias in this paper and focus on  $R^2$ , RMSE and RPD (Eq. 3) for the evaluation and comparison of different models. RMSE was calculated according to Eq. (2), where  $\hat{y}_i$  is the prediction of the spectral model for sample  $i$ , and  $y_i$  is the actual measured value for the same sample in the laboratory.

$$\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2} \quad (2)$$

RPD compares the RMSE with the standard deviation (SD, Eq. 3) of the data:

$$\text{RPD} = \frac{\text{SD}}{\text{RMSE}}. \quad (3)$$

For all model performance parameters ( $R^2$ , RMSE and RPD) of the cross-validation, we calculated the uncertainty with the standard deviation of the prediction of the hold-out folds across the five repetitions.

To classify the model performance, we combined the RPD-based classifications of Chang et al. (2001) and Zhang et al. (2018). We considered spectral models with RPD < 1.4 to be poor, models with RPD between 1.4 and 2 to be approximate, models with RPD between 2 and 3 to be accurate, and models with RPD > 3 to be excellent. Even though in spectroscopy projects relating to local extent the RMSE is the most important model performance parameter, RPD is the best parameter to compare models of different scales. Model metrics ( $R^2$ , RMSE and RPD) mentioned in the text are based on the cross-validation, and metrics for the model calibration in Table 2 are specifically labeled as  $R^2_{\text{cal}}$ ,  $\text{RMSE}_{\text{cal}}$  and  $\text{RPD}_{\text{cal}}$ .

**Table 2.** Description of applied pre-processing and model performance of the final chosen models using a partial least-squares regression. The local models (fields A to F) were evaluated with five-times-repeated 5-fold cross-validation, and the general models (all) were evaluated with five-times-repeated 10-fold cross-validation. Model metrics of cross-validation are indicated as mean with the standard deviation across the repeats in brackets. RMSE refers to root mean square error, RPD refers to ratio of performance to deviation, Refl. refers to reflectance, Abs. refers to absorbance, SG refers to Savitzky–Golay filter ( $m$  refers to order of derivative,  $w$  refers to window width), SNV refers to standard normal variate, GSD refers to gap segment derivative ( $m$  refers to derivative,  $w$  refers to window width,  $s$  refers to segment size), and MSC refers to multiplicative scatter correction.

Field	Property	Range of wavelengths/ interval (nm)	Pre-processing	Latent variable	$n$	Calibration				Cross-validation				Model performance
						$R^2_{\text{cal}}$	Bias <sub>cal</sub>	RMSE <sub>cal</sub>	RPD <sub>cal</sub>	$R^2$	Bias	RMSE	RPD	
A	SOC [g kg <sup>-1</sup> ]	410–2500/6	Abs., SG ( $m = 1, w = 35$ )	2	70	0.59	0.00	2.35	1.58	0.55 (0.14)	0.01 (0.69)	2.43 (0.55)	1.50 (0.30)	Approximate
	POXC [g kg <sup>-1</sup> ]	350–2500/2	Refl., SNV, GSD ( $m = 2, w = 31, s = 1$ )	7	70	0.81	0.00	0.05	2.28	0.64 (0.17)	0.00 (0.02)	0.07 (0.01)	1.65 (0.41)	Approximate
	Total N [g kg <sup>-1</sup> ]	370–2480/6	Refl., SG ( $m = 1, w = 21$ ), MSC	7	70	0.87	0.00	0.11	2.77	0.79 (0.11)	-0.01 (0.05)	0.14 (0.03)	2.18 (0.61)	Accurate
	Total C [g kg <sup>-1</sup> ]	390–2500/4	Abs., SNV, GSD ( $m = 2, w = 21, s = 1$ )	6	70	0.94	0.00	2.14	4.21	0.88 (0.09)	0.00 (0.84)	2.63 (0.66)	3.48 (1.41)	Excellent
	pH	410–2500/4	Abs., SG ( $m = 1, w = 35$ )	5	70	0.74	0.00	0.06	1.97	0.63 (0.21)	0.00 (0.03)	0.08 (0.02)	1.70 (0.60)	Approximate
B	SOC [g kg <sup>-1</sup> ]	360–2500/5	Abs., SG ( $m = 2, w = 21$ ), MSC	7	70	0.98	0.00	0.66	6.47	0.91 (0.05)	-0.04 (0.37)	1.26 (0.36)	3.46 (1.12)	Excellent
	POXC [g kg <sup>-1</sup> ]	360–2480/3	Abs., SG ( $m = 2, w = 21$ ), MSC	4	70	0.94	0.00	0.03	4.08	0.84 (0.12)	0.00 (0.02)	0.05 (0.01)	2.60 (0.74)	Accurate
	Total N [g kg <sup>-1</sup> ]	360–2480/5	Abs., SG ( $m = 2, w = 21$ ), MSC	4	70	0.93	0.00	0.10	3.87	0.87 (0.08)	0.00 (0.04)	0.13 (0.03)	2.85 (0.92)	Accurate
	Total C [g kg <sup>-1</sup> ]	370–2500/5	Abs., SG ( $m = 2, w = 21$ ), MSC	10	70	0.99	0.00	0.51	9.64	0.93 (0.03)	-0.05 (0.40)	1.29 (0.25)	3.65 (0.84)	Excellent
	pH	350–2500/3	Abs., SG ( $m = 1, w = 21$ ), MSC	7	70	0.98	0.00	0.07	6.64	0.83 (0.07)	0.00 (0.06)	0.19 (0.03)	2.46 (0.51)	Accurate
C	SOC [g kg <sup>-1</sup> ]	370–2480/1	Abs., SNV, GSD ( $m = 1, w = 11, s = 1$ )	7	70	0.90	0.00	1.02	3.11	0.77 (0.09)	0.03 (0.46)	1.59 (0.28)	2.05 (0.46)	Accurate
	POXC [g kg <sup>-1</sup> ]	370–2440/3	Refl., SG ( $m = 2, w = 21$ )	7	70	0.93	0.00	0.03	3.80	0.77 (0.15)	0.00 (0.01)	0.05 (0.01)	2.30 (0.81)	Accurate
	Total N [g kg <sup>-1</sup> ]	350–2460/4	Abs., SG ( $m = 2, w = 21$ ), MSC	7	70	0.97	0.00	0.05	5.87	0.90 (0.06)	0.00 (0.03)	0.09 (0.02)	3.22 (0.97)	Excellent
	Total C [g kg <sup>-1</sup> ]	350–2500/3	Refl., SG ( $m = 1, w = 21$ ), MSC	10	70	0.97	0.00	0.92	5.69	0.93 (0.03)	-0.07 (0.34)	1.44 (0.29)	3.74 (0.98)	Excellent
	pH	390–2500/5	Abs., SG ( $m = 2, w = 21$ ), MSC	6	70	0.89	0.00	0.05	3.09	0.77 (0.12)	0.00 (0.03)	0.08 (0.02)	2.00 (0.59)	Accurate
D	SOC [g kg <sup>-1</sup> ]	390–2500/3	Abs., SNV, GSD ( $m = 2, w = 21, s = 1$ )	6	70	0.97	0.00	0.81	6.01	0.95 (0.02)	-0.01 (0.35)	1.07 (0.19)	4.74 (1.23)	Excellent
	POXC [g kg <sup>-1</sup> ]	390–2460/6	Refl., SG ( $m = 2, w = 21$ )	7	69	0.95	0.00	0.03	4.72	0.92 (0.03)	0.00 (0.01)	0.05 (0.01)	3.47 (0.65)	Excellent
	Total N [g kg <sup>-1</sup> ]	370–2500/4	Abs., SG ( $m = 2, w = 21$ ), MSC	6	70	0.98	0.00	0.06	7.30	0.95 (0.04)	0.01 (0.03)	0.11 (0.02)	4.66 (1.16)	Excellent
	Total C [g kg <sup>-1</sup> ]	350–2500/2	Refl., SNV, GSD ( $m = 2, w = 21, s = 1$ )	6	70	0.97	0.00	1.15	5.44	0.93 (0.03)	0.02 (0.45)	1.61 (0.39)	4.07 (1.04)	Excellent
	pH	350–2500/6	Refl., SG ( $m = 2, w = 21$ ), MSC	9	70	0.99	0.00	0.06	9.79	0.95 (0.02)	0.00 (0.04)	0.13 (0.03)	4.83 (1.23)	Excellent
E	SOC [g kg <sup>-1</sup> ]	350–2500/3	Abs., SG ( $m = 1, w = 25$ )	3	53	0.82	0.00	1.25	2.35	0.79 (0.11)	-0.05 (0.53)	1.40 (0.42)	2.20 (0.70)	Accurate
	POXC [g kg <sup>-1</sup> ]	350–2500/4	Abs., GSD ( $m = 2, w = 21, s = 21$ )	4	53	0.82	0.00	0.05	2.41	0.82 (0.11)	0.00 (0.02)	0.05 (0.02)	2.33 (0.69)	Accurate
	Total N [g kg <sup>-1</sup> ]	350–2500/3	Abs., SG ( $m = 2, w = 21$ )	4	53	0.94	0.00	0.07	4.12	0.90 (0.04)	0.00 (0.03)	0.10 (0.02)	3.10 (0.57)	Excellent
	Total C [g kg <sup>-1</sup> ]	360–2500/3	Refl., SG ( $m = 1, w = 21$ ), MSC	6	53	0.98	0.00	1.20	7.83	0.96 (0.03)	0.04 (0.56)	1.72 (0.51)	5.27 (1.85)	Excellent
	pH	350–2500/4	Refl., SG ( $m = 1, w = 21$ ), MSC	7	53	0.98	0.00	0.10	7.15	0.95 (0.03)	0.01 (0.08)	0.16 (0.05)	4.57 (1.91)	Excellent
F	SOC [g kg <sup>-1</sup> ]	350–2500/3	Abs., SG ( $m = 1, w = 21$ ), MSC	4	53	0.66	0.00	1.59	1.73	0.51 (0.18)	0.01 (0.72)	2.00 (0.38)	1.43 (0.39)	Approximate
	POXC [g kg <sup>-1</sup> ]	380–2500/2	Refl., GSD ( $m = 2, w = 21, s = 21$ )	5	53	0.86	0.00	0.03	2.72	0.76 (0.16)	0.00 (0.01)	0.03 (0.00)	1.96 (0.60)	Approximate
	Total N [g kg <sup>-1</sup> ]	350–2500/3	Abs., SG ( $m = 1, w = 21$ ), MSC	5	53	0.92	0.00	0.06	3.47	0.83 (0.10)	0.00 (0.04)	0.09 (0.02)	2.51 (0.84)	Accurate
	Total C [g kg <sup>-1</sup> ]	370–2500/6	Abs., SG ( $m = 1, w = 21$ ), MSC	5	53	0.84	0.00	0.96	2.49	0.72 (0.18)	0.01 (0.56)	1.29 (0.24)	1.82 (0.54)	Accurate
	pH	370–2500/4	Refl., SG ( $m = 1, w = 51$ )	1	53	0.23	0.00	0.04	1.15	0.30 (0.20)	0.00 (0.02)	0.04 (0.01)	1.14 (0.19)	Poor
All	SOC [g kg <sup>-1</sup> ]	350–2500/3	Refl., SNV, GSD ( $m = 2, w = 5, s = 1$ )	7	386	0.92	0.00	1.66	3.65	0.90 (0.04)	-0.02 (0.39)	1.93 (0.32)	3.21 (0.57)	Excellent
	POXC [g kg <sup>-1</sup> ]	350–2500/1	Refl., SG ( $m = 1, w = 21$ ), MSC	8	385	0.88	0.00	0.05	2.90	0.85 (0.05)	0.00 (0.01)	0.06 (0.01)	2.60 (0.43)	Accurate
	Total N [g kg <sup>-1</sup> ]	350–2500/4	Abs., SG ( $m = 2, w = 11$ )	7	386	0.92	0.00	0.14	3.53	0.89 (0.04)	0.00 (0.03)	0.16 (0.02)	3.06 (0.46)	Excellent
	Total C [g kg <sup>-1</sup> ]	350–2500/2	Abs., SNV, GSD ( $m = 2, w = 5, s = 1$ )	6	386	0.96	0.00	2.31	5.04	0.94 (0.01)	0.00 (0.53)	2.79 (0.30)	4.16 (0.47)	Excellent
	pH	350–2500/3	Refl., SG ( $m = 2, w = 21$ ), MSC	9	386	0.95	0.00	0.12	4.40	0.90 (0.03)	0.00 (0.02)	0.15 (0.02)	3.34 (0.59)	Excellent

## 2.5 Development and evaluation of general models

In addition to the field-specific local models, we built general models for the five soil properties that included all reference samples ( $n = 386$ ) of the six fields. Even though for this sample size an independent test set would be more suitable than a cross-validation approach, we evaluated the model performance using the hold-out samples in the five-times-repeated 10-fold cross-validation, keeping, as for the local models, samples from the same GPS point and the same sampling time in the same fold. The first reason for not using an independent validation set is that the modeling approach of the general model should be similar to the one of the local models to make them comparable. The second reason is that a representative split of the dataset into a calibration and a validation set according to the spectral variability would not result in an equal number of samples per field in the validation set. Conversely, if we selected an equal sample size per field for the validation set, we would not have been able to cover the entire spectral variability. Evaluating the general models with hold-out samples of the cross-validation allowed us to calculate not only the RMSE over all samples but also the RMSE for the samples of each field individually. These field-specific RMSE values of the general model could then be compared with the RMSE values of the local models. Since the only purpose of the general models was to increase modeling efficiency for a specific combined dataset, we did not group the samples according to fields during cross-validation because the same share of samples from the same field would also be in the prediction dataset. For the general models, we cannot indicate uncertainties at a field-specific level since the folds did not always contain the same number of samples per field.

## 2.6 Model interpretation

To interpret spectral models, it is crucial to find relevant spectral features that are consistently important for a certain soil property. To identify the most important wavelength ranges in the final chosen models, we used the variable importance in projection (VIP) method first published by Wold et al. (1993) and evaluated by Chong and Jun (2005). The VIP method can deal with multicollinearity and is therefore suitable for the interpretation of spectral models as it was, for example, applied by Baumann et al. (2021). Wavelengths that have an above-average impact on the model have a VIP score above 1. We classified spectral ranges in groups of VIP scores between 1 and 1.5, 1.5 and 2, and above 2.

## 2.7 Assessment of site characteristics influencing model performance

To understand the reasons for the varying performance of the 35 developed spectral models, we studied the influence of various site characteristics on the models. To do so, we correlated the model performance parameters ( $R^2$ , RPD and

RMSE) with field size, soil texture and carbonate content and with the correlation coefficients between SOC and total N in the dataset. With six local datasets as independent variables it is hardly possible to apply statistical tests that could potentially reject a null hypothesis. Therefore, we relied on the interpretation of graphs and Pearson's moment correlation coefficients between soil properties and RMSE. Since the RMSE values are estimates with uncertainties (standard deviations; see Sect. 2.4), we used a Monte Carlo simulation and reported the mean and standard deviation of the correlation coefficients after 1000 iterations. For the identified site characteristics that showed the strongest trends in terms of model performance (carbonate content, correlation coefficient between SOC and N and variability in clay content), we looked for possible explanations in the spectral features. Thereby, we relied on the VIP analysis of the trained models, on the correlation coefficients between soil properties with spectral variables and on the correlation matrices between target variables.

## 2.8 Data organization

All analyses were performed in R version 4.0.3 (R Core Team, 2020). The spectral datasets were analyzed using the R package `simplerspec` version 0.2.0 (Baumann, 2019) in combination with the packages `prospectr` version 0.2.1 (Stevens and Ramirez-Lopez, 2020) and `caret` version 6.0–86 (Kuhn, 2020).

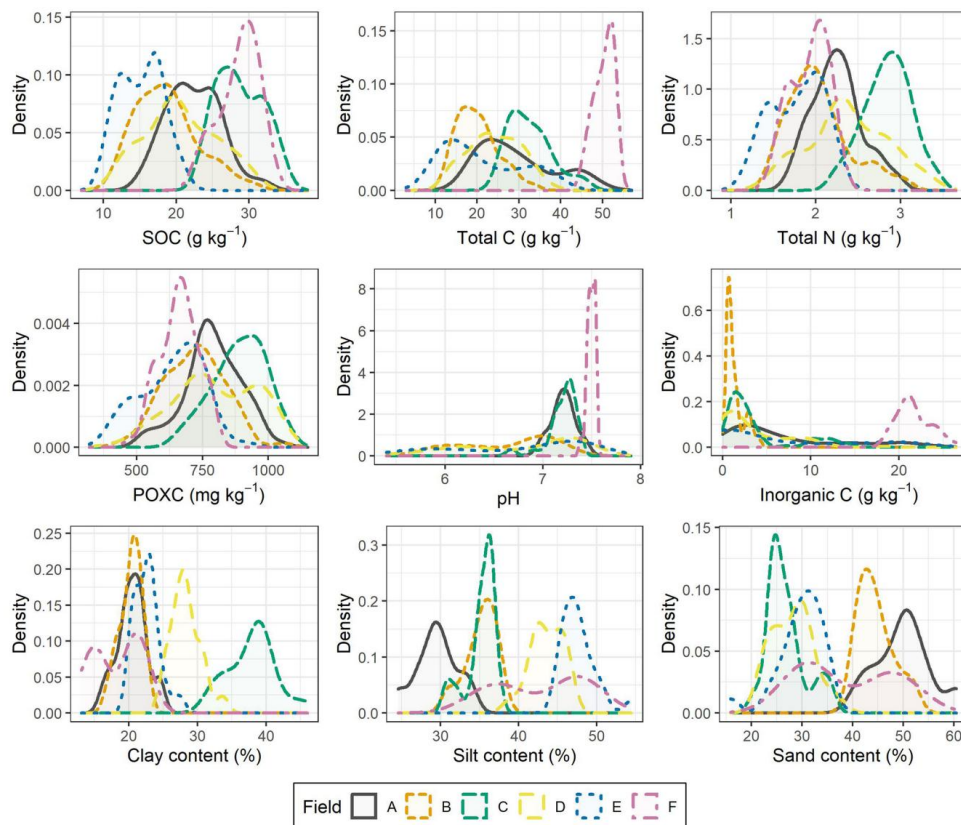
# 3 Results

## 3.1 Description of the datasets

A comparison of the data distribution between the six different fields can be seen in Fig. 1, and the corresponding statistics can be seen in Table S1 in the Supplement. The means for SOC, total N and POXC differed between the six fields, but the distribution was relatively similar for these three soil properties. The density functions for total C and pH were highly influenced by the spatial distribution of carbonate in the soil: fields B, D and E contain samples with and without carbonate, resulting in a broad distribution for both total C and pH. All soil samples of fields A and C contained carbonate in varying concentrations, resulting in a broad distribution for total C but a narrow distribution for pH. Field F showed high and only slightly varying carbonate content and therefore a very narrow distribution for total C and pH. Field C had highest mean clay content, and field A had the highest mean sand content, whereas field F showed the highest variability in soil texture.

## 3.2 Performance of spectral models

Based on RPD, 13 out of 30 local models showed an excellent performance ( $RPD > 3$ ), 11 models an accurate per-



**Figure 1.** Density plots of the reference samples for the five target properties (SOC, total C, total N, POXC and pH) and inorganic C. Fields A to D each contained 70 samples, and fields E and F each contained 53 samples. Soil texture was analyzed in 20 samples per field.

formance ( $RPD > 2$ ), 5 models an approximate performance ( $RPD > 1.4$ ), and 1 model a poor performance ( $RPD > 1.4$ ; Table 2). The six models without accurate performance were SOC, POXC and pH in fields A and F.

However, the RMSE values of the local models for pH of fields A ( $0.08 \pm 0.02$ ; mean  $\pm$  standard deviation) and F ( $0.04 \pm 0.01$ ) were similar to or smaller than the RMSE of the other three local models (between  $0.08 \pm 0.02$  and  $0.19 \pm 0.03$ ) whose performances were classified as accurate. Differently, the local models for SOC in fields A and F with only approximate performance showed a higher RMSE ( $2.43 \pm 0.55$  and  $2.00 \pm 0.38 \text{ g kg}^{-1}$ ) than the other accurately performing local models for SOC (between  $1.07 \pm 0.19$  and  $1.59 \pm 0.28 \text{ g kg}^{-1}$ ). The five general models all showed an accurate to excellent performance, with RPD values ranging from  $2.60 \pm 0.43$  to  $4.16 \pm 0.47$ .

### 3.3 Influence of pre-processing on spectral variability

For all 35 models, pre-processing improved the models compared to the raw spectra (see an example of pre-processing optimization for total C in Table S2 in the Supplement).

Although pre-processing was necessary for all models, we highlight that several pre-processing options performed similarly well within 1 standard deviation, and the differences in RMSE were often relatively small (see Table S2 in the Supplement). Figure S2 in the Supplement gives an overview of the best-performing pre-processing techniques. Most times, the first- or second-order derivatives improved the models substantially. Most models performed best when the spectra were reduced to every third wavelength and when models based on absorbance were a bit more frequently used than models based on reflectance. The combined application of SG filter and MSC was the most successful pre-processing, while a single SG filter, GSD and SNV in combination with GSD were of minor importance. Cutting of the beginning (350–400) or end of the spectra (2450–2500) sometimes improved the model performance, but since most pre-processing steps reduce the beginning and end of the spectra, it was not possible to evaluate the cutting. Similarly, it was not possible to evaluate the window width chosen in the SG filter because there is an interference with the resampling interval. A detailed list of the selected pre-processing

options of the final models and the corresponding metrics for model performance can be found in Table 2.

The sensitivity of model performance to pre-processing can be visualized with the biplots of principal component analysis (PCA). Figure 2 shows the first three biplots of the raw spectra and the spectra that were pre-processed according to the general models of the five soil properties. The raw spectra had a very high share of the explained variance (96.8 %) for the first principal component but hardly any groups according to fields could be observed with the first two principal components. All pre-processing options used for the general models decreased the explained variance for the first principal component (32.5 % to 39.6 %), and a grouping according to fields could already be seen in the biplot of the first two principal components. Thereby, in particular, field F (with the highest carbonate content) and field C (with the highest clay content) often showed clear groups. Nevertheless, in the pre-processing for pH, field E (with the highest pH variability) shows a clear group in the first biplot, and the pH variability is well represented with the first PC.

#### 3.4 Comparison of general models with local models and lab measurement error

The overall cross-validated model metrics of the general model (filled black circle in Fig. 3) indicated a good performance over all fields for all soil properties, but the field-specific model evaluation showed distinct differences among fields. The field-specific  $R^2$  of the general models of fields B, C, D and E was similar to the  $R^2$  of the local model for SOC, total C, total N and POXC (only a slight slope in Fig. 3). For pH, only fields C, D and E showed similar  $R^2$  in the local and general models, while fields A, B and F showed clearly higher  $R^2$  in the local model. On the other hand, field F had clearly lower  $R^2$  in the general model than in the local model for all soil properties except POXC. For field A,  $R^2$  was similar between the local and the general models for SOC, total C and POXC but clearly lower for total N and pH in the general model.

The field-specific RPD of the general model was, on average, 31 % lower across all soil properties compared to the local models (Fig. 3). All property–field combinations of fields B, C, D and F showed at least an approximate (RPD > 1.4) performance in the general models, whereas the seven poorly (RPD < 1.4) performing property field combinations were all from fields A and F. It can therefore be concluded that the general models could not improve the low-performing local models.

Field-specific RMSE of the general models was, on average, 47 % higher compared to the local models. However, there were substantial differences between the different fields. For field F, the field-specific RMSE values in the general models for SOC, total C, total N and pH ( $2.58 \text{ g kg}^{-1}$ ,  $0.17 \text{ g kg}^{-1}$  and  $0.09$ ) were much higher compared to those of the local model ( $2.00 \pm 0.38 \text{ g kg}^{-1}$ ,  $0.09 \pm 0.02 \text{ g kg}^{-1}$

and  $0.04 \pm 0.01$ , respectively; Fig. 3). Similarly, for total N and pH, field A had a much higher RMSE in the general model ( $0.22$  and  $0.14 \text{ g kg}^{-1}$ ) than in the local model ( $0.14 \pm 0.03$  and  $0.08 \pm 0.02$ ). On the other hand, fields C and E showed quite similar RMSE values in the local and in the general model for all soil properties except total C.

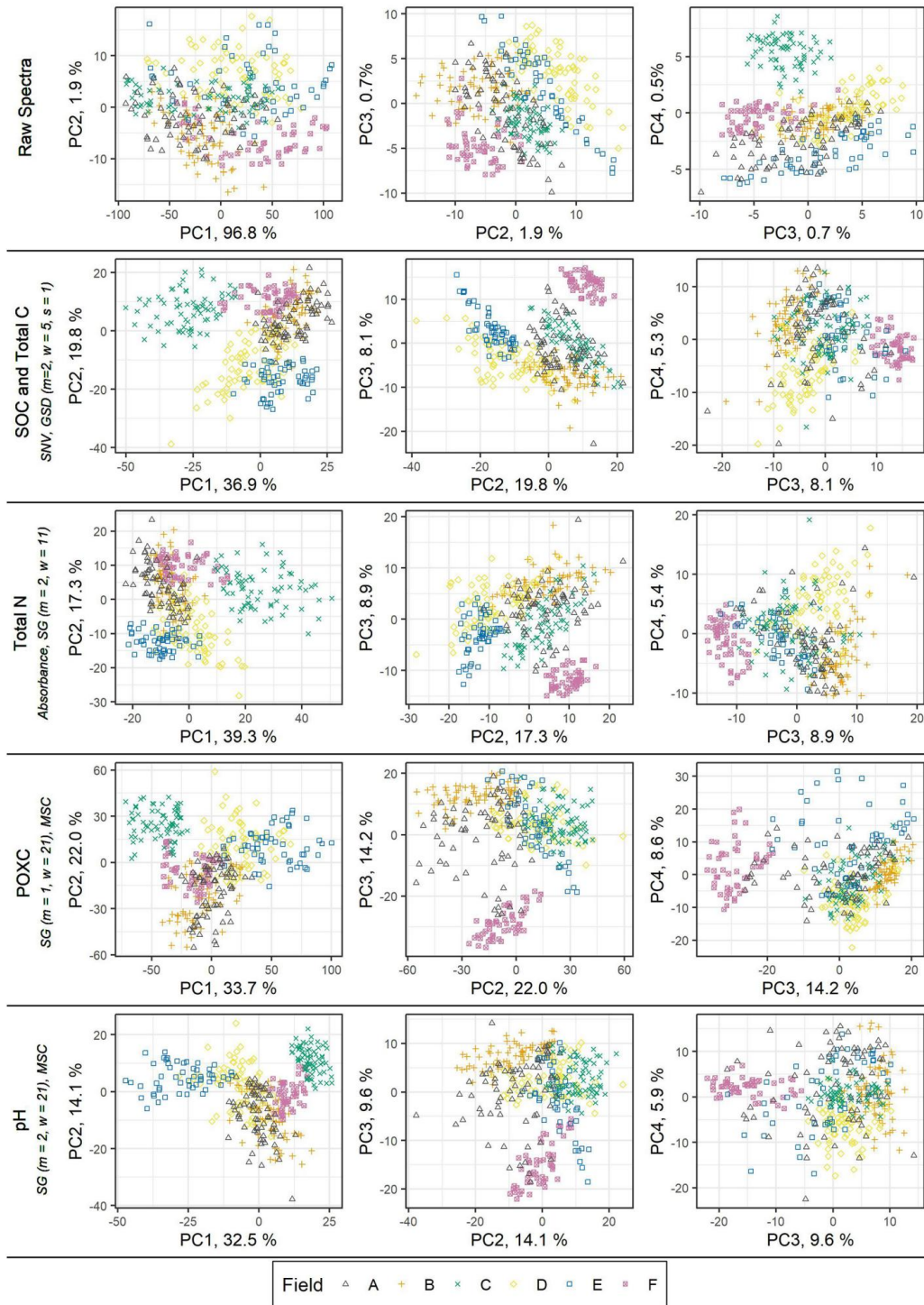
The RMSE values of the best local models were close to the overall lab measurement errors for SOC, total C and total N, a bit higher for pH, and substantially higher for POXC (Fig. 3). The RMSE values of SOC for fields B ( $1.26 \pm 0.36 \text{ g kg}^{-1}$ ) and D ( $1.07 \pm 0.19 \text{ g kg}^{-1}$ ) were within the standard deviation of the lab measurement error for SOC ( $1.01 \pm 0.40 \text{ g kg}^{-1}$ ). The overall lab measurement error for SOC was calculated from the measurement error for total C and inorganic C; therefore, for fields B and D, with only a little inorganic C, the lab measurement error for total C ( $0.83 \pm 0.25 \text{ g kg}^{-1}$ ) might be the better reference. However, the RMSE of the local spectral models of all fields exceeded the overall lab measurement errors between factors of 1.1 and 2.4 for SOC, 1.6 and 3.2 for total C, 1.3 and 2.0 for total N, 2.3 and 4.3 for POXC, and between 3.4 and 17.8 for pH. The field-specific RMSE of the general model exceeded the overall lab measurement error between factors of 1.3 and 2.3 for SOC, 2.2 and 5.2 for total C, 1.5 and 3.2 for total N, 2.8 and 4.6 for POXC, and between 8.3 and 19.9 for pH.

The VIP scores (Fig. 4) show that the most important wavelengths were dataset specific. It can be seen that in field B and, to a lower extent, in field F, the same wavelengths were important in all soil properties related to soil organic matter (SOC, total C, total N and POXC), whereas in the other fields, the VIP patterns of the different properties were more distinct from each other. However, for all the analyzed soil properties, the wavelength ranges between 400 and 750 nm (visible), as well as between 1800 and 2450 nm, were most important, while the range in between was of lower importance. Nevertheless, some models had VIP scores above 2 in the range between 750 and 1800 nm.

Prediction performance in terms of RMSE and RPD of total C for fields E and F was particularly lower in the general model than in the local model (Fig. 3). This finding can be explained with the VIP analysis (Fig. 4) that showed for the general model that the most important wavelength range was between 2150 and 2450 nm, while for the local models of fields E and F, it was in the range of 500 to 1020 nm. The local model for total N of field F showed very high VIP scores (> 2) in a small specific range between 2345 and 2369 nm, but these wavelengths were not important in the general model for total N (Fig. 4), which resulted in a much lower prediction accuracy of total N for field F in the general model compared to in the local model.

#### 3.5 Site characteristics influencing model performance

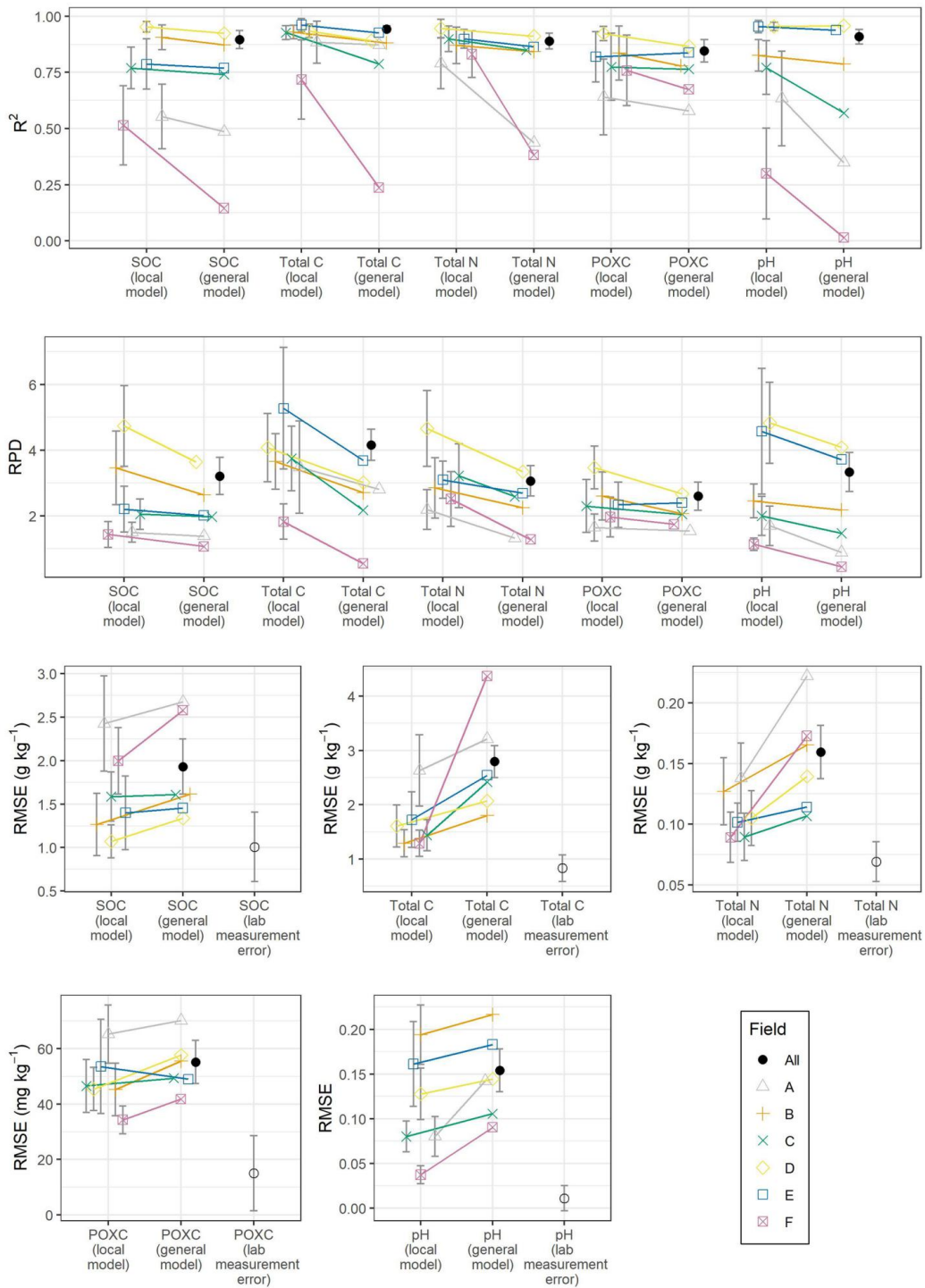
We found an order of model performance with respect to  $R^2$  and RPD that is dependent on mean carbonate content,



**Figure 2.** Biplots of principal component analysis with the first four principal components for the raw spectra and the pre-processed spectra according to the properties SOC, total C, total N, POXC and pH. The pre-processing is indicated in the figure, and, except for total N, it was conducted on reflectance spectra (SG refers to Savitzky–Golay filter ( $m$  refers to order of derivative,  $w$  refers to window width), SNV refers to standard normal variate, GSD refers to gap segment derivative ( $m$  refers to derivative,  $w$  refers to window width,  $s$  refers to segment size), and MSC refers to multiplicative scatter correction).

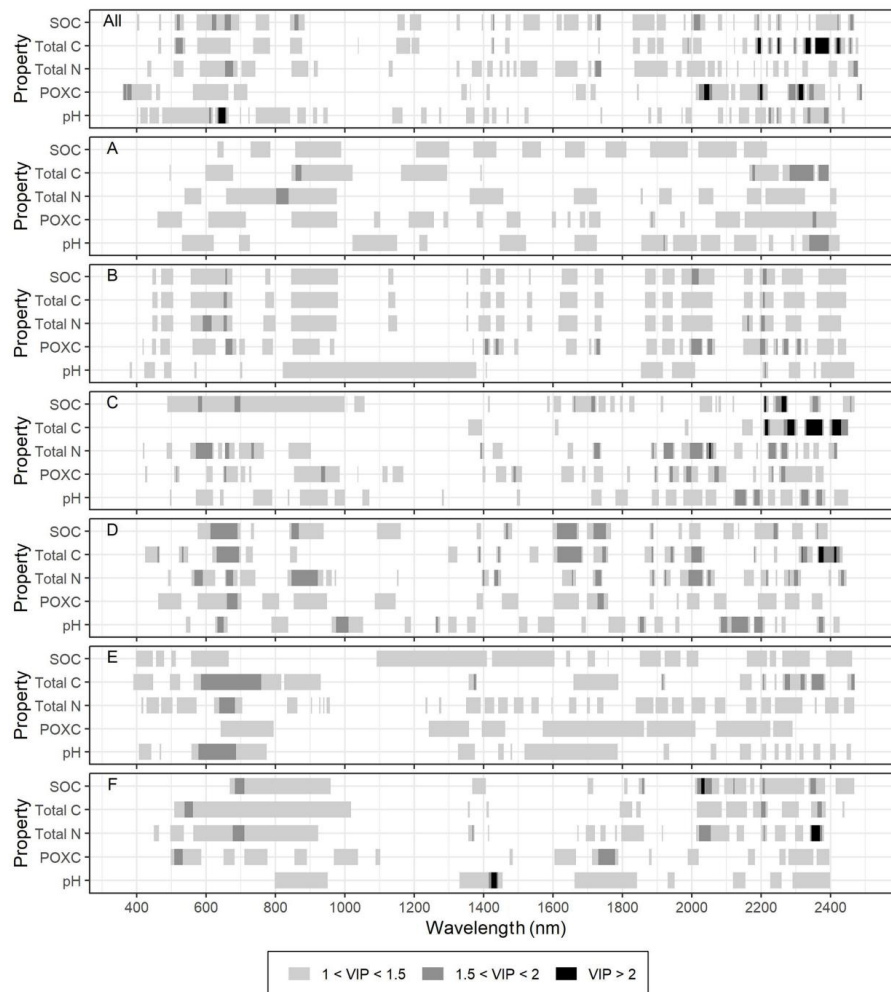
<https://doi.org/10.5194/soil-10-231-2024>

SOIL, 10, 231–249, 2024



**Figure 3.**  $R^2$ , ratio of performance to deviation (RPD) and root mean square error (RMSE) calculated from the local models and field-specifically calculated from the general model for the six fields (A–F) and the five soil properties (SOC, total C, total N, POXC and pH). The error bars for the RMSE of spectral models represent standard deviations across the repeats in the cross-validation. The overall RMSE of the general model is indicated with a filled black circle and the label “All”. The RMSE values are compared with the error of the lab measurements (mean standard error of 18 triplicates indicated with standard deviation).



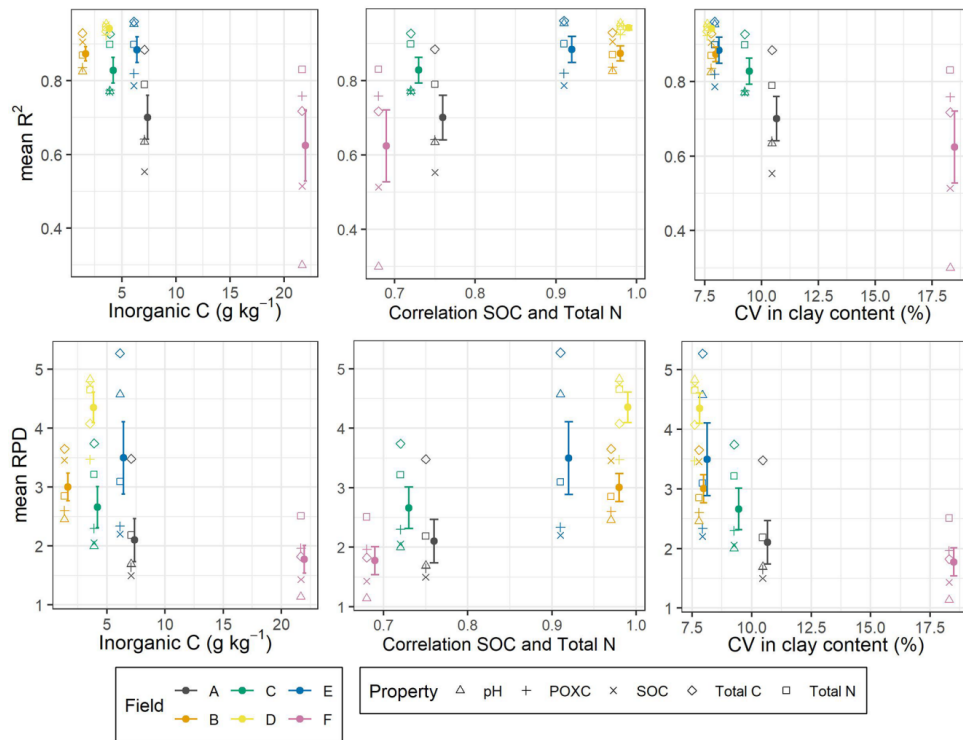


**Figure 4.** Variable importance in projection (VIP) for the local models of fields A–F and the general model that combined the datasets of all fields (All).

the correlation coefficient between SOC and total N, and the coefficient of variation in clay content (Fig. 5). Fields A and F which showed lower model performance in terms of RPD with higher carbonate content, a lower correlation coefficient between SOC and total N, and higher variability in soil texture (compare also with density plots in Fig. 1). However, in absolute prediction performance (RMSE), we only found for SOC and pH substantial correlations ( $|r| \geq 0.46$ ) between RMSE and field characteristics (Fig. 6). Compared to the three field characteristics mentioned above, we found a weaker influence of the field size; the absolute contents of sand, silt and clay; and/or the variability in the carbonate content on model performance (see Fig. S3 in the Supplement).

The influence of carbonate content on the model performance of SOC is illustrated by plotting at each wavelength the correlation coefficients between pre-processed spectral

variables and inorganic C and SOC content (Fig. 7). The correlation between SOC and spectral variables was higher in fields B, D and E than in fields A, C and F, which also explains the better model performance. In field A, SOC and carbonate content show a very similar correlation with spectral variables across the whole vis–NIR range, which makes it difficult to distinguish organic and inorganic C in field A, resulting in an excellent performance of total C but much lower performance for SOC (see Table 2). Even though the correlation between spectral variables and SOC content in field C was lower than in other fields (B, D and E), the very different correlation pattern of carbonate content still resulted in good model performance for SOC. In particular, the ranges between 600 and 1200 nm and the peaks at 1680 and 2240 nm showed different spectral features for SOC and carbonate, which corresponds to the high VIP scores at those



**Figure 5.**  $R^2$  and ratio of performance to deviation (RPD) from the local models for SOC, total C, total N, POXC and pH aggregated (mean and standard error) per field (A–F) versus mean inorganic C content, Pearson’s correlation coefficient between SOC and total N, and the coefficient of variation (CV) in clay content. The error bars represent standard deviations across the repeats in the cross-validation.

wavelengths for the SOC model in field C. In field F, correlations for both carbonate content and SOC were relatively weak, whereby carbonate content showed stronger correlations with spectral variables, which probably masked the spectral features of SOC, resulting, as for field A, in a better model for total C than SOC.

The better model performance in fields B, D and E compared to in fields A, C and F also coincided with a higher correlation between SOC and total N (Fig. 5). In general, correlation coefficients between target variables tended to be higher in fields B, D and E compared to in fields A, C and F (see Fig. 8 as an example and all correlation matrices in Fig. S4 in the Supplement).

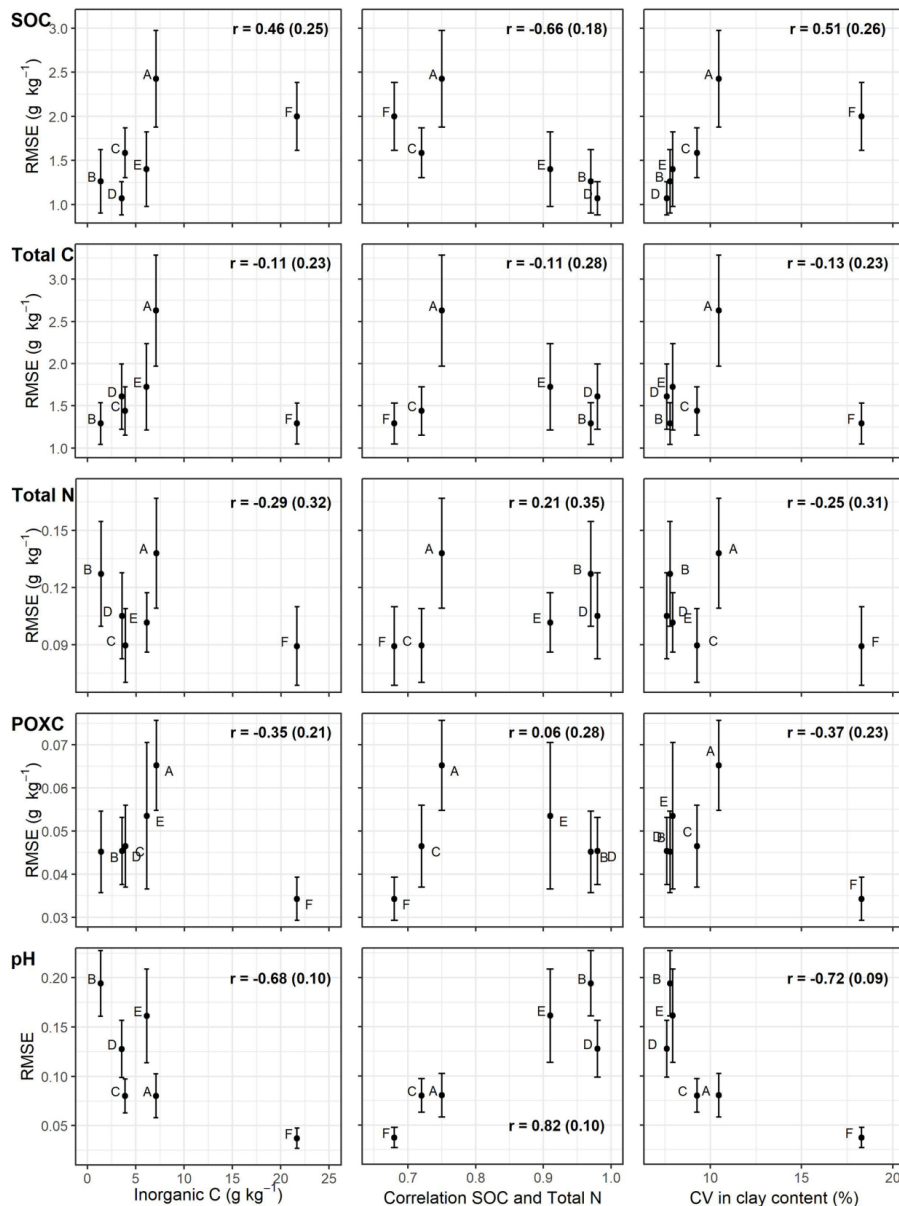
## 4 Discussion

### 4.1 Performance of local spectral models

Most of the developed local models showed an accurate performance and confirm the suitability of vis–NIR spectroscopy in projects of local or single-plot extent. The performance (based on RPD) of the two models for pH in fields A and F, which were classified as only approximate or even poor, respectively, can be explained by the low variability of

pH in these datasets (see Fig. 1) and is supported by the fact that these two models had the smallest RMSE values for pH (Fig. 3). This explanation does not hold for the other three local models that were also classified as only approximate because SOC and POXC in field A, as well as SOC in field F, showed a similar variability compared to in the other fields (Fig. 1) but higher RMSE values. However, considering the mean SOC concentration in fields A ( $22.4 \pm 3.7 \text{ g kg}^{-1}$ ) and F ( $28.6 \pm 2.7 \text{ g kg}^{-1}$ ) as well as the lab measurement error ( $1.00 \pm 0.04 \text{ g kg}^{-1}$ ), we argue that the RMSE values in fields A ( $2.43 \pm 0.55 \text{ g kg}^{-1}$ ) and F ( $2.00 \pm 0.38 \text{ g kg}^{-1}$ ) are probably, for many research projects, still acceptable, especially when taking into account that a higher sample size can be analyzed for the same costs.

In agreement with literature (Soriano-Disla et al., 2014), primary properties with a direct impact in the vis–NIR range, like SOC, total C, total N and POXC, showed an RMSE that was closer to the lab measurement error. On the other hand, pH has only an indirect impact on the spectra and thus showed a much higher RMSE compared to the lab measurement error. but the RMSE for pH in the local models (between  $0.04 \pm 0.01$  and  $0.19 \pm 0.03$ ) is probably small enough for most research purposes.

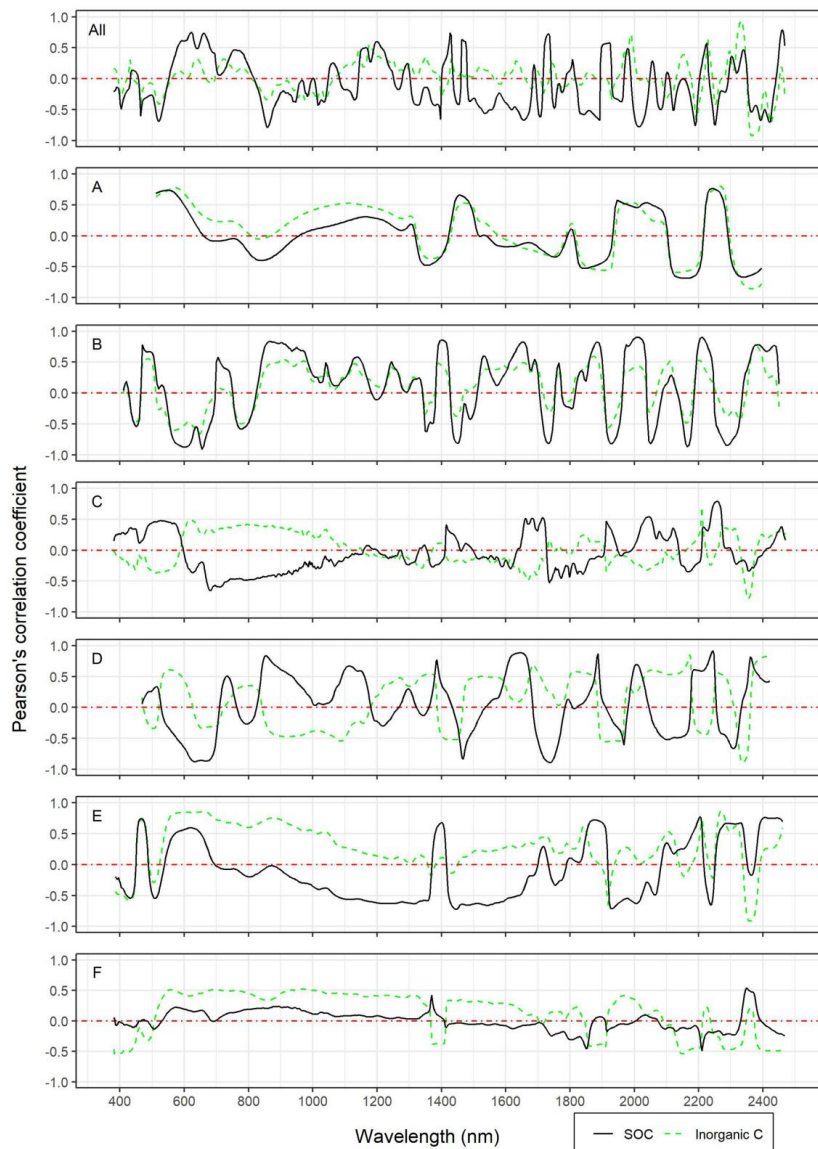


**Figure 6.** Root mean square error (RMSE) for the five target properties (SOC, total C, total N, POXC and pH) and each field (A–F) versus mean inorganic C content, Pearson’s correlation coefficient between SOC and total N, and the coefficient of variation (CV) in clay content. The error bars represent standard deviations across the repeats in the cross-validation. Pearson’s correlation coefficients are indicated as the mean and standard deviation (in parentheses) of a Monte Carlo simulation.

#### 4.2 Comparison of general models with local models

The general models could not improve the prediction of low-performing local models. This finding is especially interesting because, in this study, the general model was built with datasets of six fields that were spatially close to one another (maximal distance of 13 km) and that had the same soil type

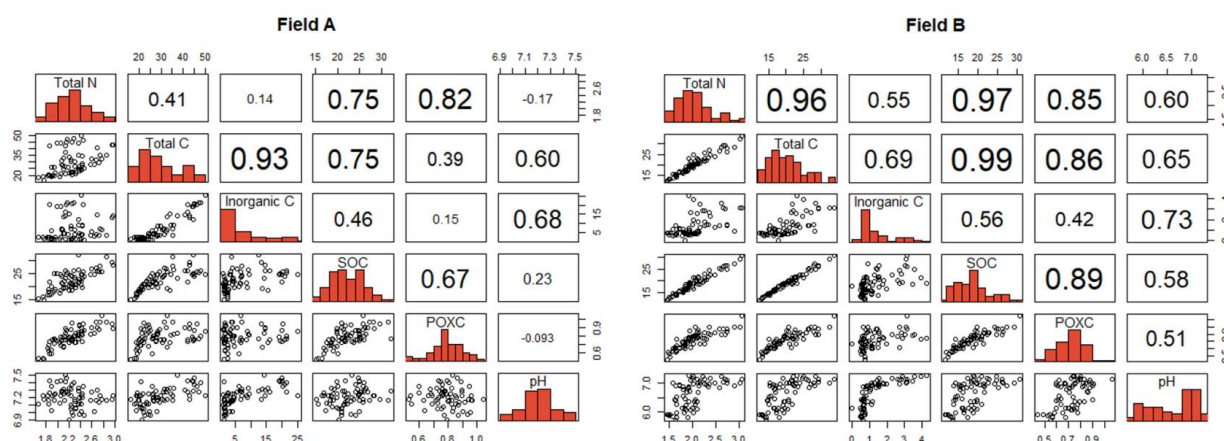
and the same parent material. However, the base moraine as a parent material can be variable, which we mainly observed in different soil textures and carbonate contents but also in the high spectral variability (see PCA biplots in Fig. 2). In this sense, we confirm the conclusions of Seidel et al. (2019) and Ng et al. (2022), who suggested that the best solution is



**Figure 7.** Correlation graphs between spectral variables at each wavelength and SOC, as well as inorganic C, for the combined dataset (All) and the individual fields (A–F). The spectra were pre-processed according to the chosen models for SOC.

always to develop a local model if enough samples (> 30) are available. This conclusion is supported in this study by the quite distinctive pattern of VIP scores between the different models (Fig. 4). The overall picture shows that the wavelengths between 2000 and 2450 nm followed by the visible range between 400 and 700 nm were most important for prediction of the investigated properties, which is in agreement with the literature (Munnaf and Mouazen, 2022; Soriano-Disla et al., 2014). Nevertheless, each local model has distinct and site-specific features that could not be at-

tributed to specific soil characteristics while being important for the model development. The development of general models where different locations are aggregated in one dataset can save costs because the number of lab analyses per location can be reduced, and less work is required for model building. Depending on the research purpose and the required measurement accuracy, the development of general models can be a very suitable and cost-effective approach. Nevertheless, this study showed that some fields (A and F) can show a poor performance in general models; hence, it is



**Figure 8.** Pairwise correlation matrices between target soil properties and inorganic C for a field with weak correlations (field A) and a field with strong correlations (field B) between the target variables. The correlation matrices for all fields can be found in the Supplement (Fig. S4).

crucial to consider what locations or datasets are being combined.

#### 4.3 Pre-processing

The selection of the optimal pre-processing scheme was crucial for model performance but was strongly dependent on the dataset. Often, MSC was the best performing pre-processing option, which was confirmed in some studies (Cambule et al., 2012; Liu et al., 2019) but disproved in others (Knox et al., 2015; Riefole et al., 2020). We therefore highly recommend considering MSC as a pre-processing option in spectral modeling but at the same time agree with Barra et al. (2021) that there is no general pre-processing solution that works for all datasets. The principal component analysis with the combined dataset of all fields (Fig. 2) illustrates this finding by the different groupings of individual field datasets due to different pre-processing. This leads to the conclusion that studies that did not optimize the pre-processing scheme for every soil property separately did eventually not make full use of the spectroscopy, which has been shown by other studies as well (Alomar et al., 2021; Rodriguez-Febereiro et al., 2022; Singh et al., 2022). Nevertheless, the property-specific optimization of spectral pre-processing is a tedious process and constrains the fast and cost-effective application of vis–NIR spectroscopy, but some progress has recently been made by Mishra et al. (2022).

#### 4.4 Site characteristics influencing model performance

We found higher model performance in fields with low carbonate content, high correlations between soil properties and low variability in clay content. We want to discuss how these identified important field characteristics influence or mask spectral features.

##### 4.4.1 Mean carbonate content

We found an influence of carbonate content, with the lowest performance of local spectral models in fields A and F. Similar observations were made by Amare et al. (2013) and McCarty et al. (2002), who argued that the absorbance bands of carbonate mask those of SOC. Looking at the correlation between spectral variables and inorganic C and SOC (Fig. 7), we can confirm this finding but have to add that, on the local scale, the relative intensity of absorption bands for carbonate and SOC varied substantially between different datasets. In this context, Reeves (2010), who showed that the spectrum of a soil sample varied greatly with its carbonate content, considered the prediction of SOC in soils with high carbonate content to be one of the open questions in vis–NIR spectroscopy research. An important point missing in this discussion is the measurement accuracy of SOC in the laboratory, which is strongly influenced by the presence of carbonate and the method used (Gojdz et al., 2009). If the soil samples contain carbonate, often two measurements must be conducted, and SOC is calculated as the difference between total C and inorganic C. Especially with a high carbonate content, the measurement error for the inorganic C content can be a substantial share of the SOC content. The higher lab measurement error with higher carbonate content might be a possible explanation for the lower model performance in soils with high carbonate content for SOC but not for the other four soil properties where model performance (in terms of RPD) still tended to be lower than in fields with little carbonate content (Fig. 5). This confirms the above-mentioned observation of spectral interference between inorganic C and organic matter and is additionally substantiated by the result that most properties of fields A and F showed a poor performance in the general models (Fig. 3). It is known that

carbonate has many more defined peaks and less interferences with organic matter in the MIR than in the vis–NIR (Reeves, 2010). Therefore, datasets that combine soil samples with high and low carbonate content might be better predicted with MIR spectroscopy. However, while all samples of field F have a high carbonate content, field A shows a broad range of carbonate contents, whereby the mean carbonate content ( $7.1 \pm 6.7 \text{ g kg}^{-1}$ ) is only slightly higher compared to the other fields. We therefore hypothesize that the lower performance of field A compared to fields B, C and D might also have additional reasons besides the field characteristics explored in this study and requires more research. The strong correlation between mean carbonate content and RMSE ( $r = -0.68 \pm 0.10$ ; Fig. 6) can be explained by the very low variability in pH in fields with high carbonate content. The narrow pH ranges in these fields consequently lead to models for pH with low RMSE but also low RPD (see Fig. 5).

#### 4.4.2 Correlations between target variables

Reflectance measured with vis–NIR spectroscopy is a combined effect of all constituents present in the soil sample (Stenberg et al., 2010), and through processing and modeling, one tries to distinguish the absorption feature of one specific soil property from the other constituents of the sample. Apart from pH, all our target variables were closely related to soil organic matter, which was, therefore, for this study, the most important soil constituent influencing the absorption features. In the case of high correlations between target variables that form part of soil organic matter, the modeling is easier because the same absorption features can be used for modeling the different properties, which was the case for field B (see VIP analysis in Fig. 4). On the other hand, a low correlation between target variables makes it more difficult to relate absorption features of organic matter to specific soil properties, which probably contributed to the lower model performance of fields A, C and F compared to fields B, D and E. The literature shows that different soil properties related to soil organic matter (e.g., SOC and total N) can show different absorption features in the vis–NIR range (Chang and Laird, 2002; Kusumo et al., 2019), which is also supported in our study (see VIP analysis in Fig. 4). However, we argue that prediction accuracy improves substantially if target variables related to soil organic matter are well correlated with each other, which was also hypothesized by Martin et al. (2002) in a one location field study.

#### 4.4.3 Variability of clay content

Unlike Stenberg et al. (2010) and Heinze et al. (2013), we did not find a better model performance with increasing mean clay content in the dataset, which might also be explained by the relatively small range in mean clay contents of between 18 % (field F) and 38 % (field C). However, we ob-

served that fields A and F, with lower model performance, also showed a higher variability in soil texture (see density plots in Fig. 1). We hypothesize that this observation is mainly an effect of our sampling design and the specific agricultural management and is therefore not generalizable. Clay and soil organic matter are claimed to be modeled with a high success rate with vis–NIR spectroscopy since they have strong absorption features (da Silva-Sangoi et al., 2022). Unfortunately, soil texture was measured using different samples than the reference dataset for the spectral modeling, so we cannot check for the correlation between soil texture and target variables. However, in this study, the correlation may be relatively low for the following reason: we took samples from different depths (0–5, 5–10 and 10–20 cm) within the past tillage layer and therefore expect that the soil texture is homogenized across the sampling depth. Since all fields are now under organic reduced-tillage management, the three soil layers show quite distinct soil organic matter contents (see Fig. S5 in the Supplement) but, very probably, similar soil textures. Therefore, a high (horizontal) variability in soil texture in a field (e.g., clay content) without a strong correlation to organic matter could have added “noise” to the spectrum, which worsened the prediction accuracy in our specific sampling design. Nevertheless, in untilled soils or more distinct depth segments, a high variability in soil texture may not be a disadvantage in vis–NIR modeling because it might also be correlated with organic matter.

## 5 Conclusions

This study investigated the impact of site characteristics on vis–NIR modeling performances and compared a local and a general modeling approach. Among the 35 models, 29 performed accurately or even excellently, whereby the RMSE was close to the lab measurement error, and achieved prediction accuracies are probably, for many research purposes, acceptable. The local models with the lowest performance were all from fields A and F, and we found three field characteristics in their datasets that interfered with model performance. Fields A and F had higher mean carbonate content, lower correlation between target soil properties and higher variability in soil texture compared to the other fields. The influence of soil texture variability was mainly an issue in this specific sampling design, whereas the influence of carbonate content and correlation between soil properties can probably be generalized due to observed spectral features and VIP analysis. Before starting a local vis–NIR project, testing for inorganic C content can be done relatively easily, but it is almost impossible to know beforehand the correlations between different soil properties. One can only be aware of the correlation issue and consider potential gradients of soil properties while designing the sampling design, which is probably more important and feasible in disturbed or agricultural soils than in natural undisturbed soils. In terms of efficiency in data

collection, we conclude that, in a region, several target sites (or agricultural fields) with low carbonate contents can be combined in a general model with only a minor reduction in model performance. A general model for multiple target sites then also allows us to reduce the number of wet chemistry analyses. Whether or not several target sites with high carbonate content can be combined in one general model using vis–NIR spectroscopy is a question that requires further research. However, since carbonates show fewer interferences with organic matter in the MIR than in the vis–NIR spectral range, soil samples from sites with high carbonate content might be better predicted with MIR spectroscopy. Yet, the application of laboratory vis–NIR spectroscopy in projects of local extent provides the opportunity to increase the spatial or temporal resolution in a sampling design cost effectively with only minor decreases in measurement accuracy.

**Code and data availability.** Data and R-codes are available on a Zenodo repository (<https://doi.org/10.5281/zenodo.10691694>, Oberholzer and Summerauer, 2024).

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**Competing interests.** The contact author has declared that none of the authors has any competing interests.

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## References

- Allory, V., Cambou, A., Moulin, P., Schwartz, C., Cannavo, P., Vidal-Beaudet, L., and Barthes, B. G.: Quantification of soil organic carbon stock in urban soils using visible and near infrared reflectance spectroscopy (VNIRS) in situ or in laboratory conditions, *Sci. Total Environ.*, 686, 764–773, <https://doi.org/10.1016/j.scitotenv.2019.05.192>, 2019.
- Alomar, S., Mireei, S. A., Hemmat, A., Masoumi, A. A., and Khademi, H.: Comparison of Vis/SWNIR and NIR spectrometers combined with different multivariate techniques for estimating soil fertility parameters of calcareous topsoil in an arid climate, *Biosys. Eng.*, 201, 50–66, <https://doi.org/10.1016/j.biosystemseng.2020.11.007>, 2021.
- Amare, T., Hergarten, C., Hurni, H., Wolfgramm, B., Yitaferu, B., and Selassie, Y. G.: Prediction of Soil Organic Carbon for Ethiopian Highlands Using Soil Spectroscopy, *ISRN Soil Sci.*, 2013, 720589, <https://doi.org/10.1155/2013/720589>, 2013.
- Angelopoulou, T., Balafoutis, A., Zalidis, G., and Bochtis, D.: From Laboratory to Proximal Sensing Spectroscopy for Soil Organic Carbon Estimation – A Review, *Sustainability-Basel*, 12, 443, <https://doi.org/10.3390/su12020443>, 2020.
- Barra, I., Haefele, S. M., Sakrabani, R., and Kebede, F.: Soil spectroscopy with the use of chemometrics, machine learning and pre-processing techniques in soil diagnosis: Recent advances – A review, *Trac.-Trend. Anal. Chem.*, 135, 116166, <https://doi.org/10.1016/j.trac.2020.116166>, 2021.
- Baumann, P.: philipp-baumann/simplerspec: Beta release simplerspec 0.1.0 for zenodo, Zenodo [code], <https://doi.org/10.5281/zenodo.3303637>, 2019.
- Baumann, P., Lee, J., Frossard, E., Schönholzer, L. P., Diby, L., Hgaza, V. K., Kiba, D. I., Sila, A., Sheperd, K., and Six, J.: Estimation of soil properties with mid-infrared soil spectroscopy across yam production landscapes in West Africa, *Soil*, 7, 717–731, <https://doi.org/10.5194/soil-7-717-2021>, 2021.
- Breure, T. S., Prout, J. M., Haefele, S. M., Milne, A. E., Hannam, J. A., Moreno-Rojas, S., and Corstanje, R.: Comparing the effect of different sample conditions and spectral libraries on the prediction accuracy of soil properties from near- and mid-infrared spectra at the field-scale, *Soil Till. Res.*, 215, 105196, <https://doi.org/10.1016/j.still.2021.105196>, 2022.
- Brown, D. J.: Using a global VNIR soil-spectral library for local soil characterization and landscape modeling in a 2nd-order Uganda watershed, *Geoderma*, 140, 444–453, <https://doi.org/10.1016/j.geoderma.2007.04.021>, 2007.
- Camargo, L. A., do Amaral, L. R., dos Reis, A. A., Brasco, T. L., and Magalhaes, P. S. G.: Improving soil organic carbon mapping with a field-specific calibration approach through diffuse reflectance spectroscopy and machine learning algorithms, *Soil Use Manage.*, 38, 292–303, <https://doi.org/10.1111/sum.12775>, 2022.
- Cambule, A. H., Rossiter, D. G., Stoorvogel, J. J., and Smaling, E. M. A.: Building a near infrared spectral library for soil organic carbon estimation in the Limpopo National Park, Mozambique, *Geoderma*, 183, 41–48, <https://doi.org/10.1016/j.geoderma.2012.03.011>, 2012.

- Chang, C. W. and Laird, D. A.: Near-infrared reflectance spectroscopic analysis of soil C and N, *Soil Sci.*, 167, 110–116, <https://doi.org/10.1097/00010694-200202000-00003>, 2002.
- Chang, C. W., Laird, D. A., Mausbach, M. J., and Hurburgh, C. R.: Near-infrared reflectance spectroscopy-principal components regression analyses of soil properties, *Soil Sci. Soc. Am. J.*, 65, 480–490, <https://doi.org/10.2136/sssaj2001.652480x>, 2001.
- Chong, I. G. and Jun, C. H.: Performance of some variable selection methods when multicollinearity is present, *Chemom. Intell. Lab. Syst.*, 78, 103–112, <https://doi.org/10.1016/j.chemolab.2004.12.011>, 2005.
- da Silva-Sangoi, D. V., Horst, T. Z., Moura-Bueno, J. M., Dalmonin, R. S. D., Sebem, E., Gebler, L., and Santos, M. D.: Soil organic matter and clay predictions by laboratory spectroscopy: Data spatial correlation, *Geoderma Reg.*, 28, e00486, <https://doi.org/10.1016/j.geoder.2022.e00486>, 2022.
- Durner, W. and Iden, S. C.: The improved integral suspension pressure method (ISP plus) for precise particle size analysis of soil and sedimentary materials, *Soil Till. Res.*, 213, 105086, <https://doi.org/10.1016/j.still.2021.105086>, 2021.
- Ellinger, M., Merbach, I., Werban, U., and Liess, M.: Error propagation in spectrometric functions of soil organic carbon, *Soil*, 5, 275–288, <https://doi.org/10.5194/soil-5-275-2019>, 2019.
- Goidts, E., Van Wesemael, B., and Crucifix, M.: Magnitude and sources of uncertainties in soil organic carbon (SOC) stock assessments at various scales, *Eur. J. Soil Sci.*, 60, 723–739, <https://doi.org/10.1111/j.1365-2389.2009.01157.x>, 2009.
- Greenberg, I., Seidel, M., Vohland, M., Koch, H. J., and Ludwig, B.: Performance of in situ vs. laboratory mid-infrared soil spectroscopy using local and regional calibration strategies, *Geoderma*, 409, 115614, <https://doi.org/10.1016/j.geoderma.2021.115614>, 2022.
- Grunwald, S., Yu, C. R., and Xiong, X.: Transferability and Scalability of Soil Total Carbon Prediction Models in Florida, USA, *Pedosphere*, 28, 856–872, [https://doi.org/10.1016/s1002-0160\(18\)60048-7](https://doi.org/10.1016/s1002-0160(18)60048-7), 2018.
- Hastie, T., Tibshirani, R., and Friedman, J. H.: The elements of statistical learning: data mining, inference, and prediction, Second edition, corrected at 12th printing 2017, Springer series in statistics, Springer, New York, NY, <https://doi.org/10.1007/978-0-387-84858-7>, 2017.
- Heinze, S., Vohland, M., Joergensen, R. G., and Ludwig, B.: Usefulness of near-infrared spectroscopy for the prediction of chemical and biological soil properties in different long-term experiments, *J. Plant Nutr. Soil Sci.*, 176, 520–528, <https://doi.org/10.1002/jpln.201200483>, 2013.
- Hutengs, C., Seidel, M., Oertel, F., Ludwig, B., and Vohland, M.: In situ and laboratory soil spectroscopy with portable visible-to-near-infrared and mid-infrared instruments for the assessment of organic carbon in soils, *Geoderma*, 355, 113900, <https://doi.org/10.1016/j.geoderma.2019.113900>, 2019.
- Kennard, R. W. and Stone, L. A.: Computer aided design of experiments, *Technometrics*, 11, 137–148, <https://doi.org/10.2307/1266770>, 1969.
- Knox, N. M., Grunwald, S., McDowell, M. L., Bruland, G. L., Myers, D. B., and Harris, W. G.: Modelling soil carbon fractions with visible near-infrared (VNIR) and mid-infrared (MIR) spectroscopy, *Geoderma*, 239, 229–239, <https://doi.org/10.1016/j.geoderma.2014.10.019>, 2015.
- Kuang, B. and Mouazen, A. M.: Calibration of visible and near infrared spectroscopy for soil analysis at the field scale on three European farms, *Eur. J. Soil Sci.*, 62, 629–636, <https://doi.org/10.1111/j.1365-2389.2011.01358.x>, 2011.
- Kuang, B. and Mouazen, A. M.: Influence of the number of samples on prediction error of visible and near infrared spectroscopy of selected soil properties at the farm scale, *Eur. J. Soil Sci.*, 63, 421–429, <https://doi.org/10.1111/j.1365-2389.2012.01456.x>, 2012.
- Kuhn, M.: caret: Classification and Regression Training, R package [code], <https://doi.org/10.18637/jss.v028.i05>, 2020.
- Kuhn, M. and Johnson, K.: Applied predictive modeling, Springer, New York, <https://doi.org/10.1007/978-1-4614-6849-3>, 2013.
- Kusumo, B. H., Sukartono, S., Bustan, B., and Purwanto, Y. A.: Total nitrogen in rice paddy field independently predicted from soil carbon using Near Infrared Reflectance Spectroscopy (NIRS), 4th Annual Applied Science and Engineering Conference (AASEC), Univ Pendidikan Indonesia, Sch Postgraduate Studies, Tech. Vocat. Educ. St., Bali, INDONESIA, IOP Publishing, <https://doi.org/10.1088/1742-6596/1402/2/022096>, 2019.
- Li, H. Y., Jia, S. Y., and Le, Z. C.: Prediction of Soil Organic Carbon in a New Target Area by Near-Infrared Spectroscopy: Comparison of the Effects of Spiking in Different Scale Soil Spectral Libraries, *Sensors*, 20, 4357, <https://doi.org/10.3390/s20164357>, 2020.
- Liu, S., Shen, H., Chen, S., Zhao, X., Biswas, A., Xiaolin, J., Shi, Z., and Fang, J.: Estimating forest soil organic carbon content using vis-NIR spectroscopy: Implications for large-scale soil carbon spectroscopic assessment, *Geoderma*, 348, 37–44, <https://doi.org/10.1016/j.geoderma.2019.04.003>, 2019.
- Lobsey, C. R., Viscarra Rossel, R. A., Roudier, P., and Hedley, C. B.: rs-local data-mines information from spectral libraries to improve local calibrations, *Eur. J. Soil Sci.*, 68, 840–852, <https://doi.org/10.1111/ejss.12490>, 2017.
- Lucas, S. T. and Weil, R. R.: Can a Labile Carbon Test be Used to Predict Crop Responses to Improve Soil Organic Matter Management?, *Agron. J.*, 104, 1160–1170, <https://doi.org/10.2134/agronj2011.0415>, 2012.
- Martin, P. D., Malley, D. F., Manning, G., and Fuller, L.: Determination of soil organic carbon and nitrogen at the field level using near-infrared spectroscopy, *Can. J. Soil Sci.*, 82, 413–422, <https://doi.org/10.4141/s01-054>, 2002.
- McCarty, G., Reeves, J., Reeves, V., Follett, R., and Kimble, J.: Mid-Infrared and Near-Infrared Diffuse Reflectance Spectroscopy for Soil Carbon Measurement, *Soil Sci. Soc. Am. J.*, 66, 640–646, <https://doi.org/10.2136/sssaj2002.6400a>, 2002.
- Mishra, P., Roger, J. M., Marini, F., Biancolillo, A., and Rutledge, D. N.: Pre-processing ensembles with response oriented sequential alternation calibration (PROSAC): A step towards ending the pre-processing search and optimization quest for near-infrared spectral modelling, *Chemom. Intell. Lab. Syst.*, 222, 104497, <https://doi.org/10.1016/j.chemolab.2022.104497>, 2022.
- Molinaro, A. M., Simon, R., and Pfeiffer, R. M.: Prediction error estimation: a comparison of resampling methods, *Bioinformatics*, 21, 3301–3307, <https://doi.org/10.1093/bioinformatics/bti499>, 2005.
- Munnaf, M. A. and Mouazen, A. M.: Removal of external influences from on-line vis-NIR spectra for predicting soil or-



- ganic carbon using machine learning, *Catena*, 211, 106015, <https://doi.org/10.1016/j.catena.2022.106015>, 2022.
- Ng, W., Minasny, B., Jones, E., and McBratney, A.: To spike or to localize? Strategies to improve the prediction of local soil properties using regional spectral library, *Geoderma*, 406, 115501, <https://doi.org/10.1016/j.geoderma.2021.115501>, 2022.
- Oberholzer, S. and Summerauer, L.: Dataset and R-codes for Publication: “Best performances of visible-near infrared models in soils with little carbonate – a field study in Switzerland” (Submission version) (v.1.0), Zenodo [code], <https://doi.org/10.5281/zenodo.10691694>, 2024.
- R Core Team: R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing [code], <https://www.R-project.org> (last access: 25 February 2024), 2020.
- Ramirez-Lopez, L., Behrens, T., Schmidt, K., Stevens, A., Dematte, J. A. M., and Scholten, T.: The spectrum-based learner: A new local approach for modeling soil vis-NIR spectra of complex datasets, *Geoderma*, 195, 268–279, <https://doi.org/10.1016/j.geoderma.2012.12.014>, 2013.
- Reeves, J. B.: Near- versus mid-infrared diffuse reflectance spectroscopy for soil analysis emphasizing carbon and laboratory versus on-site analysis: Where are we and what needs to be done?, *Geoderma*, 158, 3–14, <https://doi.org/10.1016/j.geoderma.2009.04.005>, 2010.
- Riefolo, C., Castrignano, A., Colombo, C., Conforti, M., Ruggieri, S., Vitti, C., and Buttafuoco, G.: Investigation of soil surface organic and inorganic carbon contents in a low-intensity farming system using laboratory visible and near-infrared spectroscopy, *Arch. Agron. Soil Sci.*, 66, 1436–1448, <https://doi.org/10.1080/03650340.2019.1674446>, 2020.
- Rodriguez-Feberero, M., Dafonte, J., Fandino, M., Cancela, J. J., and Rodriguez-Perez, J. R.: Evaluation of Spectroscopy and Methodological Pre-Treatments to Estimate Soil Nutrients in the Vineyard, *Remote Sens.*, 14, 1326, <https://doi.org/10.3390/rs14061326>, 2022.
- Seidel, M., Hutengs, C., Ludwig, B., Thiele-Bruhn, S., and Vohland, M.: Strategies for the efficient estimation of soil organic carbon at the field scale with vis-NIR spectroscopy: Spectral libraries and spiking vs. local calibrations, *Geoderma*, 354, 113856, <https://doi.org/10.1016/j.geoderma.2019.07.014>, 2019.
- Shen, Z. F., Ramirez-Lopez, L., Behrens, T., Cui, L., Zhang, M. X., Walden, L., Wetterlind, J., Shi, Z., Sudduth, K. A., Song, Y. Z., Catambay, K., and Rossel, R. A. V.: Deep transfer learning of global spectra for local soil carbon monitoring, *ISPRS J. Photogramm. Remote Sens.*, 188, 190–200, <https://doi.org/10.1016/j.isprsjprs.2022.04.009>, 2022.
- Singh, K., Aitkenhead, M., Fidelis, C., Yinil, D., Sanderson, T., Snoeck, D., and Field, D. J.: Optimization of spectral pre-processing for estimating soil condition on small farms, *Soil Use Manage.*, 38, 150–163, <https://doi.org/10.1111/sum.12684>, 2022.
- Soriano-Disla, J. M., Janik, L. J., Viscarra Rossel, R. A., Macdonald, L. M., and McLaughlin, M. J.: The Performance of Visible, Near-, and Mid-Infrared Reflectance Spectroscopy for Prediction of Soil Physical, Chemical, and Biological Properties, *Appl. Spectrosc. Rev.*, 49, 139–186, <https://doi.org/10.1080/05704928.2013.811081>, 2014.
- Stenberg, B., Rossel, R. A. V., Mouazen, A. M., and Wetterlind, J.: Visible and near infrared spectroscopy in soil science, edited by: Sparks, D. L., *Adv. Agron.*, 107, 163–215, [https://doi.org/10.1016/s0065-2113\(10\)07005-7](https://doi.org/10.1016/s0065-2113(10)07005-7), 2010.
- Stevens, A. S. and Ramirez-Lopez, L.: An introduction to the prospectr package, R package [code], <https://cran.r-project.org/web/packages/prospectr/vignettes/prospectr.html> (last access: 25 February 2024), 2020.
- Taubner, H., Roth, B., and Tippkötter, R.: Determination of soil texture: Comparison of the sedimentation method and the laser-diffraction analysis, *J. Plant Nutr. Soil Sci.*, 172, 161–171, <https://doi.org/10.1002/jpln.200800085>, 2009.
- Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., and Samson-Liebig, S. E.: Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use, *Am. J. Alternative Agr.*, 18, 3–17, <https://www.jstor.org/stable/pdf/44503242.pdf> (last access: 25 February 2024), 2003.
- Wetterlind, J. and Stenberg, B.: Near-infrared spectroscopy for within-field soil characterization: small local calibrations compared with national libraries spiked with local samples, *Eur. J. Soil Sci.*, 61, 823–843, <https://doi.org/10.1111/j.1365-2389.2010.01283.x>, 2010.
- Wold, S., Martens, H., and Wold, H.: The multivariate calibration problem in chemistry solved by the PLS method, *Matrix Pencils*, Berlin, Heidelberg, Springer, 286–293, <https://doi.org/10.1007/BFb0062108>, 1983.
- Wold, S., Johansson, E., and Cocchi, M.: PLS-partial least squares projections to latent structures, in: *3D QSAR in drug design*, edited by: Kubinyi, H., Folkers, G., and Martin, Y., Escom, Leiden, 523–550, <https://doi.org/10.1007/0-306-46858-1>, 1993.
- Zhang, L., Yang, X. M., Drury, C., Chantigny, M., Gregorich, E., Miller, J., Bittman, S., Reynolds, W. D., and Yang, J. Y.: Infrared spectroscopy estimation methods for water-dissolved carbon and amino sugars in diverse Canadian agricultural soils, *Can. J. Soil Sci.*, 98, 484–499, <https://doi.org/10.1139/cjss-2018-0027>, 2018.
- Zhao, D. X., Arshad, M., Wang, J., and Triantafyllis, J.: Soil exchangeable cations estimation using Vis-NIR spectroscopy in different depths: Effects of multiple calibration models and spiking, *Comput. Electron. Agric.*, 182, 105990, <https://doi.org/10.1016/j.compag.2021.105990>, 2021.

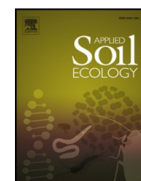
## Paper 3: Effective Microorganisms

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## No effect on biological or chemical soil properties when amended with effective microorganisms for improved cover crop decomposition

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## ABSTRACT

The implementation of cover crops into a crop rotation can contribute to a more sustainable soil management. For the improved decomposition of cover crop residues, the commercial inoculant Effective Microorganisms® (EM) is increasingly applied. Despite its extensive application, comprehensive studies on the effect of EM application on soil processes are lacking, since rarely a clean differentiation between an EM-effect (induced by living EM directly) or a substrate effect (induced by the accompanying EM substrate) is made. To determine the potential effects of EM application after cover crop integration to soil we conducted a lab incubation experiment under spring-like conditions in temperate climates and applied EM either on bare soil or on cover crops prior to soil incorporation at recommended and 100 times the recommended doses. Control groups included treatments with no EM addition and a sterilized EM solution applied at 100 times the recommended dose. Over a monitoring period of 28 days, the application of EM at the recommended dose showed no consistent effect on soil respiration, microbial bound carbon or nitrogen, soil pH, permanganate oxidizable carbon or water extractable nutrients and trace elements. Any observed effects in the treatment that received 100 times the recommended dose was attributed to the substrate introduced with the EM solution rather than the living EM themselves. Amplicon sequencing showed that certain EM taxa could be detected in soil at low abundance after EM application, but only when EM were applied at 100 times the recommended dose. We conclude that the application of EM did not produce a discernible effect on soil biological or chemical properties, nor did it influence the decomposition process of the cover crop.

### 1. Introduction

Sustainable agroecosystems aim to maintain a high level of soil fertility to minimize the external inputs. For that, periods of bare soil should be avoided because they lead to nutrient losses, soil erosion and loss in soil organic matter, leading to a decrease of soil fertility (Daryanto et al., 2018). Cover crops bridge the break time between two main crops and are therefore a key element in soil fertility and nutrient management (Thorup-Kristensen et al., 2003). However, particularly in organic farming systems with reduced tillage, where cover crops are shallowly incorporated or left on the soil surface, the management of cover crops faces major challenges. A fast decomposition of the

incorporated cover crop residues is crucial for a good seedbed preparation (Gollner et al., 2020; Vincent-Caboud et al., 2017). Yet, when environmental conditions are cold and wet, as it often happens during spring in temperate climates, the cover crop material on the soil surfaces often does not decompose properly but becomes slimy and malodorous. This largely affects the seedbed preparation and the growth of the subsequent crop. Ideally, most cover crop material should be decomposed to smaller pieces within 10 days so that residues do not disturb the sowing of the subsequent cash crop.

One increasingly used approach to accelerate the decomposition process of freshly incorporated cover crop material is the use of microbial inoculants. The most applied microbial inoculant with this purpose

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is Effective Microorganisms® (EM), a product that was developed in the 1980s and was trademarked by EMRO Japan (2023). The commercial EM product consists of a mix of up to 80 naturally occurring aerobic and anaerobic microorganisms which coexist in a liquid culture (Higa, 1991). The exact composition is not made available by the producer, but previous analyses have shown that EM solutions are mainly dominated by lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*) and yeasts (*Saccharomyces cerevisiae*, *Candida utilis*) with smaller numbers of photosynthetic bacteria (*Rhodospseudomonas palustris*, *Rhodobacter sphaeroides*), actinomycetes (*Streptomyces albus*, *Streptomyces griseus*) and fermenting fungi (*Aspergillus oryzae*; Ahn et al., 2014; Xu, 2000). Similar to other plant growth promoting rhizobacteria (PGPR), EM are applied to alter the soil microbial community towards more favorable growing conditions for the crop (Gouda et al., 2018). In practice the expectations of EM application are, among others, enhanced soil fertility, higher crop yield and quality, higher nutrient use efficiency of organic fertilizers and amendments, improved soil physical characteristics, and better pathogen control (Balogun et al., 2016; Olle and Williams, 2013). In the specific application of EM on cover crops before shallow incorporation, farmers expect to accelerate the decomposition process, improve nutrient cycling and soil organic matter formation (EM Schweiz, 2023).

The suggested mechanisms how EM might influence the decomposition of cover crop biomass or other organic matter in soil are derived from analogies of food preservation and processing of kitchen wastes through anaerobic fermentation widely practiced in Asia. For anaerobic fermentation, it is of most importance that fermenting microorganisms are dominant over putrefactive bacteria that might damage the product and lead to malodorous and potentially harmful metabolites (Rhee et al., 2011; Wang et al., 2001). Putrefaction is associated with the emission of ammonia, methane and nitrogen (N) oxides and occurs under at least partly anoxic conditions. Effective microorganisms are supposed to avoid putrefaction in periods or locations of low oxygen availability and shift the metabolic pathways towards fermentation and stabilization of organic matter (Higa and Parr, 1994). Most arable soils are mainly under oxic conditions, but anoxic microsites are always present in well aerated soils as well (Keiluweit et al., 2018; Keiluweit et al., 2017; Lacroix et al., 2022). Accordingly, EM is proclaimed to benefit the decomposition of organic matter even in well aerated soil with rather oxic conditions (Hu et al., 2018; Javaid, 2011). Lactic acid bacteria and yeasts, the dominant groups in the EM consortia, are facultative anaerobic, meaning that they can survive in an environment with oxygen and are therefore also found in natural soils (Lamont et al., 2017). The application of EM for enhanced organic matter decomposition relies thus on the assumptions that first the inoculated EM can establish themselves in the soil system and second that they play a dominant role in the decomposition process.

Up to now only a very limited number of studies surveyed the effect of EM application on critical soil properties such as soil respiration (Fatunbi and Ncube, 2009; Schenck zu Schweinsberg-Mickan and Müller, 2009; Valarini et al., 2003) or nutrient availability (Hu et al., 2018; Jusoh et al., 2013; Van Fan et al., 2018; Zhong et al., 2018). Other studies suggest, but did not demonstrate that the enhanced decomposition of organic matter via EM application could also lead to an increased availability of micronutrients (Daur, 2016), or a reduction of potential toxic trace elements (PTTEs), (Zhou et al., 2020). Unfortunately, many of the studies that tested EM failed in differentiating between i) the EM-effect (an effect that is induced by the actual living EM in the inoculant) and ii) the substrate effect (an effect that is induced by the nutrients, carbon sources and other compounds that is provided in combination with the EM inoculant solution). By not differentiating between these two effects, it is easy to reach misleading conclusions, yet, the inclusion of these critical controls quickly increases the number of necessary samples.

Given the large discrepancy between expectations and actual scientific evidence on the actual effects of EM we conducted a lab incubation study to rigorously differentiate between EM induced effects and

substrate induced effects on soil properties during cover crop decomposition. The question whether or not the decomposition of freshly incorporated cover crops can be positively influenced by EM application has a particular relevance, since the lack of adequate alternatives was identified as a major challenge that hampers the practice of cover cropping in organic reduced tillage systems (Vincent-Caboud et al., 2017). To obtain a mechanistical understanding on the potential effectiveness of EM application on soil processes we conducted a soil incubation experiment mimicking spring-like field conditions in temperate climates. Soils were incubated alone or in combination with cover crop plant material and amended with typical or 100 times the typical application dose. As control treatments, we sterilized the EM solution prior to application to rigorously differentiate between an EM effect and a substrate effect. We followed several soil biological and biochemical soil properties over the course of 28 days to determine any immediate or mid-term effect of EM application to soil properties.

## 2. Methods

### 2.1. Sampling and preparation of soil and cover crop biomass

The soil and the cover crop biomass were sampled from an agricultural field situated in the temperate climate zone in Diessenhofen, Canton Thurgau, Switzerland at 414 m elevation. The farmer has been practicing the shallow incorporation of cover crops with the application of EM in the last five years and reported positive experiences with respect to soil structure and crop yields. The sampling was conducted on 5 May 2020 when the cover crop was well established and about to be shallowly incorporated. Approximately 200 soil cores (0–10 cm) were taken randomly with an auger (2.5 cm diameter) on the field of 1.3 ha size. The cover crop aboveground biomass was cut in a representative 50 × 50 cm square on the same day. The sown cover crop was purchased (Wintergrün, Camena Samen, Germany) and contained 5 frost tolerant species: 62 % winter rye (*Secale cereale* L.), 26 % hungarian vetch (*Vicia pannonica* CRANTZ.), 10 % crimson clover (*Trifolium incarnatum* L.), 1 % winter oilseed rape (*Brassica napus* L.), 1 % winter turnip rape (*Brassica rapa* L.). In our plant sample we only collected winter rye, hungarian vetch, and crimson clover. The harvested and dried cover crop had a carbon (C) concentration of 42.2 % and a C/N ratio of 17.7.

The sampled soil (approx. 15 kg) was air dried at room temperature for three days before it could be sieved (2 mm). Remaining larger pieces of organic material were removed manually. The collected cover crop biomass was placed in the drying oven at 40 °C for a week and then cut into small pieces.

### 2.2. Effective microorganisms®

For this experiment we applied a commercial EM product called Rottelenker (EM Schweiz, Switzerland) that was specifically developed to support the shallow incorporation of cover crops. The liquid was purchased five days before the application to ensure original product quality. EM Rottelenker is recommended for application when temperatures rise >8 °C in a quantity of 100 L ha<sup>-1</sup> and to be diluted with an amount of water that suits a proper and even application (EM Schweiz, 2023). In practice, that means a dilution factor between 1 and 10 depending on the application technique. To distinguish between the effects of living EM and a pure-substrate effect, we ran a treatment with sterilized EM. For the sterilized treatments, EM solution was taken from the original container one day before the start of the incubation and was autoclaved twice at 121 °C for 20 min within 24 h. To test both, the living status of the purchased EM solution as well as the sterilization, we ran a colony forming unit analysis (CFU). For this, original and sterilized EM liquid were plated on Trypticase Soy Broth (TSB) media and TSB media amended with the fungicide cycloheximide, respectively, within 24 h of the launch of the incubation experiment. The dilution rows were done in five steps from 1 to 10<sup>-5</sup> with five replicates per sample and then

the plates were incubated at room temperature for three days. The CFU analysis showed that living microorganisms were present in the purchased solution and that no living microorganism was present in the sterilized EM solution on either of the two TSB media (for details see Fig. S1 in the supplementary material).

### 2.3. Experimental design

We conducted the 28-day soil incubation experiment with the two factors cover crop and EM-level. We chose the time span of 28 days to capture the time between shallow cover crop incorporation and sowing of spring crop (around 10 days) as well as the start of the spring crop. We tested four different levels of EM- application: no EM (EM0), EM as recommended in agricultural praxis (100 L ha<sup>-1</sup>; EM1), 100 times higher quantity (EM100), and 100 times higher quantity of sterilized EM (EM100st). In a fully orthogonal design, these four EM-levels were combined with the factor cover crop resulting in four treatments with cover crop input (CC-EM0, CC-EM1, CC-EM100, CC-EM100st) and four treatments with no cover crop input and only EM application (NCC-EM0, NCC-EM1, NCC-EM100, NCC-EM100st; Fig. 1). We imitated the process in the field with a cover crop aboveground biomass of 5 t ha<sup>-1</sup> and a topsoil (0–3 cm) bulk density of 1.3 g cm<sup>-3</sup>, which corresponds to a cover crop biomass input of 12.82 g dry matter per kg of soil. The EM application of 100 L ha<sup>-1</sup> corresponds to 0.256 mL per kg of soil for the level EM1 and accordingly 25.6 mL per kg soil for the EM100 level (Table 2).

### 2.4. Soil incubation

Three days after soil sampling, the air-dried and sieved soil was slightly rewetted to a gravimetric water content (GWC) of 0.16 g H<sub>2</sub>O g<sup>-1</sup> soil and then preincubated seven days before the start of the experiment to re-establish basal respiration. Pre-incubation was conducted at 16 °C and 80 % air humidity to prevent a peak of microbial respiration induced by the soil sieving before the onset of the experiment.

The eight soil treatments were prepared on the start day of the incubation (day 0). The pre-incubated soil was brought to a GWC of approximately 0.2 g H<sub>2</sub>O g<sup>-1</sup> soil by gently spraying Milli-Q water on top whilst constantly mixing the soil by hand wearing plastic gloves to avoid any contamination. After that, the moist soil was separated into sealable 3 L plastic bags. The different levels of EM and cover crop biomass were added whereby the EM0-level received the same amount of water. Where cover crop biomass was added, the liquid was carefully sprinkled onto the plant material before being added to the soil to imitate the incorporation of cover crops as practiced in the field. Each bag was then sealed, and the content carefully mixed by hand for multiple minutes until a homogenous mixture was achieved and then transferred to plastic beakers for the incubation experiment. The incubation was conducted at 12 °C with 80 % air humidity. The final GWC of the incubated soil was 0.23 g H<sub>2</sub>O g<sup>-1</sup> soil which corresponded to 64 % of the maximum water holding capacity of the soil. Mixed soil samples were split into three different groups for soil respiration (separate glass jars), POXC (separate corning tubes) while for all other analyses, 75 g of moist soil were placed in plastic beaker with four replicates per time point (3) and treatment (8) and covered with a paper tissue to allow gas

exchange but to avoid water loss within the incubator. These were opened on the respective sampling date and the soil was split into the different volumes and beakers for further analysis. Throughout the incubation period no signs of dried aggregates on the soil surface could be visually detected. An overview of the timeline and measurement intervals can be found in Table S1 in the supplementary material.

### 2.5. Assessment of biological soil parameters

Soil respiration was measured according to the protocol of (Alef, 1995). In brief, two small plastic cups were placed into 1 L sealable glasses, where 1 cup would hold 40 g of dry soil equivalent and the other cup 10 mL of 0.2 M NaOH to trap produced CO<sub>2</sub>. We used 36 sealable glass jars (8 treatments \* 4 replicates + 4 blanks) and 13 time points resulting in 478 measurements. At every measurement time point the jar was opened and about 4 mL (in excess) of 1 M BaCl<sub>2</sub> and few drops of phenolphthalein were added to the NaOH solution and then trapped CO<sub>2</sub> was determined by a titration with 0.1 M HCl. Each mole of dissolved CO<sub>2</sub> led to the production of 2 mol of H<sup>+</sup> which neutralize 2 mol of OH<sup>-</sup> according to formula 1:

$$\text{CO}_2[\text{mmol}] \text{ trapped in NaOH} = 0.5 * (\text{HCl}_{\text{blank}}[\text{ml}] - \text{HCl}_{\text{sample}}[\text{ml}]) * M_{\text{HCl}} \quad (1)$$

Microbial C (Cmic) and N (Nmic) were measured according to the protocol of (Vance et al., 1987) with some adaptations. We weighed moist soil equal to 10 g dry matter and used 40 mL of 0.5 M K<sub>2</sub>SO<sub>4</sub> for the extraction. The dissolved C and N in the extracts were measured with a TOC-analyzer (DIMATOC® 2100, DIMATEC Analysetechnik GmbH, Germany). We report Cmic and Nmic as chloroform labile C and N did not use any conversion factor to account for incomplete extraction efficiency.

The analysis of the microbial community in the EM solution and incubated soil was performed on treatments with cover crop addition (CC-EM0, CC-EM1, CC-EM100, CC-EM100st) at day seven of the experiment. For that, DNA was extracted from pure EM solution and approximately 0.45 g soil sample using the ‘NucleoSpin® 96 Soil’ kit (Macherey- Nagel, Düren, Germany) with lysis buffer SL2 and enhancer SX following the manufacturer’s instruction. Extracted DNA was quantified fluorometrically with the plate reader Infinite M Nano+ (Tecan, Maennedorf, Switzerland) and the Qubit dsDNA HS Assay Kit (Invitrogen by Thermo Fisher Scientific, Waltham, USA). The bacterial community was characterized using 16S rRNA amplicon sequencing using a similar protocol as Lori et al. (2022). Briefly, primers 314F and 806R (Frey et al., 2016) were used for the first PCR with Kapa Sybr fast qPCR kit Master Mix (Kapa Biosystems, Wilmington, USA) and 200 nM of each primer. Samples were used either undiluted, 1:5, 1:10 or 1:50, depending on their concentration. The cycling program consisted of 3 min initial denaturation at 95 °C, 38 cycles of 20 s denaturation at 95 °C, 20 s annealing at 58 °C and 40 s elongation at 72 °C followed by 10 min final elongation. Amplicons were purified with homemade magnetic bead solution (SpeedBead Magnetic Carboxylate Modified Particles, GE Healthcare) and visualized on agarose gel for validation. The second PCR to barcode the samples and MiSeq sequencing were performed at the Genome Quebec Innovation Center (Montreal, Canada).

The fungal community was characterized using ITS amplicon sequencing with PacBio following Bodenhausen et al. (2019). M13-tagged primers ITS1F and ITS4 were used for the first PCR with HiFi

Experimental Design (2 Factors)		EM-Level (4 levels)			
		EM0	EM1	EM100	EM100st
Cover Crop (2 levels)	Cover Crop	CC-EM0	CC-EM1	CC-EM100	CC-EM100st
	No Cover Crop	NCC-EM0	NCC-EM1	NCC-EM100	NCC-EM100st

Fig. 1. Experimental design with four EM levels and two cover crop levels.

HotStart Ready Mix (Kapa Biosystems, Roche, Basel, Switzerland) and 300 nM of each primer. The first cycling program consisted of 3 min initial denaturation at 95 °C, 25 cycles of 20 s denaturation at 98 °C, 20 s annealing at 60 °C and 60 s elongation at 72 °C followed by 5 min final elongation. 3 µl of the first PCR was used as template for the second PCR reaction with M13-tagged barcodes. The second cycling program was similar as above except after the first two cycles, the annealing temperature was increased to 65 °C and the total number of cycles was 22. After cleaning-up with homemade magnetic bead solution, PCR products were quantified NanoQuant (Tecan, Maennedorf, Switzerland) and pooled in equimolar fashion. Negative controls were included and sequenced with the other samples. The library was sequenced with PacBio at the Next Sequencing Platform of the University of Bern on a Sequel II instrument according to their standard protocols. Raw sequences were deposited at NCBI Short Read Archive (PRJNA1026363).

MiSeq reads were demultiplexed by the sequencing facility. The bioinformatics analysis of MiSeq data was performed on Scientific Computer Cluster Euler at the ETH Zurich. Briefly, USEARCH v11.0.667 (Edgar, 2013) was used to merge the reads and remove primer sequences. PRINSEQ-lite 0.20.4 was used to filter for quality (Schmieder and Edwards, 2011). After chimera removal with UPARSE (Edgar, 2013), reads were clustered into zero radius operational taxonomic units (ZOTU) with UNOISE3 (Edgar, 2016). ZOTU were further clustered at 97 % similarity with UPARSE (Edgar, 2013). Finally, taxonomy was assigned with SINTAX v11.0.667 (Robert, 2016) and the SILVA database, SILVA138\_RESRIPT.fasta (Quast et al., 2013). The bioinformatics analysis of PacBio data was similar except that lima 2.7.1 (<https://lima.how>) was used for demultiplexing and the taxonomy assignment was with the UNITE database, UNITE\_v83\_AllEukaryotes\_10.05.2021.fasta (Abarenkov et al., 2010).

Relative shares of OTUs from the pure EM solution with >50 counts served as target EM taxa and were traced during the soil incubation.

## 2.6. Assessment of chemical parameters

To measure the dynamics of easily oxidizable carbon, we determined permanganate oxidizable carbon (POXC) at several time points of the incubation. For that, 5 g of moist soil were put in 50 mL corning tubes covered with a paper tissue to allow for gas exchange but to avoid water loss within the incubator. Four replicates per treatment and sampling time point were prepared ( $n = 4 \times 8 \times 8 = 256$ ) and when the sampling date arrived, they were covered with a lid and frozen until analysis. Afterwards, POXC was then measured in one run according to the protocol of Weil et al. (2003) with 0.2 M  $\text{KMnO}_4$  as reactant and absorption measurement at 550 nm with a Spectrophotometer (UV-1800, Shimadzu corporation, Japan).

Water-soluble ions were measured by extracting soil equivalent to 8 g dry soil from the collective beakers with 40 mL of Milli-Q water for one hour on day 0, 7, 14 and 28. These samples were centrifuged (3000 rpm for 15 min) and 5 mL of the supernatant was syringe filtered (hydrophilic, 0.45 µm) and stored at 5 °C. Ion chromatography (IC) was performed in one run two weeks after the end of the incubation on a Dionex Aquion™ (Thermo Fisher Scientific Inc., Waltham, USA) to measure the concentrations of the anions fluoride ( $\text{F}^-$ ), chloride ( $\text{Cl}^-$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{PO}_4^{3-}$ ), sulfate ( $\text{SO}_4^{2-}$ ) as well as the cations sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), magnesium ( $\text{Mg}^{2+}$ ) and calcium ( $\text{Ca}^{2+}$ ).

For the analysis of water-soluble elements, 25 mL of the same supernatant as for the water-soluble ions-measurements were used. To remove dispersed clay particles in the liquid, 1 mL of 1 M  $\text{MgCl}_2$  was added, and then the samples were vigorously shaken and centrifuged (3000 rpm for 15 min). From this solution 9.8 mL were filtered (hydrophilic, 0.45 µm) and then mixed with 0.2 mL nitric acid ( $\text{HNO}_3$ , 69 %) resulting in 10 mL samples containing 1 %  $\text{HNO}_3$ . These samples were then stored at 5 °C and analyzed in one common run one month after the end of the incubation experiment on a 7700× ICP-MS from Agilent Technologies (Santa Clara, USA) measuring the concentrations

of arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), silver (Ag), aluminum (Al), phosphorus (P), vanadium (V), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and uranium (U).

To characterize the soil, the cover crop biomass and the EM solution for elemental composition we conducted a total multielement analysis in triplicates. For that, 0.2 g of soil, 0.2 g of cover crop biomass and 0.2 mL of a 121-times diluted original EM-solution were mixed with 8 mL of 69 %  $\text{HNO}_3$  and 2 mL 37 %  $\text{H}_2\text{O}_2$  and then digested in a CEM MARS 6 microwave (stage 1: 10 min at 120 °C, stage 2: 40 min at 170 °C). After, the cooled down samples were brought to 50 mL volume with Milli-Q, centrifuged (2500 rpm for 5 min) and analyzed with the above mentioned ICP-MS. The turbidity of the EM solution did not allow for an analysis of containing ions via IC analysis.

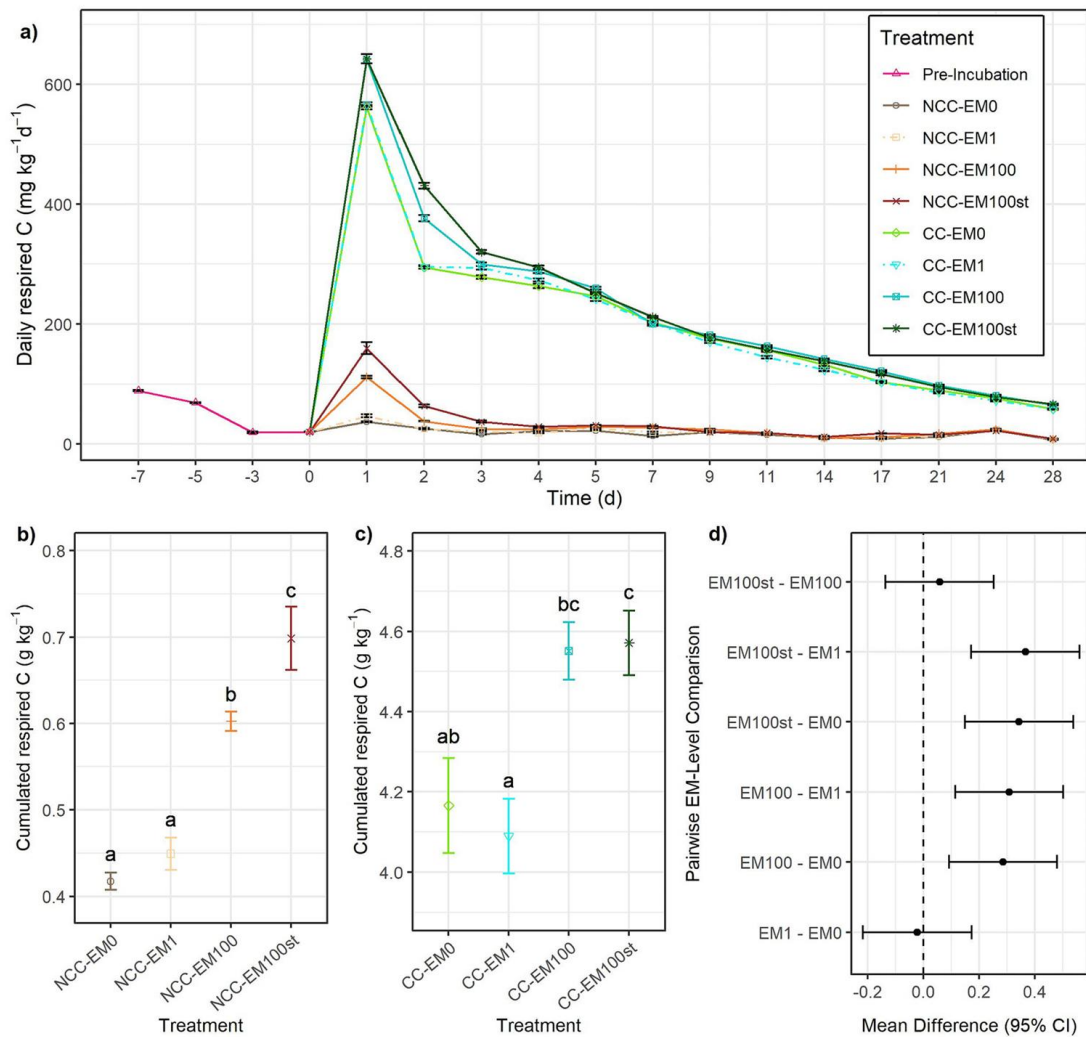
## 2.7. Statistics

Fungal and bacterial richness and Shannon diversity were assessed on the base of rarefied read counts using the vegan R package (Oksanen et al., 2019). Additionally, differences between the fungal and bacterial community composition were tested with a PERMANOVA with  $10^4$  permutations based on Bray-Curties dissimilarity matrices. All other parameters mostly fulfilled or just showed minor deviations from the requirements of normality (Shapiro-Wilk test) and homoscedasticity (Levenes' test). Therefore, we decided to use parametric tests. We tested a multiplicative analysis of variance (ANOVA) with the factors cover crop and EM-level for soil respiration, microbial biomass, pH, POXC and water extractable ions and elements. We used Tukey HSD as a post hoc test to evaluate significant differences between different EM-levels or different treatments. Only for the cumulative respiration, which was very different between treatments with and without cover crop addition, we used a separate Tukey-HSD test for the CC and NCC treatments. For the other response variables, if the ANOVA did not show a significant interaction, we only discuss the main effects of EM-level. Otherwise, if the interaction effect was significant, we discuss only the comparisons between CC-EM0 and CC-EM1, NCC-EM0 and NCC-EM1, CC-EM100 and CC-EM100st as well as NCC-EM100 and NCC-EM100st, because all other possible 24 comparisons were not of practical relevance. All analysis were performed in R version 4.2.2 (R Core Team, 2020).

## 3. Results

### 3.1. Soil respiration

The incubation experiment started with a basal respiration rate of  $20.2 \pm 0.3 \text{ mg C kg}^{-1} \text{ d}^{-1}$  (day 0), which was maintained at a similar level for the course of the soil incubation in the NCC-EM0 and NCC-EM1 treatments (Fig. 2a). Addition of living or sterilized EM in high dose on bare soil (NCC-EM100 and NCC-EM100st) caused an increase in soil respiration of up to  $159 \pm 10 \text{ mg C kg}^{-1} \text{ d}^{-1}$  on day 1 and basal soil respiration was reached again latest by day four. The addition of cover crop biomass clearly had the strongest effect on soil respiration, peaking at  $647 \pm 8 \text{ mg C kg}^{-1} \text{ d}^{-1}$  for CC-EM100st at day 1. After that, soil respiration rates continuously decreased, but CC treatments did not reach basal soil respiration rates during the whole incubation period. Differences between the different EM-levels mainly occurred during the first 4 days. During the 28-day incubation the cumulated respiration summed up between  $0.42 \pm 0.01$  and  $0.7 \pm 0.04 \text{ g C kg}^{-1}$  for the NCC treatments and between  $4.09 \pm 0.9$  and  $4.57 \pm 0.08 \text{ g C kg}^{-1}$  for the CC treatments (Fig. 2b and c). The addition of cover crop biomass as well as the addition of EM in high dose (Fig. 2d) increased the cumulated respired C. However, we did not see any effect on cumulated respired C by the combination of cover crops with any level of EM application (no significant interaction in the multiplicative ANOVA between factors cover crop and EM-level,  $p$ -value = 0.13, see Table S2 in the supplementary material). The differences in cumulated respired C among the EM-levels were more pronounced in the NCC (Fig. 2b) than in the CC



**Fig. 2.** a) Daily respiration rates. The value of CC-EM100 at day 1 is based only on one replicate, since for the other three replicates the NaOH trap was already completely saturated, suggesting even higher overall respiration rates in this treatment. b) Cumulated mean soil respiration after 28 days of incubation for treatments without cover crop addition. c) Cumulated mean soil respiration after 28 days of incubation for treatments with cover crop addition. d) Tukey's mean difference with 95 % confidence interval for the factor EM-level in the two-way ANOVA with cumulated respiration at the end of the incubation (day 28) as response variable. The panels a, b and c show the mean of four replicates with error bars indicating the standard error.

treatments (Fig. 2c). Pairwise comparison within the NCC treatments also showed that the cumulative respiration was significantly higher for NCC-EM100st than for NCC-EM100 (Fig. 2b). Addition of EM at recommended dose had no effect on soil respiration as we did not find any significant difference between the EM1 and the EM0 level.

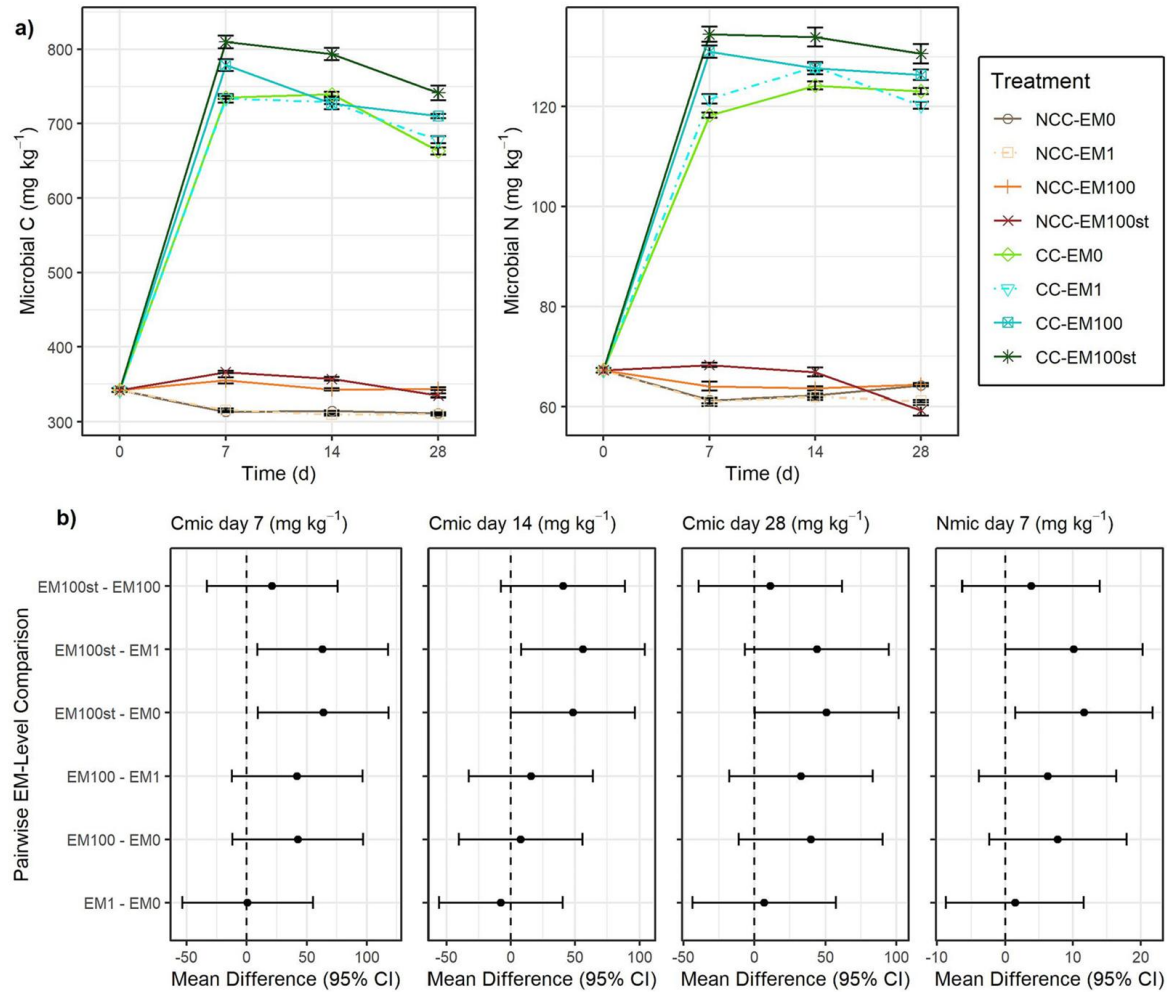
### 3.2. Microbial biomass

At the start of the incubation experiment (day 0), soil microbial biomass contained  $342 \pm 5$  mg C kg<sup>-1</sup> and  $67 \pm 1$  mg N kg<sup>-1</sup> (Fig. 3a). In the NCC treatments, there were only minor changes in microbial C and N over time, with most times highest values in the NCC-EM100st treatment ( $366 \pm 14$  mg C kg<sup>-1</sup> and  $68 \pm 2$  mg N kg<sup>-1</sup>) followed by the NCC-EM100 treatment. In contrast, microbial C and N almost doubled in all CC treatments with highest values in the CC-EM100st treatment ( $810 \pm 34$  mg C kg<sup>-1</sup> and  $134 \pm 6$  mg N kg<sup>-1</sup>) followed by the CC-EM100 treatment. There was no significant interaction at any day between the two factors cover crop and EM-level for the response variables Cmic

and Nmic (lowest p-value for the interaction term was  $p = 0.33$  for Nmic at day 28). Independent of cover crop input, application of EM in high dose led to slightly higher Cmic and Nmic but only the EM100st level showed on some days significantly higher Cmic and Nmic than the EM1 or EM0 level (Fig. 3b). Independent of cover crop addition, no effect of the addition of EM at the recommended dose existed as there was no significant difference between the EM1 and EM0 level.

### 3.3. Identifying and tracing EM taxa

Taxonomic identification of bacterial and fungal taxa within the applied EM solution showed domination of fungal taxa by OTU5, which made up >90 % of fungal OTUs and was assigned to the Order of *Saccharomycetales*. Other identified fungal taxa within the EM solution include OTUs assigned to the orders of *Mortierellales*, *Filobasidiales* and *Hypocreales* but they comprise only a small fraction of the inoculated fungal community (Table 3). Bacteria taxa on the EM solution were dominated by OTUs assigned to the genus of *Lactobacillus*. Five different



**Fig. 3.** a) Microbial C and N during the 28-day incubation experiment with 4 measurement time points. Means of four replicates and standard errors are shown. b) Tukey's mean differences between EM-levels for the ANOVA microbial C (or N) ~ Cover crop \* EM-level. Only days with significant EM-level effect are presented. Mean differences are indicated with 95 % confidence interval (CI) and significances are marked based on alpha = 0.05.

OTUs of this genus were observed, jointly accounting for >99 % of applied bacterial OTUs. *Acetobacteraceae* and *Clostridiaceae* were identified in negligible amounts. Bacterial OTUs 4440 and 4994 were most abundant with 72.6 and 16.4 % of applied bacterial OTUs (Table 3). After 7 days of incubation the structure of soil bacterial and microbial communities was compared via permanova, revealing a weak effect on bacterial community structure ( $p = 0.046$ ) and no effect on fungal community structure ( $p = 0.816$ ), based on Bray-Curties dissimilarities matrices. For the bacterial community, pairwise permanova further revealed significant difference between CC-EM0 and CC-EM100 ( $p = 0.032$ ), while no other treatment pair significantly differed from each other. Principally, bacterial community structure was dominated by *Actinobacteriota* and *Proteobacteria*, while fungal communities mainly comprised *Mortierellamycolota* (Fig. 4). Neither fungal and bacterial richness nor Shannon diversity showed a significant effect of experimental treatments seven days after incubation (Table S3 in the supplementary material).

OTUs identified within the EM solution were traced within the identified bacterial and fungal communities (Fig. 5). While the recommended dose of EM application did not yield an observable increases of inoculated EM taxa, a slight increase could be detected in the 100 times the recommended application dose for bOTU4440 and bOTU4994. Still

relative abundances of these OTUS were below 1 %. For fungal communities, there was no effect in the treatments except for the CC-EM1 treatment where the recommended application dose increased fOTU5 (Fig. 5).

### 3.4. Soil pH

The initial soil pH of  $7.12 \pm 0.02$  was influenced by the different treatments. Addition of acidic solutions of EM1 (pH = 3.98), EM100 (pH = 3.55) and EM100st (pH = 3.58) decreased soil pH only when added in combination with cover crop input (Fig. 6). By day 28, soil pH increased to about 7.2 in all treatments, except for the NCC-EM0 ( $7.04 \pm 0.06$ ) treatment, which was significantly lower than the NCC-EM100 and NCC-EM100st treatment.

### 3.5. Permanganate oxidizable C

Concentrations of POXC decreased from  $544 \pm 3 \text{ mg kg}^{-1}$  on day zero to values between 445 and  $510 \text{ mg kg}^{-1}$  within the first days of the incubation experiment and remained stable from day seven onwards (Fig. S2). The CC-EM100st treatment showed a larger decrease within the first four days but also stabilized after day seven in the same range as



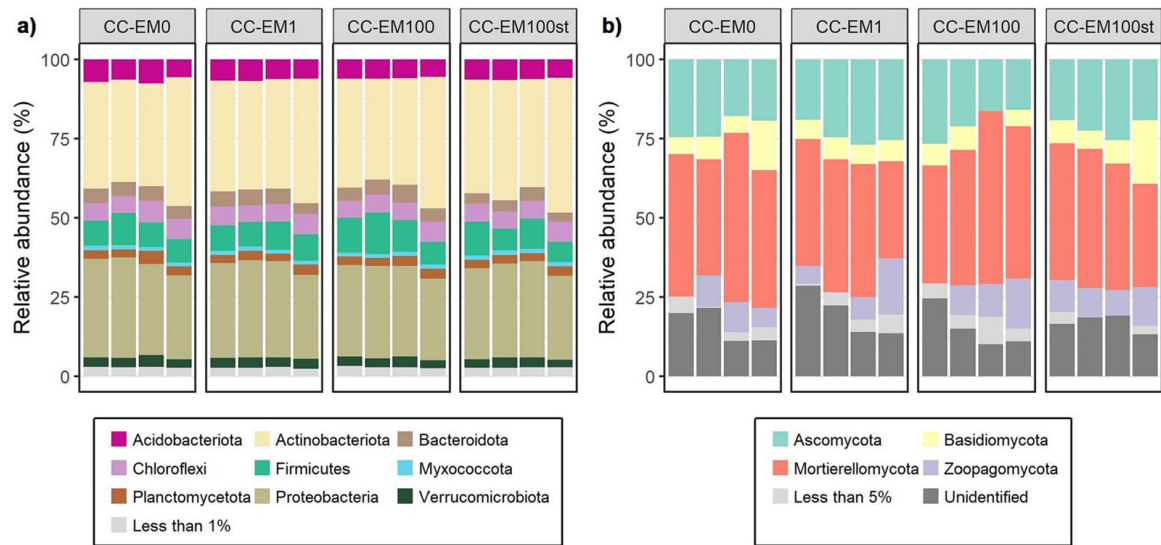


Fig. 4. Relative abundance of bacterial (a) and fungal (b) phyla after 7 days of incubation in all four replicates per treatment.

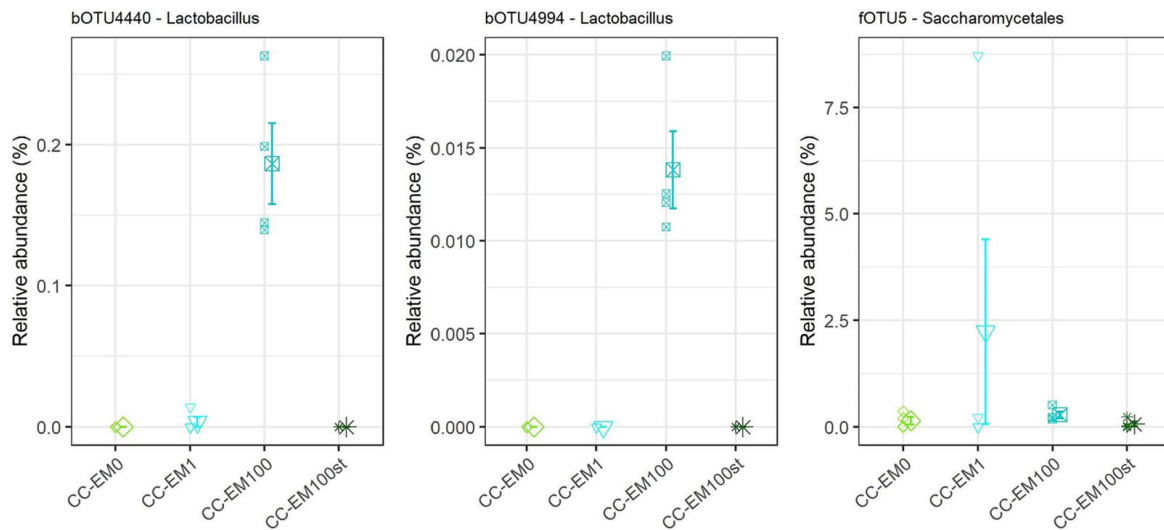


Fig. 5. Relative abundance of bOTU4440 Lactobacillus, bOTU4994, fOTU5 Saccharomycetales of the four treatments with cover crop addition seven days after the start of the incubation. Average and standard errors as well as the values of the four replicates per treatment are indicated.

the other treatments. For POXC, the factors cover crop and EM-level showed significant interactions on all measurement days except day 14 and day 28. Nevertheless, the significantly different treatments as indicated by the Tukey test were not consistent over time.

### 3.6. Water-soluble ions

The concentrations of the analyzed water-soluble ions were influenced by EM and cover crop addition (Fig. 7 and corresponding statistics in Table S4 in the supplementary material). For  $F^-$ ,  $Cl^-$ ,  $Na^+$ ,  $K^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  we observed significantly higher concentrations in the treatments with cover crop biomass input. This effect was in general clearer at the beginning (day 7) of the incubation and decreased towards the end (day 28).  $Cl^-$ ,  $SO_4^{2-}$ ,  $Na^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  were often significantly higher in the EM100 and EM100st than in the EM0 and EM1 levels. We only observed a few differences between the EM1 and EM0 level

suggesting that the application of EM at the recommended dose did not influence the concentration of water-soluble ions. However, CC-EM1 showed higher  $Mg^{2+}$  concentration on day 7 but lower  $K^+$  concentration on day 28 than CC-EM0. More consistent was the difference between the EM100 and the EM100st level. The CC-EM100st treatment showed at least at one time point higher ion concentrations than the CC-EM100 treatment for  $F^-$ ,  $Cl^-$  and  $SO_4^{2-}$ . For  $Cl^-$  this effect was also observed for the NCC treatments and NCC-EM100st showed significantly higher concentrations than NCC-EM100. For  $NO_3^-$ , we found higher concentrations in NCC-EM0 and NCC-EM1 treatments than in all other treatments.

### 3.7. Water-soluble elements

The inputs of water-soluble elements through cover crop biomass or EM addition can be seen in Table 4 in absolute numbers and relative to

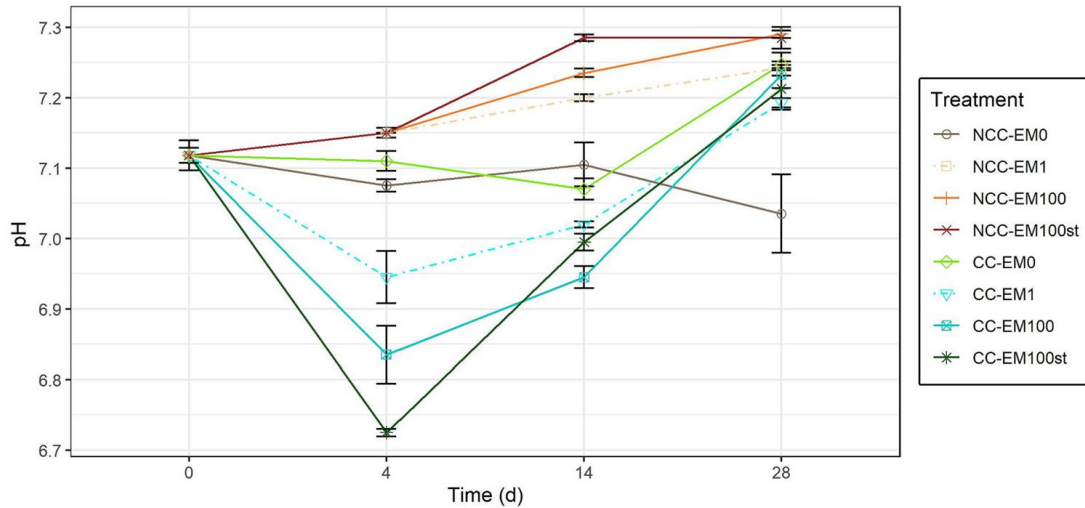


Fig. 6. Soil pH during the 28-day incubation experiment with four measurement time points. Values show means of four replicates (except for day 4 with only 2 replications per treatment). Error bars indicate the standard error.

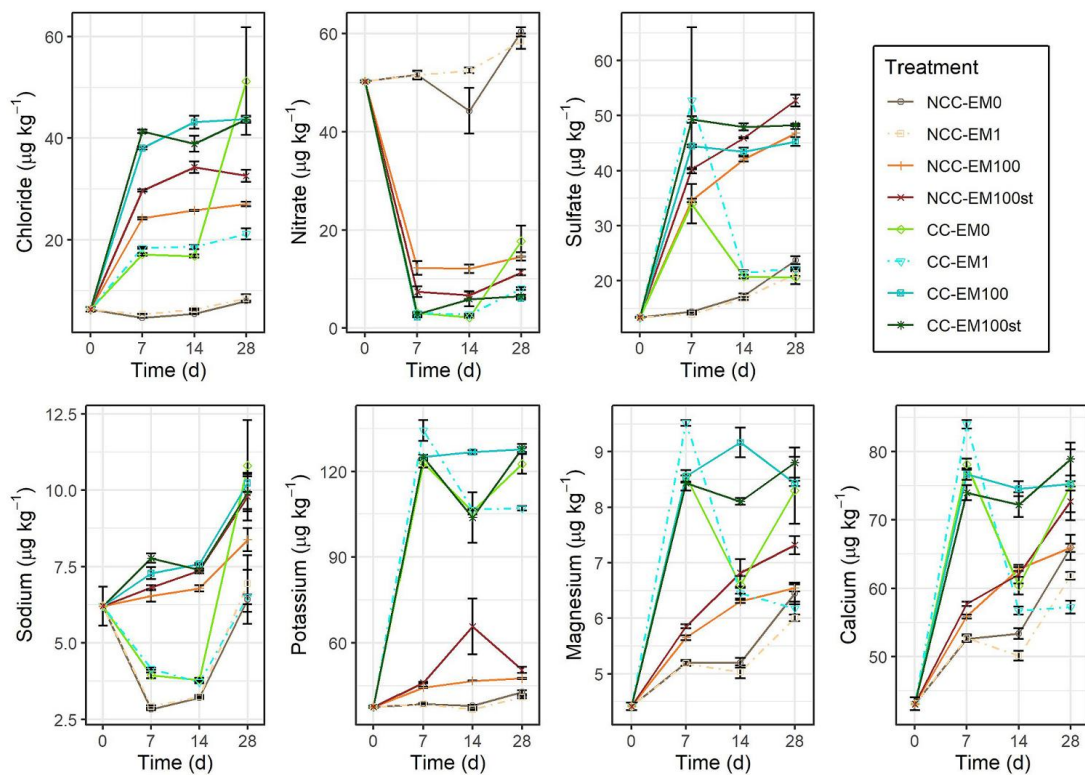
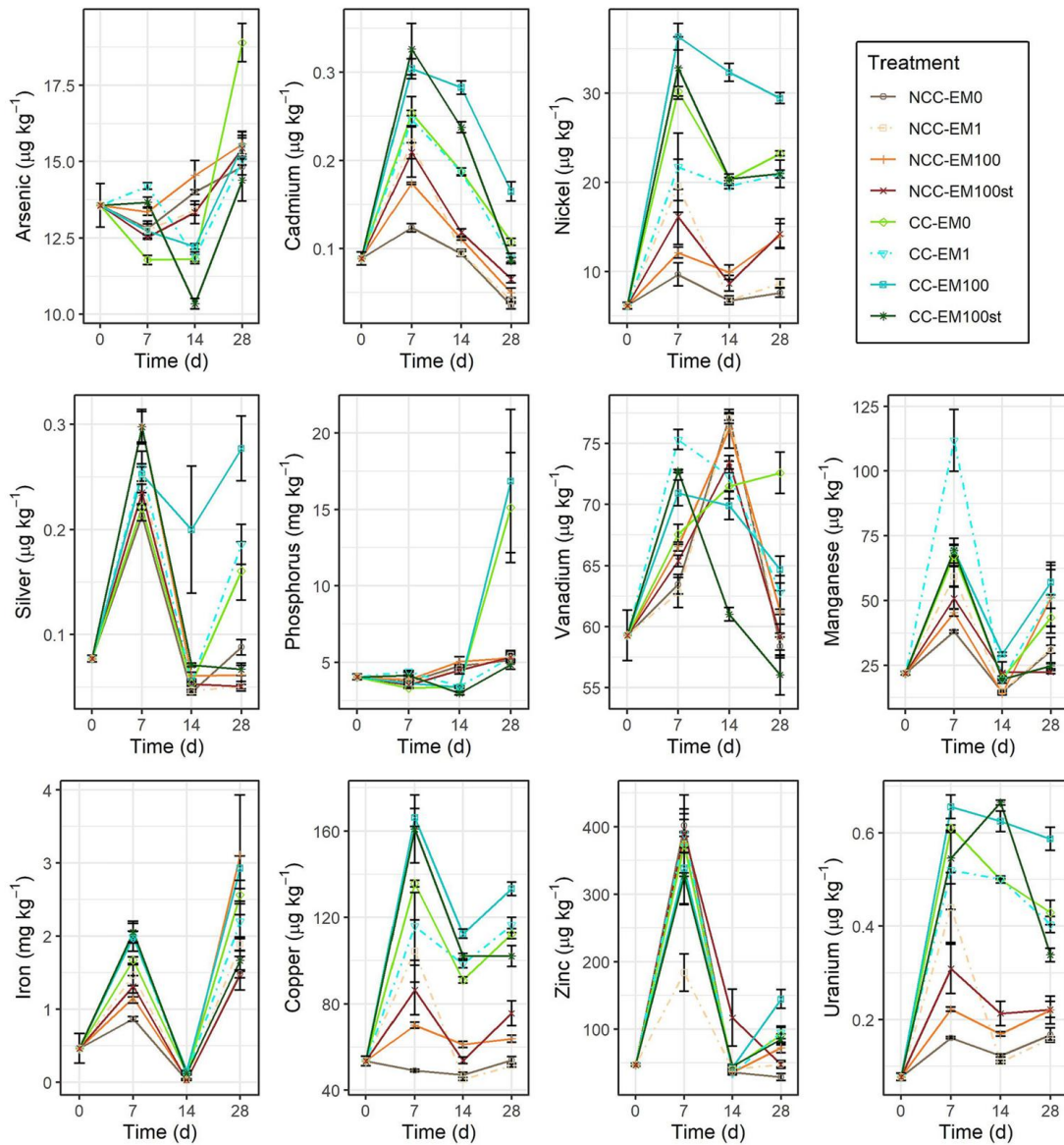


Fig. 7. Concentration ( $\mu\text{g}$  per  $\text{kg}$  soil) of water-soluble ions that showed a significant main effect of EM-level (chloride, nitrate, sulfate, sodium, potassium, magnesium, and calcium) during the 28-day incubation experiment with 4 measurement time points. Values show mean of four replicates and error bars the standard error.

the initial water-soluble concentration in the soil. For all analyzed elements the input through the cover crop biomass was higher than through the EM addition. The concentrations of the analyzed water-soluble elements were influenced by EM and cover crop addition at least at one of the four measurement time points (Fig. 8 and corresponding statistic in Table S5 in the supplementary material). Cover

crop input significantly increased concentrations of water soluble Pb, Cd, Cr, Ni, Al, Ag, Mn, Fe, Cu, Zn and U compared to the NCC treatments at least at one timepoint. There were only minor and non-systematic differences in measured concentrations of water-soluble elements between EM-levels and they only occurred in the treatments with cover crop addition. The application of EM in high dose (EM100, EM100st) did



**Fig. 8.** Concentration ( $\mu\text{g}$  or  $\text{mg kg}^{-1}$  soil) of water-soluble elements that showed a significant main effect of EM-level (arsenic, cadmium, nickel, silver, phosphorus, vanadium, manganese, iron, copper, zinc, and uranium) during the 28-day incubation experiment with 4 measurement time points. Error bars indicate the standard error.

not consistently influence the concentration of water-soluble elements during the soil incubation experiment. The only effects of EM1 compared to EM0 that were statistically significant were on day 7, where the CC-EM1 treatment showed higher concentrations in As and P than the CC-EM0 treatment. The comparison between sterilized and living EM revealed at least at one timepoint significantly higher concentrations of Cd, Ni, Ag, P, Cu and U in the CC-EM100 compared to the CC-EM100st treatment. In the NCC treatments, no significant difference was identified between NCC-EM1 and NCC-EM0 or NCC-EM100 and NCC-EM100st.

## 4. Discussion

### 4.1. EM application at recommended dose (EM1)

Application of EM at the recommended dose (EM1) showed no effect on critical soil properties such as soil respiration (Fig. 2) or the development of microbial biomass (Fig. 3) compared to the control treatments (EM0), in both the CC as well as the NCC treatments. This is in line with findings of Schenck zu Schweinsberg-Mickan and Müller (2009) who did not observe any influence of living EM addition on soil respiration compared to a sterilized EM control treatment. The lack of observed results was confirmed by the absence of traced EM-taxa in the soil, as only 1 out of 4 replicates in the CC-EM1 treatment showed slightly higher relative abundance in *botu4440* (*Lactobacillus*) and *fOTU5* (*Saccharomycetales*) than the CC-EM0 treatment. The few statistically

significant differences in water-soluble ions and elements that occurred at specific time points during the incubation experiment were inconsistent over time. In other studies, (Hu et al., 2018) observed higher available phosphorus and potassium contents in EM-compost while other studies reported a slightly higher N content in EM-compost compared to traditional compost (Daur, 2016; Jusoh et al., 2013; Van Fan et al., 2018; Zhong et al., 2018), yet, these investigations were up to now missing for soil. Furthermore, we did not observe any consistent change in POXC throughout the incubation experiment suggesting that EM addition had no effect on already existing labile soil organic matter in the soil that could cause additional release of nutrients of PTTEs. Similarly, a recent review (Safwat and Matta, 2021) also found little evidence to confirm the beneficial effects of EM on composting of organic matter.

The soil incubation mimicked spring-like field conditions in temperate climates (12 °C and 0.2 g H<sub>2</sub>O g<sup>-1</sup> soil) that would be relevant for enhanced cover crop decomposition through EM application. Nevertheless, soil temperature, moisture and water filled pore space during spring are typically highly variable. This would consequently also affect the establishment of EM, that might require very specific conditions for their establishment. On the field scale some experiments have reported higher yield and nutrient efficiency when green manure, farmyard manure or chemical fertilizer were applied in combination with EM under mainly under subtropical climates (Hu and Qi, 2013; Hussain et al., 1999; Javaid and Bajwa, 2011; Khaliq et al., 2006; Youssef et al., 2021). However, in regions with temperate climates, the few existing field studies could not determine any effects on crop yields or soil quality that could be traced to the application of EM to soil (Mayer et al., 2010; Pranagal et al., 2020). This was supported by the results of our study, demonstrating no effect of EM addition at typical application rates on soil properties.

#### 4.2. EM application in high dose (EM100, EM100st)

The addition of EM at 100 times higher than recommended dose showed some effects on soil properties, e.g., on soil respiration (Fig. 2) or microbial C (Fig. 3). However, these changes took place regardless of whether the solution was sterilized (EM100st) or not (EM100), and can thus clearly be assigned to a substrate effect, and not an actual EM effect. The amount of carbon added with the EM100 and EM100st application level was about 0.2 g C per kg of soil (Table 2). This closely matched the difference in cumulated respired C compared to the EM0 level in both the CC and the NCC treatments (Fig. 2b). Since the EM solution was acidic (pH 3.6; Table 1), some of the released CO<sub>2</sub> may have originated from the dissolution of carbonates in the alkaline soil. Yet, assuming that all the added acid of the EM solution was buffered by CaCO<sub>3</sub> and released as CO<sub>2</sub>, this would equal to a C release of only 1.6 mg C kg<sup>-1</sup> soil, i.e., to negligible amounts compared to basal soil respiration (Fig. 2a). The amount of C added at the EM100 treatment (0.2 g C per kg of soil) was much lower than C added with cover crop biomass (5.4 g C per kg soil). Cover crop addition also caused a slight increase in microbial biomass and likely resulted in an immobilization of NO<sub>3</sub><sup>-</sup>, which explains the significantly higher NO<sub>3</sub><sup>-</sup> concentration in the treatments without high-dose EM or cover crop biomass (NCC-EM0 and NCC-EM1; see Fig. 7 and Table S4). Additionally, at certain time points, higher concentrations of Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> were observed in EM100 and EM100st levels compared to the EM1 and EM0 levels, regardless of cover crop input (Fig. 7, Table S4), suggesting that these ions were part of the EM solution. This suggests that the higher concentrations in those water-soluble ions were also a result of the substrate effect, even though this cannot be fully confirmed since the original EM solution could not be analyzed for these water-soluble ions due to high organic impurities. In contrast, the analysis of the original EM solution for a wide range of water-soluble elements (Table 4) showed that these inputs at the 100 times application level were still minor compared to the inputs by the cover crop biomass. Inputs of potentially harmful

**Table 1**

Characteristics of the arable soil used in this study. Means and standard deviation are presented.

Property	Unit	Value (SD)
Sand <sup>†</sup>	mass %	50
Silt <sup>†</sup>	mass %	29
Clay <sup>†</sup>	mass %	21
Maximum water holding capacity <sup>‡</sup>	g water per g soil	0.36
pH (CaCl <sub>2</sub> )		7.12 (0.09)
Total C <sup>§</sup>	g C kg <sup>-1</sup> soil	28.6 (0.1)
Inorganic C <sup>§</sup>	g C kg <sup>-1</sup> soil	9.04 (0.19)
Organic C <sup>§</sup>	g C kg <sup>-1</sup> soil	19.5 (0.3)
Permanganate oxidizable C <sup>#</sup>	mg C kg <sup>-1</sup> soil	543 (12)
microbial C <sup>††</sup>	mg C kg <sup>-1</sup> soil	342 (18)
Total N <sup>§</sup>	g N kg <sup>-1</sup> soil	2.12 (0.01)
microbial N <sup>††</sup>	mg N kg <sup>-1</sup> soil	67.2 (3.8)
Magnesium <sup>††</sup>	g kg <sup>-1</sup> soil	6.02 (0.16)
Aluminum <sup>††</sup>	g kg <sup>-1</sup> soil	9.44 (0.63)
Phosphorus <sup>††</sup>	g kg <sup>-1</sup> soil	1.32 (0.66)
Manganese <sup>††</sup>	g kg <sup>-1</sup> soil	0.88 (0.02)
Iron <sup>††</sup>	g kg <sup>-1</sup> soil	17.8 (0.9)
Copper <sup>††</sup>	mg kg <sup>-1</sup> soil	43.2 (0.8)
Zinc <sup>††</sup>	mg kg <sup>-1</sup> soil	69.9 (2.7)
Lead <sup>††</sup>	mg kg <sup>-1</sup> soil	38.6 (1.5)

<sup>†</sup> Improved integral suspension pressure method (ISP+) (Durner and Iden, 2021).

<sup>‡</sup> The maximum water holding capacity was determined gravimetrically after a water saturated sample lost all gravitational water.

<sup>§</sup> Dry combustion with CNS analyzer. For the determination of inorganic C, the samples were first ignited at 550 °C.

<sup>#</sup> According to Protocol of (Weil et al., 2003).

<sup>††</sup> Chloroform fumigation according to the protocol of (Vance et al., 1987).

<sup>‡‡</sup> Extracted from soils using nitric acid microwave digestion and measured using an inductively coupled plasma - mass spectrometer.

elements from the EM solution into the soil system can therefore be ruled out.

#### 4.3. Microbial composition and establishment in soil upon addition to soil

The application of EM in high dose, accompanied by a sterilized

**Table 2**

Description of the levels for the factor cover crop (CC, NCC) and factor EM-level (EM0, EM1, EM100, EM100st) that were combined to a fully orthogonal experimental design.

Level	Input g (kg soil) <sup>-1</sup>	C input g (kg soil) <sup>-1</sup>	N input mg (kg soil) <sup>-1</sup>	Dilution factor	pH	Remarks
CC	12.8 (dry matter)	5.4	300			Dried and cut to 2 mm pieces
NCC	0	0	0			
EM0	0	0	0	Only water	7.00	
EM1	0.256	0.002	0.075	1: 121	3.98	Living EM applied at recommended dose
EM100	25.6	0.2	7.5	1: 1.21	3.55	Living EM applied in 100 times higher quantity than recommended dose
EM100st	25.6	0.2	7.5	1: 1.21	3.58	Sterilized EM applied in 100 times higher quantity than recommended dose

**Table 3**  
Taxonomy and relative share of fungal and bacterial OTUs within the applied EM solution.

	Kingdom	Phylum	Class	Order	Family	Genus	Mean relative abundance (%)	SE
fZOTU5	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales			93.3	0.8
fZOTU375	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	NA	1.9	0.3
ffZOTU1425	unidentified						1.3	0.1
fZOTU9	unidentified						0.7	0.0
fZOTU1533	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales			0.6	0.1
fZOTU66	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicocozyma	0.5	0.2
fZOTU23	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Gibberella	0.5	0.1
fZOTU941	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae		0.4	0.1
fZOTU1975	Fungi	Ascomycota	Sordariomycetes				0.4	0.0
fZOTU2087	Fungi						0.3	0.0
bZOTU4440	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	72.6	0.0
bZOTU4994	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	16.4	1.9
bZOTU3653	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	5.8	1.2
bZOTU3325	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	3.9	1.0
bZOTU2664	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acetobacter	0.6	0.2
bZOTU7663	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	0.5	0.0
bZOTU2304	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium_sensu_stricto_1	0.2	0.1

**Table 4**  
Concentration of water-soluble elements in cover crop biomass and effective microorganisms and the applied concentrations in the incubation experiment as absolute numbers and as percentage of the initial water-soluble concentration in the soil (Standard deviation of three measurements in brackets).

Unit	Cover crop			Effective microorganisms			
	Concentration in dry matter	Added concentration to soil	Added concentration compared to initial water-soluble concentration in soil (day 0)	Concentration in purchased liquid	Added concentration to soil in EM1-level	Added concentration to soil in EM100-level	Added concentration of EM100 compared to initial water-soluble concentration in soil (day 0)
	[mg kg <sup>-1</sup> ]	[µg kg <sup>-1</sup> ]	[%]	[µg L <sup>-1</sup> ]	[ng kg <sup>-1</sup> ]	[ng kg <sup>-1</sup> ]	[%]
As	0.044 (0.005)	0.558 (0.06)	4.1 (0.4)	2.69 (0.04)	0.687 (0.011)	68.7 (1.1)	0.5 (7.8)
Pb	0.204 (0.007)	2.62 (0.08)	241.4 (7.7)	Below detection limit			
Cd	0.011 (0.000)	0.146 (0.003)	165.1 (3.82)	Below detection limit			
Cr	4.31 (0.16)	55.2 (2)	2429.1 (89.5)	7.5 (0.25)	1.91 (0.07)	191.9 (6.5)	8.5 (0.3)
Ni	0.635 (0.03)	8.15 (0.38)	132.4 (6.2)	12.7 (1.4)	3.26 (0.36)	326 (36)	5.3 (0.6)
Ag	0.013 (0.000)	0.163 (0.000)	211.6 (0.1)	2.94 (0.09)	0.751 (0.024)	75.1 (2.4)	97.3 (3.1)
Al	35.4 (2.1)	454 (27)	18.4 (1.1)	173 (33)	44.3 (8.3)	4430 (833)	0.18 (0.03)
P	Not measured						
V	0.079 (0.002)	1.01 (0.02)	1.7 (0.0)	7.05 (0.23)	1.80 (0.05)	181 (6)	0.3 (0.01)
Mn	35.2 (0.7)	451 (9)	2056.3 (39.8)	96.3 (1.5)	24.7 (0.4)	2466 (38)	11.2 (0.2)
Fe	Not measured						
Cu	7.7 (0.24)	99 (3)	184.8 (5.7)	11.9 (0.5)	3.05 (0.13)	305.0 (13)	0.57 (0.02)
Zn	Not measured						
U	0.003 (0.001)	0.036 (0.009)	47.0 (12.3)	0.704 (0.017)	0.180 (0.004)	18.0 (0.4)	23.5 (0.6)

control, enabled the identification of potential effects caused by living microorganisms and distinguish them from substrate effects. In our study, three main organisms were traced and identified from the EM solution. Among them, two *Lactobacillus*-taxa (bOTU4440 and bOTU4994) showed a much higher relative abundance in the CC-EM100 treatment and were not found in the treatments without living EM. However, their presence constituted <1 % of the total bacterial community.

Effective microorganisms are distributed worldwide, multiplied and processed into various end products with different additives. This variability poses challenges in comparing different EM studies because the inoculant itself might vary (Dos Santos et al., 2020) and, in most studies, the microbial community was not analyzed. In our study, we analyzed the EM solution via amplicon sequencing of taxonomic marker genes, which revealed bacterial and fungal OTUs assigned to the bacterial genus *Lactobacillus* and fungal order of *Saccharomycetales*. However, we did not identify photosynthetic bacteria or highly abundant *Ascomycota* within the applied EM solution, although these taxa were described as part of the EM consortia (Ahn et al., 2014). Nevertheless, since *Lactobacillus* and *Saccharomycetales* have the potential to conduct anaerobic fermentation, which is the main suggested mechanism through which EM influences the decomposition of organic matter (Higa and Parr, 1994), we conclude that we tested a representative product

within this study.

## 5. Conclusion

The addition of EM at the recommended application dose (EM1) to soil, with or without cover crop biomass, did not lead to any consistently effect on any of the monitored biological or chemical soil properties. When applied at a dose 100 times higher than recommended (EM100), an increased soil respiration and microbial biomass was observed, however, similar effects were observed in the sterilized control treatments (EM100st) and can thus be fully explained by a substrate induced effect. The soil microbial community remained largely unaffected upon EM addition. The analysis of ten water-soluble ions did not reveal any significant effect from the addition of EM solution on the mineralization of organic matter or the release of nutrients. Furthermore, the analysis of 14 water-soluble nutrients and elements showed that none of the analyzed compounds contained in the EM solution are present at harmful concentrations when applied at the recommended doses. However, there was also no significant effect in mobilizing or immobilizing selected compounds in the soil. We therefore conclude that added EM solution themselves did not alter the cover crop decomposition nor any other soil process beyond the carbon, nutrients or other substances added with the EM solution.

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## CRedit authorship contribution statement

**Simon Oberholzer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Christa Herrmann:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Natacha Bodenhausen:** Writing – review & editing, Methodology, Investigation, Data curation. **Hans-Martin Krause:** Writing – review & editing, Methodology, Investigation, Data curation. **Adrien Mestrot:** Writing – review & editing, Supervision, Project administration. **Chinwe Ifejika Speranza:** Writing – review & editing, Supervision, Funding acquisition. **Klaus A. Jarosch:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2024.105358>.

## References

- Abarenkov, K., Nilsson, R.H., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S., Hoiland, K., Kjoller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F.S., Tedersoo, L., Ursing, B.M., Vralstad, T., Liimatainen, K., Peintner, U., Koljalg, U., 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytol.* 186, 281–285. <https://doi.org/10.1111/j.1469-8137.2009.03160.x>.
- Ahn, K., Lee, K.B., Kim, Y.J., Koo, Y.M., 2014. Quantitative analysis of the three main genera in effective microorganisms using qPCR. *Korean J. Chem. Eng.* 31, 849–854. <https://doi.org/10.1007/s11814-013-0274-6>.
- Alef, K., 1995. 5 - estimation of microbial activities. In: Alef, K., Nannipieri, P. (Eds.), *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, pp. 193–270. <https://doi.org/10.1016/B978-012513840-6/50020-3>.
- Balogun, R.B., Ogbu, J.U., Umeokechukwu, E.C., Kalejaiye-Matti, R.B., 2016. Effective Micro-organisms (EM) as sustainable components in organic farming: Principles, applications and validity. In: Nandwani, D. (Ed.), *Organic Farming for Sustainable Agriculture*. Springer International Publishing, Cham, pp. 259–291. [https://doi.org/10.1007/978-3-319-26803-3\\_12](https://doi.org/10.1007/978-3-319-26803-3_12).
- Bodenhausen, N., Somerville, V., Desiro, A., Walser, J.C., Borghi, L., van der Heijden, M. G.A., Schlaeppli, K., 2019. Petunia- and Arabidopsis-specific root microbiota responses to phosphate supplementation. *Phytobiomes J.* 3, 112–124. <https://doi.org/10.1094/phybiomes-12-18-0057-r>.
- Daryanto, S., Fu, B.J., Wang, L.X., Jacinthe, P.A., Zhao, W.W., 2018. Quantitative synthesis on the ecosystem services of cover crops. *Earth Sci. Rev.* 185, 357–373. <https://doi.org/10.1016/j.earscirev.2018.06.013>.
- Daur, I., 2016. Study of commercial effective microorganism on composting and dynamics of plant essential metal micronutrients. *J. Environ. Biol.* 37, 937–941.
- Dos Santos, L.F., Lana, R.P., Da Silva, M.C.S., Veloso, T.G.R., Kasuya, M.C.M., Ribeiro, K. G., 2020. Effective microorganisms inoculant: diversity and effect on the germination of palisade grass seeds. *An. Acad. Bras. Cienc.* 92 <https://doi.org/10.1590/0001-3765202020180426>.
- Durner, W., Iden, S.C., 2021. The improved integral suspension pressure method (ISP plus) for precise particle size analysis of soil and sedimentary materials. *Soil Tillage Res.* 213, 105086 <https://doi.org/10.1016/j.still.2021.105086>.
- Edgar, R.C., 2013. UPPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10. <https://doi.org/10.1038/nmeth.2604>, 996–+.
- Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*, 081257. <https://doi.org/10.1101/081257>.
- EMRO Japan, 2023. Effective microorganisms. <https://emrojapan.com/>.
- Fatunbi, O., Ncube, L., 2009. Activities of effective microorganism (EM) on the nutrient dynamics of different organic materials applied to soil. *Am.-Eurasian J. Agron.* 2, 26–35.
- Frey, B., Rime, T., Phillips, M., Stierli, B., Hajdas, I., Widmer, F., Hartmann, M., 2016. Microbial diversity in European alpine permafrost and active layers. *FEMS Microbiol. Ecol.* 92 <https://doi.org/10.1093/femsec/fiw018>.
- Gollner, G., Fohraflner, J., Friedel, J.K., 2020. Winter-hardy vs. freeze-killed cover crop mixtures before maize in an organic farming system with reduced soil cultivation. *Org. Agric.* 10, 5–11. <https://doi.org/10.1007/s13165-020-00294-3>.
- Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S., Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiol. Res.* 206, 131–140. <https://doi.org/10.1016/j.micres.2017.08.016>.
- Higa, T., 1991. Effective microorganisms: A biotechnology for mankind. In: Parr, J.F., Hornick, S.B., Whitman, C.E. (Eds.), *First International Conference on Kyusei Nature Farming*. USA, Washington DC, pp. 8–14.
- Higa, T., Parr, J.F., 1994. Beneficial and effective microorganisms for a sustainable agriculture and environment, in: Center, I.N.F.R.
- Hu, C., Qi, Y.C., 2013. Long-term effective microorganisms application promote growth and increase yields and nutrition of wheat in China. *Eur. J. Agron.* 46, 63–67. <https://doi.org/10.1016/j.eja.2012.12.003>.
- Hu, C., Xia, X.G., Chen, Y.F., Han, X.M., 2018. Soil carbon and nitrogen sequestration and crop growth as influenced by long-term application of effective microorganism compost. *Chil. J. Agric. Res.* 78, 13–22. <https://doi.org/10.4067/s0718-58392018000100013>.
- Hussain, T., Javid, T., Parr, J.F., Jilani, G., Haq, M.A., 1999. Rice and wheat production in Pakistan with effective microorganisms. *Am. J. Altern. Agric.* 14, 30–36. <https://doi.org/10.1017/S0889189300007980>.
- Javid, A., 2011. Effects of biofertilizers combined with different soil amendments on potted Rice plants. *Chil. J. Agric. Res.* 71, 157–163.
- Javid, A., Bajwa, R., 2011. Field evaluation of effective microorganisms (EM) application for growth, nodulation, and nutrition of mung bean. *Turk. J. Agric. For.* 35, 443–452. <https://doi.org/10.3906/tar-1001-599>.
- Jusoh, M.L.C., Abd Manaf, L., Latiff, P.A., 2013. Composting of rice straw with effective microorganisms (EM) and its influence on compost quality. *Iranian J. Environ. Health Sci. Eng.* 10, 17. <https://doi.org/10.1186/1735-2746-10-17>.
- Keilueit, M., Wanzek, T., Kleber, M., Nico, P., Fendorf, S., 2017. Anaerobic microsites have an unaccounted role in soil carbon stabilization. *Nat. Commun.* 8, 1771. <https://doi.org/10.1038/s41467-017-01406-6>.
- Keilueit, M., Gee, K., Denney, A., Fendorf, S., 2018. Anoxic microsites in upland soils dominantly controlled by clay content. *Soil Biol. Biochem.* 118, 42–50. <https://doi.org/10.1016/j.soilbio.2017.12.002>.
- Khalid, A., Abbasi, M.K., Hussain, T., 2006. Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresour. Technol.* 97, 967–972. <https://doi.org/10.1016/j.biortech.2005.05.002>.
- Lacroix, E.M., Mendillo, J., Gomes, A., Dekas, A., Fendorf, S., 2022. Contributions of anoxic microsites to soil carbon protection across soil textures. *Geoderma* 425, 116050. <https://doi.org/10.1016/j.geoderma.2022.116050>.
- Lamont, J.R., Wilkins, O., Bywater-Ekegard, M., Smith, D.L., 2017. From yogurt to yield: potential applications of lactic acid bacteria in plant production. *Soil Biol. Biochem.* 111, 1–9. <https://doi.org/10.1016/j.soilbio.2017.03.015>.
- Lori, M., Armengot, L., Schneider, M., Schneidewind, U., Bodenhausen, N., Mader, P., Krause, H.M., 2022. Organic management enhances soil quality and drives microbial community diversity in cocoa production systems. *Sci. Total Environ.* 834 <https://doi.org/10.1016/j.scitotenv.2022.155223>.

- Mayer, J., Scheid, S., Widmer, F., Fließbach, A., Oberholzer, H.R., 2010. How effective are 'Effective microorganisms (R) (EM)'? Results from a field study in temperate climate. *Appl. Soil Ecol.* 46, 230–239. <https://doi.org/10.1016/j.apsoil.2010.08.007>.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szocs, E., Wagner, H., 2019. Vegan: community ecology package [software]. <http://CRAN.R-project.org/package=vegan>.
- Olle, M., Williams, I.H., 2013. Effective microorganisms and their influence on vegetable production - a review. *J. Hortic. Sci. Biotechnol.* 88, 380–386. <https://doi.org/10.1080/14620316.2013.11512979>.
- Pranagal, J., Ligeza, S., Smal, H., 2020. Impact of effective microorganisms (EM) application on the physical condition of haplic Luvisol. *Agronomy-Basel* 10, 1049. <https://doi.org/10.3390/agronomy10071049>.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. <https://doi.org/10.1093/nar/gks1219>.
- R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>.
- Rhee, S.J., Lee, J.E., Lee, C.H., 2011. Importance of lactic acid bacteria in Asian fermented foods. *Microb. Cell Factories* 10. <https://doi.org/10.1186/1475-2859-10-s1-s5>.
- Robert, C.E., 2016. SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *bioRxiv*, 074161. <https://doi.org/10.1101/074161>.
- Safwat, S.M., Matta, M.E., 2021. Environmental applications of effective microorganisms: a review of current knowledge and recommendations for future directions. *J. Eng. Appl. Sci.* 68, 48. <https://doi.org/10.1186/s44147-021-00049-1>.
- Schenck Zu Schweinsberg-Mickan, M., Müller, T., 2009. Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth. *J. Plant Nutr. Soil Sci.* 172, 704–712. <https://doi.org/10.1002/jpln.200800021>.
- Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 27, 863–864. <https://doi.org/10.1093/bioinformatics/btr026>.
- Schweiz, E.M., 2023. Rottelenker. <https://www.em-schweiz.ch/rottelenker-25-1>.
- Thorup-Kristensen, K., Magid, J., Jensen, L.S., 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. In: Sparks, D.L. (Ed.), *Adv. Agron.*, pp. 227–302. [https://doi.org/10.1016/s0065-2113\(02\)79005-6](https://doi.org/10.1016/s0065-2113(02)79005-6).
- Valarini, P.J., Alvarez, M.C.D., Gasco, J.M., Guerrero, F., Tokeshi, H., 2003. Assessment of soil properties by organic matter and EM-microorganism incorporation. *Rev. Bras. Cienc. Solo* 27, 519–525. <https://doi.org/10.1590/s0100-06832003000300013>.
- Van Fan, Y., Lee, C.T., Klemes, J.J., Chua, L.S., Sarmidi, M.R., Leow, C.W., 2018. Evaluation of effective microorganisms on home scale organic waste composting. *J. Environ. Manag.* 216, 41–48. <https://doi.org/10.1016/j.jenvman.2017.04.019>.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for Measuring soil microbial biomass-C. *Soil Biol. Biochem.* 19, 703–707. [https://doi.org/10.1016/0038-0717\(87\)90052-6](https://doi.org/10.1016/0038-0717(87)90052-6).
- Vincent-Caboud, L., Peigne, J., Casagrande, M., Silva, E.M., 2017. Overview of organic cover crop-based no-tillage technique in europe: farmers' practices and research challenges. *Agriculture-Basel* 7. <https://doi.org/10.3390/agriculture7050042>.
- Wang, Q., Yamabe, K., Narita, J., Morishita, M., Ohsumi, Y., Kusano, K., Shirai, Y., Ogawa, H.I., 2001. Suppression of growth of putrefactive and food poisoning bacteria by lactic acid fermentation of kitchen waste. *Process Biochem.* 37, 351–357. [https://doi.org/10.1016/s0032-9592\(01\)00217-5](https://doi.org/10.1016/s0032-9592(01)00217-5).
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. *Am. J. Altern. Agric.* 18, 3–17. <https://doi.org/10.1079/AJAA200228>.
- Xu, H.-L., 2000. Effects of a microbial inoculant and organic fertilizers on the growth, Photosynthesis and yield of sweet corn. *J. Crop. Prod.* 3, 183–214. [https://doi.org/10.1300/J144v03n01\\_16](https://doi.org/10.1300/J144v03n01_16).
- Youssef, M.A., Yousef, A.F., Ali, M.M., Ahmed, A.I., Lamlom, S.F., Strobel, W.R., Kalaji, H.M., 2021. Exogenously applied nitrogenous fertilizers and effective microorganisms improve plant growth of stevia (*stevia rebaudiana* Bertoni) and soil fertility. *AMB Express* 11, 133. <https://doi.org/10.1186/s13568-021-01292-8>.
- Zhong, Z.K., Bian, F.Y., Zhang, X.P., 2018. Testing composted bamboo residues with and without added effective microorganisms as a renewable alternative to peat in horticultural production. *Ind. Crop. Prod.* 112, 602–607. <https://doi.org/10.1016/j.indcrop.2017.12.043>.
- Zhou, H.B., Shen, Y.J., Li, R., Meng, H.B., Zhang, X., Wang, J., Cheng, H.S., Dong, S.S., Song, L.Q., Ding, J.T., Cheng, Q.Y., 2020. Heavy metals and community structure of microorganism changes during livestock manure composting with inoculation of effective microorganisms. *Int. J. Agric. Biol. Eng.* 13, 125–132. <https://doi.org/10.25165/j.ijabe.20201306.5674>.

## **Paper 4: Policy Brief Effective Microorganisms**

**Authors:** Simon Oberholzer, Christa Herrmann, Natacha Bodenhausen, Hans-Martin Krause, Adrien Mestrot, Chinwe Ifejika Speranza and Klaus A. Jarosch

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[Plant production](#)

Agroscope, FiBL, University of Bern

## Can Effective Microorganisms Influence Green-Manure Decomposition?

[Oberholzer S.](#), Herrmann C., Bodenhausen N., Krause H.-M., Mestrot A., Ifejika Speranza C., [Jarosch K.A.](#)

04.06.24

Photo: Simon Oberholz  
Universität Br

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**Although effective microorganisms (EM) are frequently used to shallowly incorporate green manures (cover crops) in the field, almost no scientific research has been conducted on them to date. This study simulated the decomposition of green manures with EM in the laboratory.**

### Why EM with green manures?


Green vegetation is the ideal soil cover for preventing erosion and nutrient losses, which is why winter-hardy cover crops are the best option for bridging the period between two main crops from a soil-protection perspective. Ploughing-in a green intercrop in spring can be a challenge, however, since the risk of second growth is relatively high. This applies in particular to organic farms that have embraced no-till. In such systems green manures are often only incorporated shallowly, to promote soil life with the rapidly decomposable plant material ('surface rotting' system). However, environmental conditions in spring vary a great deal from year to year, and cold and damp conditions in particular can inhibit the decomposition of plant biomass. The incompletely decomposed smeary plant biomass can therefore make seedbed preparation substantially more difficult. The use of EM attempts to accelerate the decomposition of the incorporated plant biomass whilst promoting soil life and humus formation.

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### Effects of EM on soil parameters

An in-depth laboratory experiment was conducted into the effects of adding EM to support surface rotting. In early May we brought a portion of both the top layer of soil and the plant material of a patch sown with rye and vetch into the laboratory and simulated the rotting process under controlled conditions at 12 °C.

The processes with and without EM both exhaled the same amount of carbon dioxide, suggesting the same microbial activity in both processes. The addition of EM did not affect the solubility of nutrients and trace elements in the soil, nor did it influence microbial biomass. Moreover, genetic analyses of the soil microbiome showed that the processes with and without EM did not differ in terms of their microbiological composition. Seven days after the addition of EM to the soil, lactic acid bacteria were the only identifiable EM components; however, this effect was negligible at a normal rate of application (120 litres/ha), and only measurable at 100 times this application rate. The study therefore found no evidence that the addition of EM influenced the decomposition of green manure materials.

### Conclusions

- The addition of EM produced no consistent effects on microbial activity in the soil.
- No differences were found between the processes with and without EM in terms of nutrient and trace-element solubility.
- Only very minor differences were found in the microbial composition of the two processes.
- Lactic acid bacteria, the primary constituent of EM, were detected in the soil, but only at a substantially increased application rate.

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## **Paper 5: Soil survey in northern Spain**

**Authors:** Maja Schneider, Simon Oberholzer and Chinwe Ifejika Speranza

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## Revegetation is key for soil organic carbon sequestration on abandoned and degraded land in northern Spain

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### ABSTRACT

Agricultural land abandonment is a major land use change in the Mediterranean region, especially affecting marginal areas. The fields of the abandoned village Sierra Estronad (Aragón, Spain), experienced heavy impact treatments (bulldozing) after which half of the fields were kept open and tilled without planting any crop and the other half of the fields were left fallow. From these two treatments and the surrounding natural forest 483 soil samples were collected in addition to corresponding vegetation data at 162 GPS referenced sampling points. Soil samples were analyzed using predictive models based on visible and near-infrared spectroscopy for Soil Organic Carbon (SOC), total Nitrogen, and Permanganate Oxidizable Carbon.

Comparing the fallow fields, which have had a 15-year recovery period to the tilled fields, a SOC sequestration rate of  $0.64 \text{ Mg ha}^{-1} \text{ y}^{-1}$  was found. On tilled fields however, even after a recovery period of 5 years, very few plants were able to colonize the area, resulting in a sparse soil cover and significantly lower SOC and total N stocks.

These results show the interdependence of soil fertility proxies (SOC and /total Nitrogen) and the degree of vegetation cover, and how practices of preventing former agricultural fields from revegetating have a long-lasting impact of soil degradation, even after their termination. However, if left fallow, abandoned fields do have the potential to support a secondary succession and serve as a carbon sink thus contributing to soil fertility and climate change mitigation.

### 1. Introduction

Agricultural land abandonment has been the most important change in Mediterranean ecosystems over the last centuries (Novara et al., 2017) and is considered the most important land use change since the agricultural expansion over 10'000 years ago (Petanidou et al., 2008).

Spain is especially affected with approximately 5% of the total agricultural land projected to be abandoned by 2030 corresponding to about 23 million hectares (Perpiña Castillo et al., 2020). Within the European Union (EU), this rate is extraordinarily high compared to an EU average forecast of 3% (Perpiña Castillo et al., 2018). The advancement of mechanization, chemical fertilization, and increased irrigation, modernizing farming practice and international market developments have led to agricultural land use intensifications on easily accessible agro-ecologically favorable fields and the abandonment of more marginal fields on steeper slopes and with less fertile soils. This phenomenon was accompanied by depopulation ("rural exodus") (Chauchard et al., 2007; Lasanta et al., 2017; Mottet et al., 2006;

Strijker, 2005). Furthermore, policies like the "set-aside"-policy established by the EU Common Agricultural Policy (CAP) in 1992, encouraged the withdrawal of cultivated land, by subsidizing fallows (García-Ruiz and Lana-Renault, 2011). Such fallows could be seeded with non-food crops or remain unseeded as was most commonly practiced in semiarid regions. Thus, to receive subsidies, the land had to be ploughed continuously to prevent plant colonization (García-Ruiz and Lana-Renault, 2011).

The evolution of fields after abandonment is complex and depends on many different factors, such as soil, lithology, topography, climate, and post-abandonment management (Romero-Díaz et al., 2017). However, the factor which possibly explains soil erosion and degradation rates after land abandonment best, is the vegetation cover (Lasanta et al., 2019; Thornes, 1985). Policies which foster the practice of preventing former agricultural fields from revegetating, therefore most likely increased the area at risk of severe erosion (Van Leeuwen et al., 2019).

Without any anthropogenic disturbances and with favorable climatic

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conditions where a vegetational cover can be sustained, soil characteristics may improve after abandonment through the accumulation of organic matter, an increasing floral and faunal activity, increased water infiltration rates and lower erosion rates (Dunjó et al., 2003; Kosmas et al., 2000; López-Bermúdez et al., 1996; Pardini et al., 2002; Piché and Kelting, 2015; Wertebach et al., 2017).

Thus, the potential of former agricultural land to sequester soil organic carbon (SOC) and serve as carbon sinks has become a research focus. Many studies (Bell et al., 2021; Djuma et al., 2020; Gabarrón-Galeote et al., 2015b; Lasanta et al., 2020; Lesschen et al., 2008; Nadal-Romero et al., 2016; Navas et al., 2012, among others) have investigated the accumulation of SOC in the Mediterranean region (Bell et al., 2021), examining the influence of many environmental and land management factors. Several authors have reported very slow SOC accumulation rates or even decreases in SOC (Bell et al., 2021; Djuma et al., 2020; Lesschen et al., 2008; Martínez-Duro et al., 2010; Nadal-Romero et al., 2016; Navas et al., 2012, among others).

However, in a literature review, covering 113 publications, 80% of the studies reported increases in SOC after land abandonment (Bell et al., 2021). Bell et al. (2021) found an average accumulation rate of  $+2.3\% \text{ y}^{-1}$ .

SOC accumulation post-abandonment can be explained by the increased organic matter input from leaf litter and the increased root biomass from the vegetation cover emerging through natural revegetation processes (Kalbitz and Kaiser, 2008; Zhao et al., 2015). Furthermore, through a denser vegetational coverage the microclimate improves through increased light absorption, decreased surface temperatures and evaporation rates, resulting in better conditions for microbial communities, enhancing carbon sequestration (Novara et al., 2014). Additionally, secondary succession contributes to the formation of soil aggregates, which promote SOC stabilization and accumulation (An et al., 2010; Nadal-Romero et al., 2016; Raiesi, 2012).

Although there is a consensus, that the vegetation cover is a key component in the development of abandoned lands, the spatial resolution of vegetation data differs greatly among field studies, assessing changes in physico-chemical soil characteristics. Most studies describe the vegetation cover of an entire study site, not considering small spatial heterogeneities (Alberti et al., 2011; Deng et al. 2013 and 2016; Emran et al., 2012; Guidi et al., 2014; Nadal-Romero et al., 2016; Nadal-Romero et al., 2021; Navas et al., 2012; Novara et al. 2014 and 2013; La Mantia et al., 2013; Pellis et al., 2019; Raiesi, 2012; Tommaso et al., 2018; Zhao et al., 2015). Other studies work with areal data to describe the land cover of the study sites, without recording field observations (Bell et al., 2021; Gabarrón-Galeote et al., 2015a; Trigalet et al., 2016; Wertebach et al., 2017). Only few studies recorded vegetation with a high spatial resolution (Bonet, 2004; Romero-Díaz et al., 2017; Knops and Tilman, 2000; Li et al., 2020; Martínez-Duro et al., 2010; Baeva et al., 2019) or work with representative subsites in which the vegetation is recorded (Foote and Grogan, 2010; Lasanta et al., 2020; Lesschen et al., 2008; Spohn et al., 2016).

In this study high spatial resolution vegetation data is used in addition to SOC as a proxy for soil fertility on areas surrounding the village of Sierra Estronad (Spain). The village has experienced land abandonment in the 1950s, however agricultural activities were temporarily resumed in the years after. This included high impact management practices such as bulldozing in 1998, after which half of the fields were tilled and half of them left fallow. In addition to the comparison of these two treatments we include the surrounding forests as a control representing the most natural landscape.

Driven by land management policies, many areas in Spain have undergone similar developments, which makes it important to study the recovery potential of soil fertility on such severely degraded croplands, to generate insights for future policy making. Methodologically, this study contributes insights into the application of visible and near-

infrared (vis-NIR) spectroscopy to soil spectral data analysis.

This study contributes to a better understanding of the impacts of land abandonment and the recovery potential of vegetation cover and soil fertility in a semiarid landscape.

## 2. Methods

### 2.1. Study area

The study area in Sierra Estronad ( $42^{\circ}15'54.95''\text{N}$  and  $0^{\circ}47'23.29''\text{W}$ ) lies in the municipality of Santa Eulalia de Gállego in the province of Zaragoza, Aragón, Spain. Aragón is influenced by the western Mediterranean climate having little precipitation all year round, cool winters and hot summers. Low precipitation rates combined with strong winds cause 70% of Aragón to be characterized as semi-arid (Cherlet et al., 2018). Geologically, Aragón is predominantly made up of calcareous materials and Tertiary and Quaternary sediments. The Calcaric Regosol (Siltic) soil type is distributed homogeneously over the entire study area. However, the southeast part has a different soil type, which is why these areas were excluded from the study area.

Sierra Estronad is surrounded by fields, pastures, and forests. After the village was abandoned in the mid-1950s, the land (approximately 120 ha) was lightly grazed by sheep for about 20 years but was not further cultivated. In 1973, former fields were cleared and enlarged with a bulldozer and cultivated for a short time before being abandoned again. In 1998, the current owners of the village took over the land and bulldozed all the fields once more.

In this study three different treatments are compared, including two types of abandoned fields: After the second time of bulldozing in 1998 'tilled fields' (16.25 ha) were tilled and cropped with barley until 2017. The tillage was done with a cultivator, with a working depth of about 10–15 cm. After 2017 the fields were no longer tilled nor cropped. 'Fallow fields' (14.90 ha) were left fallow after being bulldozed in 1998. Additionally, approximately the same amount of forest area (14.97 ha), as the most natural landscape, was added to the study area. The distribution of the included forest area was strongly determined by its accessibility, as many of the surrounding forests are on steep slopes.

Thus, the last bulldozing in 1998 created a common basis, after which tilled and fallow fields have experienced different treatments. The forest serves as a natural reference.

### 2.2. Sampling and sample preparation

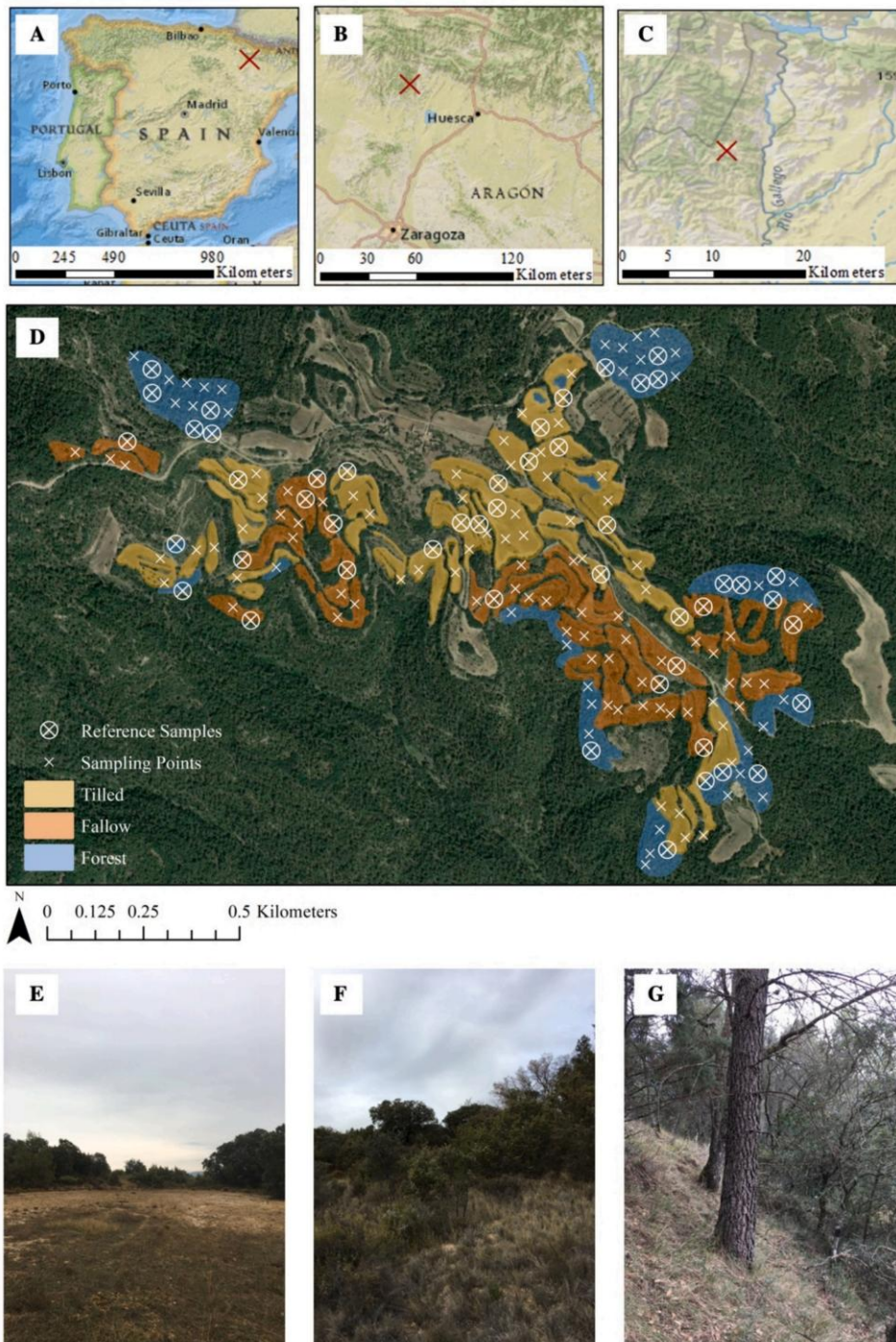
Within each treatment, 54 GPS (Global Positioning System) referenced sampling points were set in an unaligned sampling design (Webster and Lark, 2012) (see Fig. 1D). Per sampling point 3 samples were taken randomly in a  $2 \times 2$  m square with an auger of 2.5 cm diameter. The samples were then combined according to the depths 0–5 cm, 5–15 cm, 15–25 cm. In total 486 samples (3 treatments, 3 depths, 54 replications) were collected within the first two weeks of March 2022.

For the calculation of bulk density an additional 27 samples were taken (three per treatment and depth) with a  $100 \text{ cm}^3$  steel ring.

All samples were dried in the oven at  $40^{\circ}\text{C}$  to constant weight (around 72 h). Then the samples were gently crushed and sieved to 2 mm.

To determine the elemental composition of the Rock an X-ray Fluorescence Spectroscopy (XRF) Analysis was conducted on a rock sample.

Additionally, the vegetation coverage of the  $2 \times 2$  m square within which the soil samples were being taken, was estimated. Categories for vegetation coverage are 0–20%, 20–40%, 40–60%, 60–80% and 80–100%. For the same area the three most dominant vegetation types were recorded. Here the classes of Herbs, Gras, Shrubs, and Trees were used and for each class the most dominant species were identified.



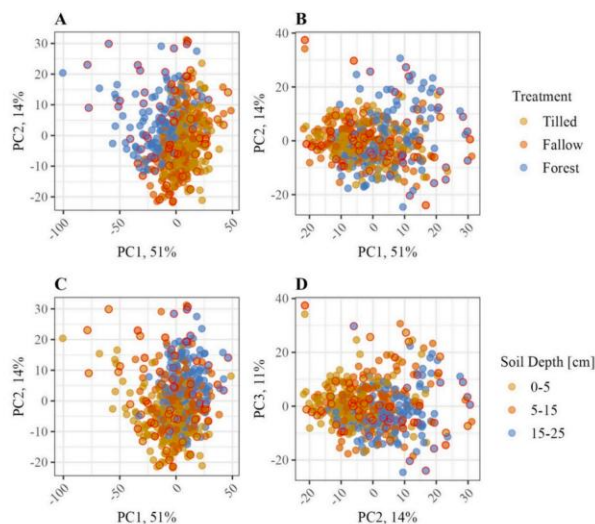
**Fig. 1.** A-C: Maps with a red mark on Sierra Estronad (Service Layer Credits: Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp). D: Map showing the study area. The colors indicate the treatments, and the crosses show the sampling points (Service Layer Credits: Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community). E-G: Photographs of the study area. E shows a tilled field, F shows a fallow field, and the photograph G was taken in the forest (Photos taken by Maja Schneider, March 2022).

### 2.3. Visible and near-infrared spectroscopy and selection of reference samples

All dried and sieved samples were analyzed with visible and near-infrared (vis-NIR) spectroscopy. The samples were measured using a Field-Spec PRO FR spectrometer (FieldSpec 4 Hi-Res, Malvern Panalytical, USA) measuring 2151 wavelengths from 350 to 2500 nm with a resolution of 1 nm. The sampling interval was 1.4 nm for wavelengths in between 350 and 1000 nm and 1.1 nm in between 1000 and 2500 nm. Each sample was measured three times, filling a new petri dish each time. Each measurement consisted of 30 scans, which were averaged by the RS3 software. Between measurements of different samples, the contact probe was cleaned with water and ethanol and the spectrometer was calibrated using a 100% reflectance white reference panel (Spectralon, 12 × 12 cm, Labsphere, USA).

The spectral data helped selecting as representative as possible subset of 57 samples (≈12%), which was used for the wet chemistry analysis. For this a principal component analysis (PCA) in combination with the Kennard-Stones algorithm, a technique for selecting a representative subset from a larger dataset, was conducted (Fig. 2). This algorithm starts with a random sample and then successively adds samples that are the most distant from those already chosen, based on Euclidean distance. This ensures that the selected samples are evenly distributed throughout the dataset (Kennard and Stone, 1969). The Kennard-Stones algorithm was run with 2 to 7 principal components for raw spectral data as well as spectral data which had previously been treated with two different preprocessing procedures: the Savitzky-Golay (SG) correction of different orders (m), with different window sizes (w) and once combined with the Multiple Scatter Correction (MSC) (SG (m = 2, w = 21)). The SG smoothing technique is used to enhance the signal-to-noise ratio of data without significantly distorting the signal. It works by fitting successive subsets of data points with a polynomial and using this polynomial to estimate the smoothed value of each point, effectively preserving the original shape and features of the signal (Savitzky and Golay, 1964). The MSC reduces the effects of scattering by normalizing the spectra (Isaksson and Næs, 1988).

The best performing combination of number of components, preprocessing procedures, and window size was selected, which covered most of the spectral variance and resulted in reference samples from all depths and treatments and are spatially well distributed.



**Fig. 2.** Scatterplots generated by the PCA with different colors indicating different treatments (A, B) or depths (C, D). The red outlines indicate the selected reference samples.

### 2.4. Wet chemistry analysis

Total C and total N concentrations were analyzed on a ground aliquot with a CNS element analyzer (vario MICRO tube, Elementar, Germany). Inorganic carbon was measured with the Scheibler method through the dissolution of carbonate in 10% HCl-solution and the measurement of the volume of the evolved CO<sub>2</sub>. SOC was calculated from the difference between total C and inorganic C. To measure Permanganate-Oxidizable Carbon (POXC) the protocol of Weil et al. (2003) modified by Lucas and Weil (2012) was followed. For this, 2.5 g of soil were mixed with 2.0 mL of 0.2 M KMnO<sub>4</sub> followed by 10 min of reaction time. Afterwards, a Spectrophotometer (UV-1800, Shimadzu Corporation, Japan) was used to measure the absorbance of the resulted liquid at 550 nm.

For the texture analysis, the samples were further prepared: Organic material was oxidized by adding H<sub>2</sub>O<sub>2</sub> and the samples were dispersed with MgCl<sub>2</sub>. The texture was then measured using laser radiation by the Mastersizer 2000.

### 2.5. Spectral models

In a following step, the results from the spectroscopy analysis and the wet chemistry analysis were combined to build predictive models which helped computing the measured proxies for all samples.

We optimized preprocessing for each soil property separately using the following techniques: SG preprocessing of different orders with different window sizes and sometimes in combination with MSC were used. Also, first and second Gap-Segment (GS) derivatives were applied, sometimes in combination with a Standard Normal Variate (SNV) correction.

The predictive models for each parameter (SOC, POXC, total N, etc.) were calibrated with the machine learning technique Partial Least Squares Regression (PLSR) (Wold et al., 1983). Random Forest (RF) and Cubist (CUB) were also tested, however leading to less accurate models. The measured values of SOC, POXC, total N etc. served as response variables for each covariate in form of differently preprocessed spectrum data.

For the PLSR the maximum number of components was set at 12 to avoid model overfit. To determine the appropriate number of components to be extracted, a 10-fold cross-validation was used (Baumann et al., 2021; Kuhn and Johnson, 2013; Molinaro et al., 2005). Again, in the attempt of avoiding model overfit, the number of components was determined by choosing the lowest number of components with a RMSE not exceeding one standard error of the lowest RMSE (Hastie et al., 2017). The hold-out folds of the cross validation served the final assessment of the model performance. The one-standard error rule was also applied during the optimization of preprocessing to avoid model overfitting.

Finally, for each measured parameter the best fitting model was selected considering different preprocessing steps, absorbance and reflectance, the number of PLSR components, different intervals, and wavelength ranges. The accuracy of each model was evaluated based on the RMSE, the ratio of performance to interquartile distance index (RPIQ) and the coefficient of determination (R<sup>2</sup>).

With the respective models, sand and clay were predicted while values for silt were obtained by subtraction. This way the shares of all three components add up to 100%.

### 2.6. Predictive models

The best performing models are listed in the table below (Table 1). The PLSR reached the most accurate models for all parameters.

**Table 1**

Table describing model performance of the chosen models. For all models the mathematical procedure PLSR (partial Least Square Regression) was applied. Both reflectance (ref.) and absorbance (abs.) were considered. Preprocessing procedures included the Savitzky-Golay filter (SG, m = order of derivative, w = window width), gap segment derivative (GSD, m = derivative, w = window width, s = segment size) and multiplicative scatter correction (MSC). The models were evaluated with 5 times repeated 10-fold cross-validation. Model metrics of Cross-validation are indicated as mean (ncomp = number of components,  $R^2$  = coefficient of determination, RMSE = Root mean square error, RPIQ = Ratio of Performance to Inter Quartile distance).

	Mathematical Procedure	Abs./Ref.	Preprocessing	Interval	Window Size	ncomp	Bias	$R^2$	RMSE	RPIQ
<b>SOC</b>	PLSR	A	SG (m = 1, w = 5)	3	350–2400	1	−0.11	0.88	6.45	3.83
<b>Total C</b>	PLSR	A	SG (m = 1, w = 21) MSC	3	350–2400	6	0.28	0.86	6.17	2.55
<b>POXC</b>	PLSR	A	GSD (m = 2, w = 91, s = 91)	3	370–2440	2	0.00	0.46	0.21	1.49
<b>Total N</b>	PLSR	A	SG (m = 1, w = 21) MSC	9	350–2430	5	0.00	0.9	0.26	3.34
<b>PH</b>	PLSR	A	GSD (m = 4, w = 21, s = 21)	3	360–2500	5	−0.00	0.8	0.11	1.73
<b>Sand</b>	PLSR	R	GSD (m = 2, w = 51, s = 51)	3	350–2490	7	−1.38	0.6	83.48	2.29
<b>Clay</b>	PLSR	A	GSD (m = 2, w = 91, s = 1)	6	350–2320	3	−0.34	0.32	25.01	1.45

### 2.7. Reference samples

The best performance was found using the Savitzky-Golay (SG) filter, first order derivative (m), window size (w) 11 (SG (m = 1, w = 11)), whereas the first three principal components described most of the variance (PC1: 51%, PC2: 14%, PC3: 10%). The selected samples were then compared considering absorbance and reflectance. Looking at the preprocessing SG (m = 1, w = 11) and the first three components, the selected samples are best distributed among treatments as well as depths when considering the reflectance. Lastly, the spatial distribution of the selected samples was assessed visually and considered balanced (see Fig. 1D).

### 2.8. Statistical analysis

The statistical analysis was conducted with the predicted values, unless the sample was part of the reference sample subset, in which case the predicted values were replaced with the measured ones.

According to the Shapiro-Wilk test, the data did not show a normal distribution. Therefore, non-parametric tests were used for the statistical analysis. In place of an analysis of variance (ANOVA), which assumes that the data follows a normal distribution, the Kruskal-Wallis test was used to examine if differences among treatments and depths could be found. This was followed by a Dunn's post-hoc test with the Bonferroni correction to test which pairings show statistically significant differences ( $p < 0.05$ ). Compared to other post-hoc tests the Bonferroni correction is rather conservative and effective at reducing type I errors (false positives) (Bland and Altman, 1995).

Measured concentrations can be turned into stocks, by multiplying them with the measured bulk density (BD) of the respective soil depth. This calculation however can be biased, when bulk density varies spatially, and stocks of a fixed depth contain different soil masses. Therefore, the equivalent soil mass (ESM) approach with fixed depth (FD) corrections was applied. Here a reference soil mass is used to work with the same soil masses in creating stocks for all sampling areas. With the minimum ESM method, the reference soil mass was adjusted to the lowest soil mass across all treatments for every layer. All other stocks were adjusted to an equivalent soil mass (Ellert and Bettany, 1995).

Values are presented as mean followed by  $\pm$  standard deviation.

## 3. Results

### 3.1. Characterization of the study area

The texture shows a relatively homogeneous distribution among the three treatments. The biggest shares are made up by silt (Tilled:  $62 \pm 5\%$ , Fallow:  $62 \pm 6\%$ , Forest  $57 \pm 9\%$ ) followed by sand (Tilled:  $26 \pm 6\%$ , Fallow:  $27 \pm 7\%$ , Forest  $33 \pm 9\%$ ) and clay (Tilled:  $12 \pm 2\%$ , Fallow:  $11 \pm 1\%$ , Forest:  $10 \pm 1\%$ ) (see Appendix 1).

pH values in the top 0–5 cm range from  $7.5 \pm 0.2$  in the forest to  $7.7 \pm 0.1$  on tilled and fallow fields. This corresponds to the inorganic C

**Table 2**

Table showing the most dominant species found within the vegetation classes of herbs, grass, shrubs, and trees.

Vegetation class	Most Dominant Species		
	Latin	Spanish	English
Trees	<i>Quercus ilex</i>	Encina	Holm Oak
	<i>Pinus halapensis</i>	Pino de Alepo / Pino Carrasco	Aleppo Pine
	<i>Arbutus unedo</i>	Madroño	“Strawberry Tree”
Shrubs	<i>Pistacia terebinthus</i>	Cornicabra	Turpentine Tree
	<i>Rosmarinus officinalis</i>	Romero	Rosemary
	<i>Dorycnium pentaphyllum</i>	Escobón	Badassi
	<i>Genista scorpius</i>	Aliaga	Broom
	<i>Juniperus oxycedrus</i>	Enebro	Juniper
	<i>Brachypodium retusum</i>	Lastón Ramoso	Mediterranean False Brome
Herbs	<i>Potentilla neumanniana</i>	Flor del hambre	Cinquefoil
	<i>Plantago lanceolata</i>	Llantén Menor / Siete Venas	Ribwort Plantain
	<i>Sanguisorba hybrida L.</i>		Burnet

concentrations of the top 0–5 cm, which are slightly lower in the forest ( $31.0 \pm 12 \text{ g kg}^{-1}$ ) than on tilled ( $40.7 \pm 6.4 \text{ g kg}^{-1}$ ) or fallow fields ( $39.5 \pm 7.6 \text{ g kg}^{-1}$ ) (see Appendix 1).

The most dominant species found within each vegetation class can be seen in Table 2.

### 3.2. Carbon stocks and CO<sub>2</sub> equivalent

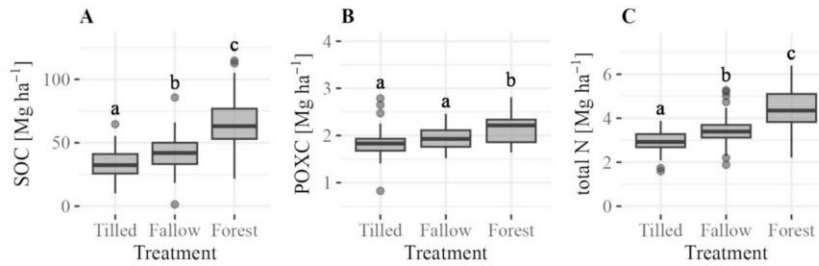
Total carbon stocks were estimated for a depth of 0–25 cm. They were lowest on tilled fields at  $32.4 \text{ Mg ha}^{-1}$ . On average, from tilled to fallow fields the stocks increased by 27.1% to  $41.9 \text{ Mg ha}^{-1}$ . From fallow fields to the forest treatment, the stocks increased by another 54.6%. Here, carbon stocks were highest at  $63.1 \text{ Mg ha}^{-1}$ . In comparing the fields, fallow fields were able to store  $9.6 \text{ Mg ha}^{-1}$  more SOC than tilled fields (Fig. 3).

When extrapolating the SOC stocks to the whole study area, tilled fields, with an area of 16.3 ha, store 526.1 Mg, fallow fields, with an area of 14.9 ha, store 624.9 Mg, and on 15.0 ha of forests, 944.4 Mg of organic carbon are stored.

Within 15 years, fallow fields were able to gain  $9.6 \text{ Mg ha}^{-1}$  more carbon than tilled fields. This equals a carbon sequestration rate of  $0.64 \text{ Mg ha}^{-1} \text{ y}^{-1}$  or  $+2.76\% \text{ y}^{-1}$ .

The CO<sub>2</sub> equivalent could be calculated from the carbon stocks. It is highest in the forest treatment with a mean value of  $231.3 \text{ Mg ha}^{-1}$  ( $\pm 17.9 \text{ Mg ha}^{-1}$ ) equivalent to 3462.9 Mg, for the whole study area. This is followed by the fallow fields, with a mean value of  $153.8 \text{ Mg ha}^{-1}$  ( $\pm$



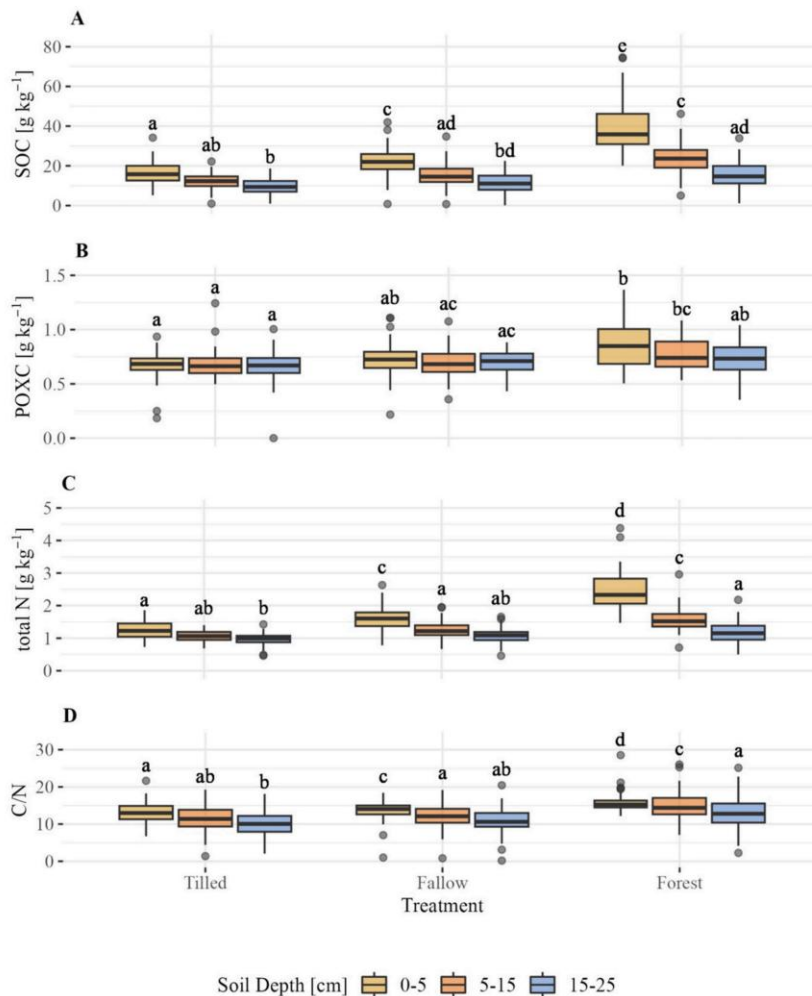


**Fig. 3.** Boxplots showing the total stocks of SOC (A), POXC (B), and total N (C). The letters above the boxplots represent significant differences between the different treatments ( $p < 0.05$ ).

53.9 Mg ha<sup>-1</sup>) equivalent to 2291.3 Mg for all fallow fields. The lowest CO<sub>2</sub> equivalent was found on tilled fields, with a mean value of 118.7 Mg ha<sup>-1</sup> ( $\pm 41.9$  Mg ha<sup>-1</sup>) equivalent to 1929.1 Mg on all tilled fields.

The additional amount of CO<sub>2</sub> that could be stored in fallow fields in comparison to tilled fields corresponds to about 1047.3 € y<sup>-1</sup> or 70.3 € ha<sup>-1</sup> y<sup>-1</sup>, according to the OECD carbon rates (27.6 € Mg<sup>-1</sup>) (OECD, 2021).

Total stocks at a depth of 0–25 cm were also estimated for POXC (mean: 1.91 Mg ha<sup>-1</sup>  $\pm$  0.31 Mg ha<sup>-1</sup>) and total N (mean: 3.44 Mg ha<sup>-1</sup>  $\pm$  0.94 Mg ha<sup>-1</sup>). The comparison between treatments shows descending values from forest over fallow to tilled fields for all parameters. According to Dunn's test, these differences are statistically significant for SOC and total N. POXC stocks showed no significant differences between tilled and fallow fields.



**Fig. 4.** Boxplots showing the concentrations of SOC (A), POXC (B), total N (C), and C/N (D). Letters above the boxplots indicate significant differences among different depths and treatments ( $p < 0.05$ ).

### 3.3. Differences along the soil profile

Highest SOC and total N concentrations were found in the top 0–5 cm layer and the lowest in 15–25 cm. In the top 0–5 cm, concentrations are significantly lower on tilled fields, followed by fallow fields and are highest in the forest treatment. The rates of decrease in concentrations in an increasing depth are lowest on tilled fields and highest in the forest treatment. Here (forest treatment), all three measured layers are significantly different from each other (Fig. 4).

Highest POXC concentrations were found in the top 0–5 cm. In those top 0–5 cm, concentrations are significantly lower on tilled fields compared to the forest. POXC concentrations do not show statistically significant differences among the three measured depths in any of the treatments.

The C/N ratio was found to be lowest on tilled fields (mean:  $11.24 \pm 3.00$ ), followed by fallow fields (mean:  $12.47 \pm 2.83$ ) and highest in the forest treatment (mean:  $14.46 \pm 3.03$ ).

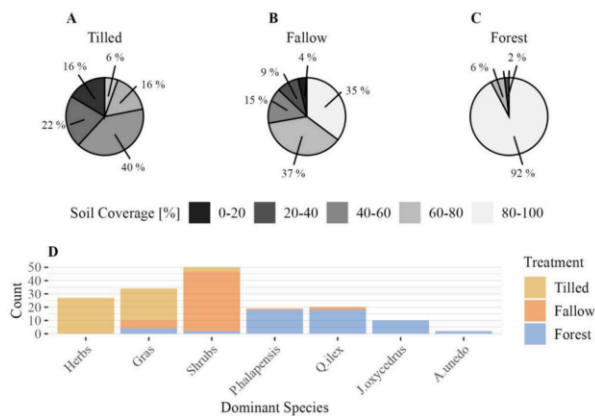
Looking at the top 0–5 cm, this increase is significant for all treatments. In 5–15 cm only the forest treatment has a significantly higher C/N ratio than the other two treatments.

### 3.4. Soil cover and most dominant vegetation

The degree of soil coverage differs among all treatments. Highest soil covers were found in the forest treatment, followed by fallow fields, while tilled fields were most often classified in the 40–60% covered category followed by 20–40% (Fig. 5A–C).

The most dominant species differ among treatments, whereas tree species can mostly be found in the forest treatments, fallow fields are often colonized by shrubs and tilled fields mostly have a cover of herbs and grass (Fig. 5D).

Within the three treatments, the influence of the degree of soil cover on different soil fertility proxies were tested using the stocks of the upper 0–15 cm. An increase in stocks with an increasingly dense soil cover could be seen for SOC, total N. However, significant differences were only found on tilled and fallow fields. SOC and total N stocks were significantly higher on tilled fields with a cover of 60–80% compared to 0–40%. The samples taken in spots with a cover of 80–100% were



**Fig. 5.** A: One pie chart for each treatment, where the colors indicate the different soil coverage classes. B: Bar plot showing how often the different plant species (Herbs, Grass, Shrubs, *Pinus halapensis*, *Quercus ilex*, *Juniperus oxycedrus*, *Arbutus unedo*) were found as the most dominant one. The different colors show the different treatments.

slightly lower and did not differ from stocks measured in 0–60% covered areas. On fallow fields there were less than three samples with a soil cover of 0–20% which is why this class could not be compared in the statistical tests. In the other classes a similar pattern like on tilled fields was observed, for SOC. Total N stocks on fallow fields are significantly higher with a soil cover of 40–60% and 80–100% compared to 20–40%. However, stocks in areas with 60–80% soil cover did not differ from any other soil cover classes (Fig. 6).

No significant differences in any of the treatments among any of the soil cover classes were found for POXC stocks and the C/N ratio.

Considering the most dominant plant species, the stocks of SOC, total N, and POXC as well as the C/N ratio were examined for a depth of 0–15 cm. In the forest treatment, the SOC and total N stocks were significantly higher where *Quercus ilex* (Holm Oak) was the most dominant species than where *Pinus halapensis* (Aleppo Pine) was the most dominant species. When considering the stocks over the whole depth (0–25 cm) these differences can no longer be seen (Fig. 7).

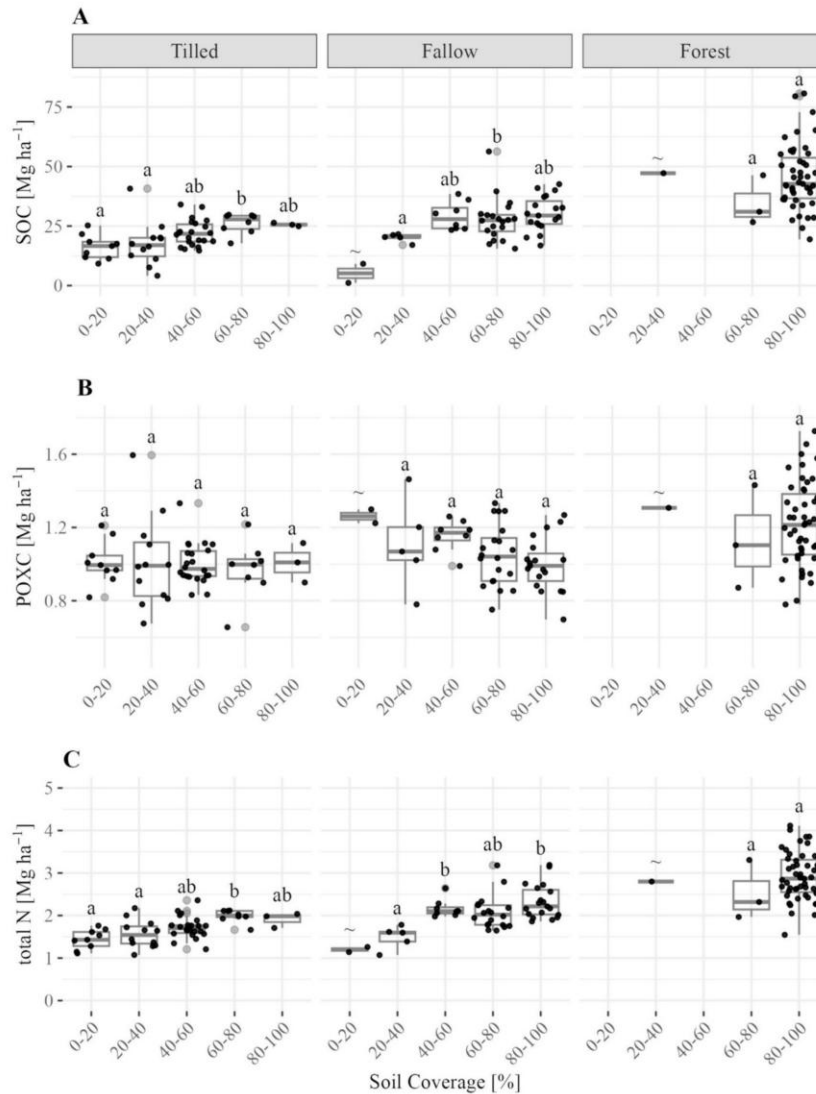
## 4. Discussion

### 4.1. Predictive models based on vis-NIR spectroscopy

In this study, as in works by Fernández et al. (2016), Levi et al. (2020), Da Silva-Sangoi et al. (2022), Serrano et al. (2021), and Zornoza et al. (2008), the vis-NIR approach was found to be an effective method in evaluating soil characteristics. The best predictive model for SOC was obtained by using partial least square regression (PLSR) and the Savitzky-Golay (SG) preprocessing. This is in accordance with findings reported by Da Silva-Sangoi et al. (2022), Moura-Bueno et al. (2019) and Vasques et al. (2008). The models built for SOC were found to be performing well, with  $R^2$  of 0.88, RMSE of 0.64% and RPIQ of 3.83. Similar  $R^2$  values were reported by Chang and Laird (2002) ( $R^2$ : 0.89, RMSE: 0.036%), Leone et al. (2012) ( $R^2$ : 0.84–0.93, RMSE: 0.34–1.74%), and Wetterlind et al. (2010) ( $R^2$ : 0.9, RMSE: 0.28%).

Of all predictive models, the ones for POXC were the least accurate ( $R^2$ : 0.46, RMSE of 0.21, RPIQ of 1.49). In contrast, Calderón et al. (2017) achieved good predictions of POXC using PLSR basing on NIR spectral data, with  $R^2$  0.66–0.76. In their research, predictions of POXC were even more accurate than for SOC, with slightly higher accuracy using MIR ( $R^2$  0.77–0.81) as compared to NIR.

Since the bias (see Table 1) was for all soil properties very low, the spectral models did not show any systematic under- or overestimation but rather increased the variability (indicated by RMSE) in the measured values. The additional error introduced by the spectral models would theoretically reduce the statistical power and make significant differences between treatments less probable. On the other hand, the application of spectroscopy increased the sample size drastically which would theoretically improve the statistical power. We conducted statistical tests with all samples and only with the reference samples, leaving out the step of predicting any data (see Fig. 8). The combination of the three land cover categories and the three soil depths results in nine different treatments that can be compared in 36 possible combinations. Looking at SOC for example, 24 out of 36 possible treatment combinations showed a statistically significant difference. However, considering only the reference samples, 5 combinations showed a statistically significant difference. This demonstrates how by including the predicted values basing on the spectroscopy data, and thus increasing the sample size, the number of significant pairings increased dramatically. We conclude therefore, that the effect of the higher sample size overcompensated the reduced measurement accuracy and led to an increased statistical power.



**Fig. 6.** Boxplot with jitter showing the stocks within the top 0–15 cm layer of SOC (A), POXC (B) and total N (C). The dots show the sample size. The X position of each dot was randomly computed to assist the readability. The letters above the boxplots represent significant differences between the different soil cover classes within the same treatment ( $p < 0.05$ ). The '~' sign appears with a sample size lower than 3 and indicates the lack of statistical comparability.

#### 4.2. Soil organic carbon sequestration rates post agricultural land abandonment

In comparing tilled and fallow fields, an addition of 9.55 Mg ha<sup>-1</sup> SOC was found. Over the 15-year period in which the fallow fields were able to recover after the last bulldozing, while tilled fields were further cultivated, this corresponds to a yearly sequestration rate of 0.64 Mg ha<sup>-1</sup> y<sup>-1</sup> and + 2.76% y<sup>-1</sup>. This is slightly higher than the average SOC sequestration rate of +2.3% y<sup>-1</sup> which Bell et al. (2020) identified from their review of previously published study sites and the sampling of three new sites in northeastern Spain. Both higher and lower sequestration rates were found by Novara et al. (2017), who worked with previously published field studies in addition to collecting their own

samples in Sicily. They reported increases in SOC stocks of the upper 0–30 cm soil layer after croplands were abandoned for 20 years with an average of 0.45 Mg ha<sup>-1</sup> y<sup>-1</sup> of SOC. Their values ranged from 0.27 Mg ha<sup>-1</sup> y<sup>-1</sup> to 1.34 Mg ha<sup>-1</sup> y<sup>-1</sup>, depending on soils and bioclimate. One possible explanation for the slightly higher sequestration rates in our study site as compared to the average rate found by Bell et al. (2020) is the lithology. Soils on calcareous lithologies, as in Sierra Estroñad, were found to have higher OM accumulation rates post-abandonment as compared to marly lithologies (Robledano-Aymerich et al., 2014). Furthermore, our study site lies within the range of annual precipitation rates, which according to Bell et al. (2020) are optimal for carbon sequestration.

However, also higher sequestration rates were reported within a

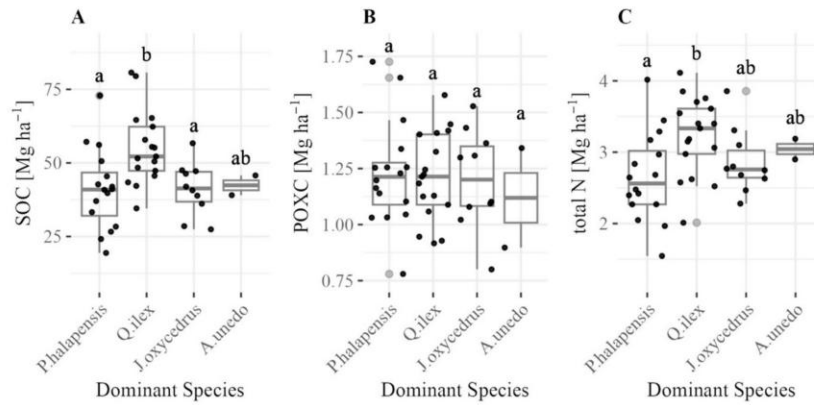


Fig. 7. Boxplot showing the SOC stocks within the top 0–15 cm layer of the forest treatment. No effects were found considering the whole depth of 0–25 cm. The letters above the boxplot represent significant differences with different dominant species ( $p < 0.05$ ).

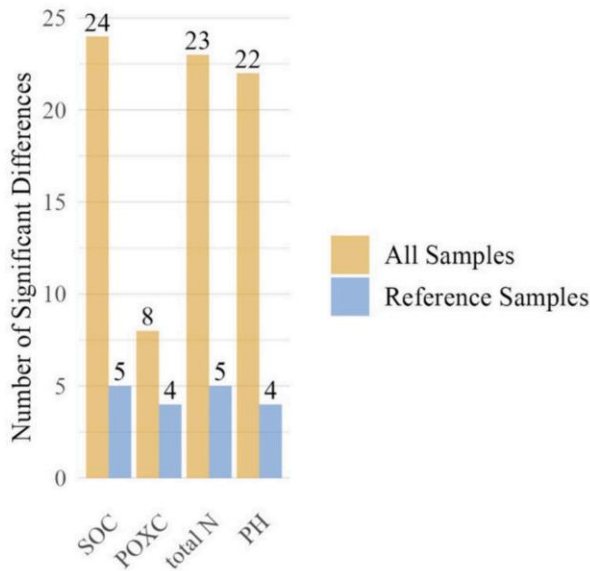


Fig. 8. Bar plot showing the number of statistically significant differences among depths and treatments when using all samples ( $n = 483$ ) in yellow and when only considering the reference samples ( $n = 57$ ) in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Mediterranean climate, for example by Tommaso et al. (2018), who found sequestration rates of  $1.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$  in a 14-year period in a depth of 30 cm in the Alps of Italy. The fact that the sequestration rate, measured in our study, is lower, could be due to the high impact cultivation through bulldozing, which depleted soils and most likely slowed down the revegetation process of both tilled and fallow fields (Bonet, 2004; Robledano-Aymerich et al., 2014).

However, it is important to note that the measured sequestration rate is not expected to last infinitely. De Baets et al. (2013) argue that C

accumulation is rapid over the first 10–50 years after abandonment, then stabilizing after that. As the current shrub vegetation on fallow fields might eventually evolve into young and finally mature forest, SOC stocks are likely to stabilize. With a SOC sequestration rate of  $0.64 \text{ Mg ha}^{-1} \text{ y}^{-1}$ , it would take 33 years for fallow fields to reach SOC stocks like the ones found in the forest of Sierra Estronad.

#### 4.3. Development of vegetation cover and soil properties post agricultural land abandonment

All fields in the study area experienced very high intensity interventions (bulldozing) and some of the fields were tilled for 19 years afterwards. On those fields many spots were so far degraded, that even after a 5-year recovery period no vegetation could establish. By recording small spatial heterogeneities in soil cover within one treatment and comparing them to the SOC and total N stocks, it could be shown, that an increased soil cover did correspond to higher SOC and total N stocks. Similarly, also on fallow fields, areas were observed, where a vegetation cover was hardly able to establish, however fewer as on tilled fields. Also here, the SOC and total N stocks tend to increase with an increasing soil cover leading to overall significantly higher SOC and total N stocks. This could be due to higher erosion rates on tilled fields as compared to fallow fields. In a similar environment, Cerdà et al. (2018) compared two treatments in eastern Spain, where all plots were tilled for two years and in one treatment, plots were abandoned afterwards. Even though, at first, erosion rates increased after abandonment, already after two years runoff rates were lower on abandoned fields and after nine years abandoned fields had 21 times less sediment yield than the continuously tilled fields.

Furthermore, it can be assumed, that the overall denser vegetation cover, observed on fallow fields, led to an increased OM input from leaf litter (Kalbitz and Kaiser, 2008; Zhao et al., 2015). This can also be seen in SOC and total N concentration which increased from tilled to fallow fields and decreased with increasing soil depth. The differences between treatments were highest in the topsoil layer. La Mantia et al. (2013) explained how the litter input “causes the upper mineral soil layers to respond more rapidly than deeper layers to changes in aboveground C and N inputs” (La Mantia et al., 2013, p. 243).

Even though labile SOC has been found to be more responsive to management change than total SOC (Rocci et al., 2021) difference in

POXC concentrations could not be found comparing tilled and fallow fields. Trigalet et al. (2016) found an initial increase of labile carbon along secondary succession on abandoned croplands in southern Spain. This increase however only occurred in the first 10 years after abandonment and followed the colonization by grasses. When grasslands turned into shrublands approximately 22 years after abandonment, “the particulate organic matter concentration decreased again to its initial level under cropland” (Trigalet et al., 2016, p. 19). With a similar development, this decrease in labile carbon, including POXC, could have already taken place on the fallow fields, which were most often covered by shrubs. Also, in this study, the predictive models for POXC had a lower performance than the ones for SOC and total N, leading overall to only few statistical significances (see Fig. 8).

As in Dunjó et al. (2003), C/N ratios were higher than 10, the optimal ratio for OM incorporation, and increased from tilled to fallow fields and the forest. A similar trend was also observed by Bell et al. (2021), Navas et al. (2012), and Deng et al. (2013) who all reported an increasing C/N ratio with time after land abandonment. This could be explained by the different vegetation coverages, whereas from tilled fields over fallow fields to the forest treatment the number of woody plants increase and so do the input of woody materials and dead leaves, which are known to increase the C/N ratio and slow down decomposition rates (Akratos, 2017). This would also explain why the difference among all treatments can only be seen in the top 0–5 cm, where plant litter is most important.

Plant litter also seems to have influenced the SOC and total N stocks within the upper 0–15 cm which were significantly higher where *Quercus ilex* (Holm Oak) was the most dominant species than where *Pinus halapensis* (Aleppo Pine) was the most dominant species. Contrary to these results, D’Orazio et al. (2014) found higher SOC stocks in soils underlying *P.halapensis*. However, they also reported faster decomposition rates of litters from *P.halapensis* as compared to *Q.ilex* litters. This can also be seen in the slightly higher C/N ratio found under *Q.ilex*, which was however not significant. Due to the therefore larger litter layer under *Q.ilex*, this could have led to more litter being sampled here, resulting in higher SOC values measured in the topsoil, while no effects were found considering the whole depth of 0–25 cm.

#### 4.4. Management implications

At the national level of Spain, one important driver of recent land abandonment was the EU CAP policy, which supported agricultural land being set-aside through subsidies. In our study area, due to low productivity and low potential for long-term farming, this led to “on and off farming”, which showed to have a negative impact on soil fertility. On tilled fields, even after five years in which the tillage was terminated, very few plants were able to colonize the area, resulting in a very sparse soil cover. This led to significantly lower SOC and total N stocks than in fallow fields, which were not cultivated since the last high impact intervention 24 years ago. This supports the argument of Van Leeuwen et al. (2019), that such policies like the set-aside policy of the EU CAP can expose areas to a severe risk of erosion. Therefore, the set-aside policy should have been accompanied by preventive measures making it impossible to reopen overgrown land for crop production.

As an alternative, low intensity grazing could be introduced. This would allow for a management in which the complex and traditional cultural landscapes of the Mediterranean mountain areas, with pastures, shrublands and forests, could be maintained (Lasanta et al., 2020). Such

a mosaic landscape could promote “biodiversity, soil quality, carbon sequestration and the availability of agricultural and livestock resources to keep the villages alive” (Lasanta et al., 2020, p. 2841). In terms of carbon storage, land abandonment followed by passive management (secondary succession), or active management (such as reforestation) is the most effective strategy (Bell et al., 2020). Thus, options to improve the set-aside policy needs to be considered, regarding the additional amount of CO<sub>2</sub> that could be stored in fallowed fields and their monetary value 70.3 € ha<sup>-1</sup> y<sup>-1</sup>, based on the OECD carbon rates (27.6 € Mg<sup>-1</sup>) (OECD, 2021), as well regarding the other landscape effects of fallowed fields such as low-intensity grazing, increased biodiversity and vegetation cover and climate mitigation potential.

## 5. Conclusion

In this study, the impact on soil fertility proxies of policy-driven land management strategies on abandoned marginal lands in northern Spain have been investigated. On a small scale but with a high spatial resolution, the interdependence of soil fertility proxies (SOC and total N) and the degree of vegetation cover could be shown, with higher SOC and total N stocks on fields with higher vegetation covers.

Data on soil properties was gained through wet chemistry analysis and extended through models basing on vis-NIR spectroscopy data, combined with multivariate data analysis. This is a low-cost approach which still allows for a large sample size to be analyzed. As in many other studies, this method was found to be very effective, leading to reliable predictive models and a higher statistical power gained through a large sample size.

A local carbon sequestration rate of 0.64 Mg ha<sup>-1</sup> y<sup>-1</sup> following the secondary succession on former agricultural fields could eventually lead to the whole study area storing 2’909.9 Mg of SOC equaling 10’668.8 Mg of CO<sub>2</sub> on 46.1 ha of forest. In terms of soil fertility, it can therefore be concluded, that policies which encourage land abandonment but ask for former agricultural fields to be kept from revegetation, do in fact foster the degradation of soils. The maintenance of a vegetation cover should be pursued, to hinder erosional processes and promote carbon sequestration, thus contributing to soil fertility and climate change mitigation.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

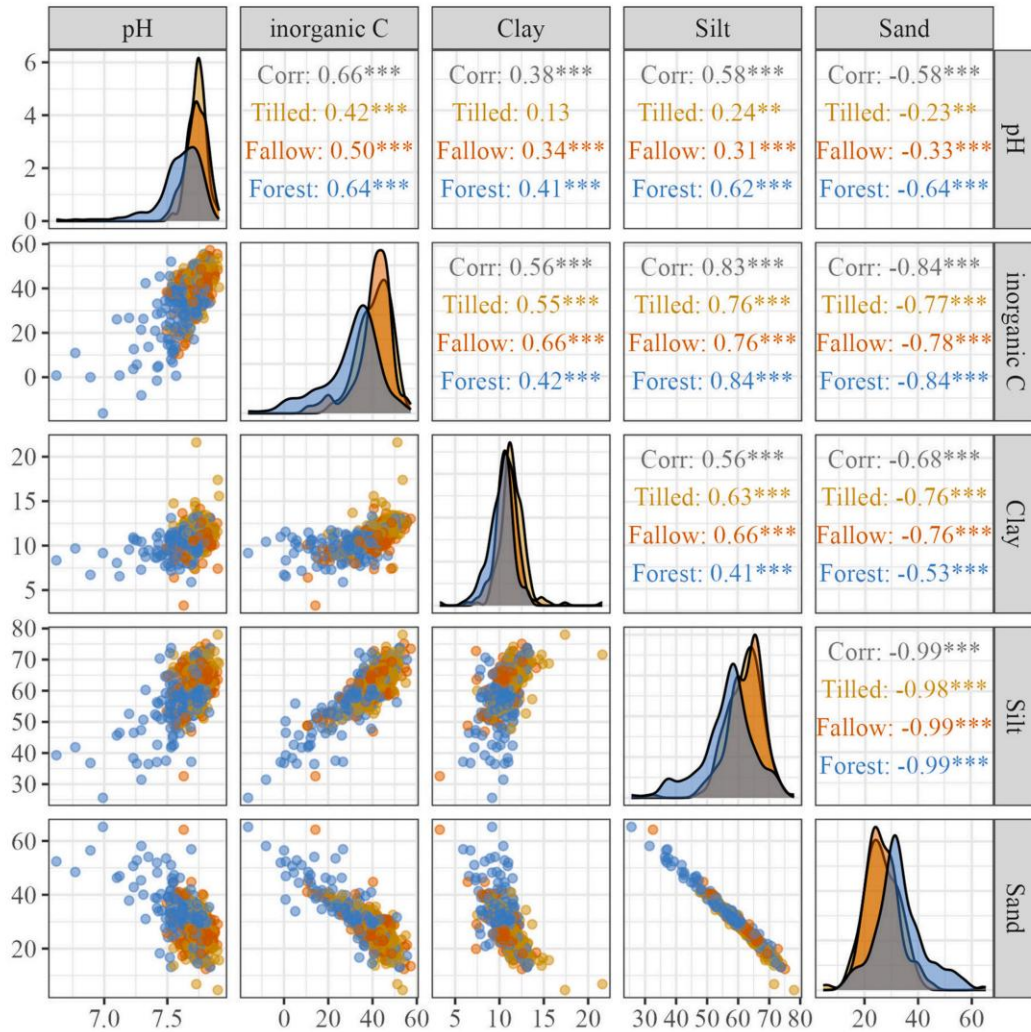
#### Data availability

Data will be made available on request.

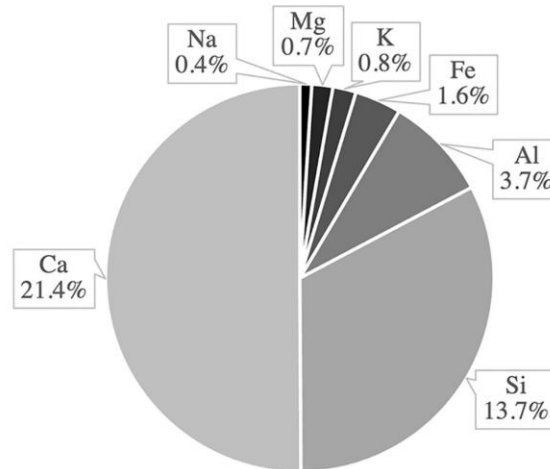
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**Appendix 1.** Graph showing the correlations between pH, inorganic C, clay, silt, and sand. Inorganic C concentrations are shown in  $\text{g kg}^{-1}$ . Clay, silt, and sand are shown in percentages. The colors indicate the different treatments. On the bottom left there are scatter plots, separated through diagonally arranged density plots from the Pearson Correlation coefficients listed on the top right



**Appendix 2.** Pie chart showing the major elemental components measured in the XRF Analysis. The shares in this plot do not add up to 100% since there were 49 more elements found which make up the remaining 58%



## References

- Akratos, C.S., 2017. Chapter 8—Cocomposting of Olive Mill Waste for the Production of Soil Amendments.
- Alberti, G., Leronna, V., Piazzì, M., Petrella, F., Mairota, P., Peressotti, A., Piusi, P., Valentini, R., Cristina, L., La Mantia, T., Novara, A., Rühl, J., 2011. Impact of woody encroachment on soil organic carbon and nitrogen in abandoned agricultural lands along a rainfall gradient in Italy. *Reg. Environ. Chang.* 11 (4), 917–924. <https://doi.org/10.1007/s10113-011-0229-6>.
- An, S., Mentler, A., Mayer, H., Blum, W.E.H., 2010. Soil aggregation, aggregate stability, organic carbon and nitrogen in different soil aggregate fractions under forest and shrub vegetation on the loess plateau. *China CATENA* 81 (3), 226–233. <https://doi.org/10.1016/j.catena.2010.04.002>.
- Baeva, Yu.I., Kurganova, I.N., Lopes de Gerenyu, V.O., Telesnina, V.M., Chernykh, N.A., Prokofeva, T.V., Morel, J.L., Ananyeva, N.D., 2019. Abandonment of arable lands triggers the recovery of native vegetation and organic carbon content in soils. In: Vasenev, V., Dovletyarova, E., Cheng, Z. (Eds.), *Urbanization: Challenge and Opportunity for Soil Functions and Ecosystem Services*. Springer International Publishing, pp. 89–96. [https://doi.org/10.1007/978-3-319-89602-1\\_12](https://doi.org/10.1007/978-3-319-89602-1_12).
- Baumann, P., Lee, J., Frossard, E., Schönholzer, L.P., Diby, L., Hgaza, V.K., Kiba, D.I., Sila, A., Sheperd, K., Six, J., 2021. Estimation of soil properties with mid-infrared soil spectroscopy across yam production landscapes in West Africa. *SOIL* 7 (2), 717–731. <https://doi.org/10.5194/soil-7-717-2021>.
- Bell, S.M., Barriocanal, C., Terrer, C., Rosell-Melé, A., 2020. Management opportunities for soil carbon sequestration following agricultural land abandonment. *Environ. Sci. Pol.* 108, 104–111. <https://doi.org/10.1016/j.envsci.2020.03.018>.
- Bell, S.M., Terrer, C., Barriocanal, C., Jackson, R.B., Rosell-Melé, A., 2021. Soil organic carbon accumulation rates on Mediterranean abandoned agricultural lands. *Sci. Total Environ.* 759, 143535. <https://doi.org/10.1016/j.scitotenv.2020.143535>.
- Bland, J.M., Altman, D.G., 1995. Multiple significance tests: the Bonferroni method. *BMJ* 310 (6973), 170. <https://doi.org/10.1136/bmj.310.6973.170>.
- Bonet, A., 2004. Secondary succession of semi-arid Mediterranean old-fields in South-Eastern Spain: insights for conservation and restoration of degraded lands. *J. Arid Environ.* 56 (2), 213–233. [https://doi.org/10.1016/S0140-1963\(03\)00048-X](https://doi.org/10.1016/S0140-1963(03)00048-X).
- Calderón, F.J., Culman, S., Six, J., Franzluebbers, A.J., Schipanski, M., Beniston, J., Grandy, S., Kong, A.Y.Y., 2017. Quantification of soil permanganate Oxidizable C (POXC) using infrared spectroscopy. *Soil Sci. Soc. Am. J.* 81 (2), 277–288. <https://doi.org/10.2136/sssaj2016.07.0216>.
- Cerdà, A., Rodrigo-Comino, J., Novara, A., Brevik, E.C., Vaezi, A.R., Pulido, M., Giménez-Morera, A., Keesstra, S.D., 2018. Long-term impact of rainfed agricultural land abandonment on soil erosion in the Western Mediterranean basin. *Prog. Phys. Geography: Earth Environ.* 42 (2), 202–219. <https://doi.org/10.1177/0309133318758521>.
- Chang, C.-W., Laird, D.A., 2002. Near-infrared reflectance spectroscopic analysis of soil C and N. *Soil Sci.* 167 (2), 110–116. <https://doi.org/10.1097/00010694-200202000-00003>.
- Chauchard, S., Carcaillet, C., Guibal, F., 2007. Patterns of land-use abandonment control tree-recruitment and forest dynamics in Mediterranean Mountains. *Ecosystems* 10 (6), 936–948. <https://doi.org/10.1007/s10021-007-9065-4>.
- Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., Von Maltitz, G., 2018. *World Atlas of Desertification*; Publication Office of the European Union: Luxembourg, 2018.
- Da Silva-Sangoi, D.V.D., Horst, T.Z., Moura-Bueno, J.M., Dalmolin, R.S.D., Sebem, E., Gebler, L., Da Silva Santos, M., 2022. Soil organic matter and clay predictions by laboratory spectroscopy: data spatial correlation. *Geoderma. Reg.* 28, e00486. <https://doi.org/10.1016/j.geodrs.2022.e00486>.
- De Baets, S., Meersmans, J., Vanacker, V., Quine, T.A., Van Oost, K., 2013. Spatial variability and change in soil organic carbon stocks in response to recovery following land abandonment and erosion in mountainous drylands. *Soil Use Manag.* 29 (1), 65–76. <https://doi.org/10.1111/sum.12017>.
- Deng, L., Wang, K.-B., Chen, M.-L., Shangguan, Z.-P., Sweeney, S., 2013. Soil organic carbon storage capacity positively related to forest succession on the loess plateau, China. *CATENA* 110, 1–7. <https://doi.org/10.1016/j.catena.2013.06.016>.
- Deng, L., Wang, K., Tang, Z., Shangguan, Z., 2016. Soil organic carbon dynamics following natural vegetation restoration: evidence from stable carbon isotopes ( $\delta^{13}C$ ). *Agric. Ecosyst. Environ.* 221, 235–244. <https://doi.org/10.1016/j.agee.2016.01.048>.
- Djuma, H., Bruggeman, A., Zissimos, A., Christoforou, I., Eliades, M., Zoumidis, C., 2020. The effect of agricultural abandonment and mountain terrace degradation on soil organic carbon in a Mediterranean landscape. *CATENA* 195, 104741. <https://doi.org/10.1016/j.catena.2020.104741>.
- D’Orazio, V., Traversa, A., Senesi, N., 2014. Forest soil organic carbon dynamics as affected by plant species and their corresponding litters: A fluorescence spectroscopy approach. *Plant Soil* 374 (1–2), 473–484. <https://doi.org/10.1007/s11104-013-1897-4>.
- Dunjó, G., Pardini, G., Gispert, M., 2003. Land use change effects on abandoned terraced soils in a Mediterranean catchment. *NE Spain CATENA* 52 (1), 23–37. [https://doi.org/10.1016/S0341-8162\(02\)00148-0](https://doi.org/10.1016/S0341-8162(02)00148-0).
- Ellert, B.H., Bettany, J.R., 1995. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. *Can. J. Soil Sci.* 75 (4), 529–538. <https://doi.org/10.4141/cjss95-075>.
- Emran, M., Gispert, M., Pardini, G., 2012. Patterns of soil organic carbon, glomalin and structural stability in abandoned Mediterranean terraced lands. *Eur. J. Soil Sci.* 63 (5), 637–649. <https://doi.org/10.1111/j.1365-2389.2012.01493.x>.
- Fernández, S., Peón, J., Recondo, C., Calleja, J.F., Guerrero, C., 2016. Spatial modelling of organic carbon in burned mountain soils using hyperspectral images, field datasets, and NIR spectroscopy (Cantabrian range; NW Spain). *Land Degrad. Dev.* 27 (5), 1479–1488. <https://doi.org/10.1002/ldr.2452>.
- Foote, R.L., Grogan, P., 2010. Soil carbon accumulation during temperate Forest succession on abandoned low productivity agricultural lands. *Ecosystems* 13 (6), 795–812. <https://doi.org/10.1007/s10021-010-9355-0>.
- Gabarrón-Galeote, M.A., Trigalet, S., Van Wesemael, B., 2015a. Effect of land abandonment on soil organic carbon fractions along a Mediterranean precipitation gradient. *Geoderma* 249–250, 69–78. <https://doi.org/10.1016/j.geoderma.2015.03.007>.
- Gabarrón-Galeote, M.A., Trigalet, S., Wesemael, B.V., 2015b. Soil organic carbon evolution after land abandonment along a precipitation gradient in southern Spain. *Agric. Ecosyst. Environ.* 199, 114–123. <https://doi.org/10.1016/j.agee.2014.08.027>.

- García-Ruiz, J.M., Lana-Renault, N., 2011. Hydrological and erosive consequences of farmland abandonment in Europe, with special reference to the Mediterranean region – A review. *Agric. Ecosyst. Environ.* 140 (3–4), 317–338. <https://doi.org/10.1016/j.agee.2011.01.003>.
- Guidi, C., Magid, J., Rodeghiero, M., Gianelle, D., Vesterdal, L., 2014. Effects of forest expansion on mountain grassland: changes within soil organic carbon fractions. *Plant Soil* 385 (1–2), 373–387. <https://doi.org/10.1007/s11104-014-2315-2>.
- Hastie, T., Tibshirani, R., Friedman, J., 2017. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*. Springer, Springer series in statistics.
- Isaksson, T., Nas, T., 1988. The effect of multiplicative scatter correction (MSC) and linearity improvement in NIR spectroscopy. *Appl. Spectrosc.* 42 (7), 1273–1284. <https://doi.org/10.1366/0003702884429869>.
- Kalbitz, K., Kaiser, K., 2008. Contribution of dissolved organic matter to carbon storage in forest mineral soils. *J. Plant Nutr. Soil Sci.* 171 (1), 52–60. <https://doi.org/10.1002/jpln.200700043>.
- Kennard, R.W., Stone, L.A., 1969. Computer aided Design of Experiments. *Technometrics* 11 (1), 137–148. <https://doi.org/10.1080/00401706.1969.10490666>.
- Knops, J.M.H., Tilman, D., 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology* 81 (1), 88–98. [https://doi.org/10.1890/0012-9658\(2000\)081\[0088:DOSNAC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[0088:DOSNAC]2.0.CO;2).
- Kosmas, C., Gerontidis, S., Marathianou, M., 2000. *The Effect of Land Use Change on Soils and Vegetation over Various Lithological Formations on Lesvos, Greece*.
- Kuhn, M., Johnson, K., 2013. *Applied Predictive Modeling*. Springer. <https://doi.org/10.1007/978-1-4614-6849-3>.
- La Mantia, T., Gristina, L., Rivaldo, E., Pasta, S., Novara, A., Rühl, J., 2013. The effects of post-pasture woody plant colonization on soil and aboveground litter carbon and nitrogen along a bioclimatic transect. *iForest - Biogeosci. Forest.* 6 (5), 238–246. <https://doi.org/10.3832/for0811-006>.
- Lasanta, T., Armáez, J., Pascual, N., Ruiz-Flaño, P., Errea, M.P., Lana-Renault, N., 2017. Space-time process and drivers of land abandonment in Europe. *CATENA* 149, 810–823. <https://doi.org/10.1016/j.catena.2016.02.024>.
- Lasanta, T., Armáez, J., Nadal-Romero, E., 2019. Soil degradation, restoration and management in abandoned and afforested lands. In: *Advances in Chemical Pollution, Environmental Management and Protection*, vol. 4. Elsevier, pp. 71–117. <https://doi.org/10.1016/bs.apmp.2019.07.002>.
- Lasanta, T., Sánchez-Navarrete, P., Medrano-Moreno, L.M., Khorchani, M., Nadal-Romero, E., 2020. Soil quality and soil organic carbon storage in abandoned agricultural lands: effects of revegetation processes in a Mediterranean mid-mountain area. *Land Degrad. Dev.* 31 (18), 2830–2845. <https://doi.org/10.1002/ldr.3655>.
- Leone, P.A., Viscarra-Rossel, A., Amenta, P., Buondonno, A., 2012. Prediction of soil properties with PLSR and Vis-NIR spectroscopy: application to Mediterranean soils from southern Italy. *Curr. Anal. Chem.* 8 (2), 283–299. <https://doi.org/10.2174/157341112800392571>.
- Lesschen, J.P., Cammeraat, L.H., Kooijman, A.M., Van Wesemael, B., 2008. Development of spatial heterogeneity in vegetation and soil properties after land abandonment in a semi-arid ecosystem. *J. Arid Environ.* 72 (11), 2082–2092. <https://doi.org/10.1016/j.jaridenv.2008.06.006>.
- Levi, N., Karnieli, A., Paz-Kagan, T., 2020. Using reflectance spectroscopy for detecting land-use effects on soil quality in drylands. *Soil Tillage Res.* 199, 104571. <https://doi.org/10.1016/j.still.2020.104571>.
- Li, J., Li, M., Dong, L., Wang, K., Liu, Y., Hai, X., Pan, Y., Lv, W., Wang, X., Shangguan, Z., Deng, L., 2020. Plant productivity and microbial composition drive soil carbon and nitrogen sequestrations following cropland abandonment. *Sci. Total Environ.* 744, 140802. <https://doi.org/10.1016/j.scitotenv.2020.140802>.
- López-Bermúdez, F., Romero-Díaz, A., Martínez-Fernández, J., 1996. *The El Ardal field site: soil and vegetation cover. Mediterranean Desertificat. Land Use* 169–188.
- Lucas, S.T., Weil, R.R., 2012. Can a labile carbon test be used to predict crop responses to improve soil organic matter management? *Agron. J.* 104 (4), 1160–1170. <https://doi.org/10.2134/agronj2011.0415>.
- Martínez-Duro, E., Ferrandis, P., Escudero, A., Luzuriaga, A.L., Herranz, J.M., 2010. Secondary old-field succession in an ecosystem with restrictive soils: does time from abandonment matter? *Appl. Veg. Sci.* 13 (2), 234–248. <https://doi.org/10.1111/j.1654-109X.2009.01064.x>.
- Molinero, A.M., Simon, R., Pfeiffer, R.M., 2005. Prediction error estimation: A comparison of resampling methods. *Bioinformatics* 21 (15), 3301–3307. <https://doi.org/10.1093/bioinformatics/bti499>.
- Mottet, A., Ladet, S., Coqué, N., Gibon, A., 2006. Agricultural land-use change and its drivers in mountain landscapes: A case study in the Pyrenees. *Agric. Ecosyst. Environ.* 114 (2–4), 296–310. <https://doi.org/10.1016/j.agee.2005.11.017>.
- Moura-Bueno, J.M., Dalmolin, R.S.D., Ten Caten, A., Dotto, A.C., Dematté, J.A.M., 2019. Stratification of a local VIS-NIR-SWIR spectral library by homogeneity criteria yields more accurate soil organic carbon predictions. *Geoderma* 337, 565–581. <https://doi.org/10.1016/j.geoderma.2018.10.015>.
- Nadal-Romero, E., Cammeraat, E., Pérez-Cardiel, E., Lasanta, T., 2016. How do soil organic carbon stocks change after cropland abandonment in Mediterranean humid mountain areas? *Sci. Total Environ.* 566–567, 741–752. <https://doi.org/10.1016/j.scitotenv.2016.05.031>.
- Nadal-Romero, E., Rubio, P., Kremyda, V., Absalah, S., Cammeraat, E., Jansen, B., Lasanta, T., 2021. Effects of agricultural land abandonment on soil organic carbon stocks and composition of soil organic matter in the central Spanish Pyrenees. *CATENA* 205, 105441. <https://doi.org/10.1016/j.catena.2021.105441>.
- Navas, A., Gaspar, L., Quijano, L., López-Vicente, M., Machín, J., 2012. Patterns of soil organic carbon and nitrogen in relation to soil movement under different land uses in mountain fields (south Central Pyrenees). *CATENA* 94, 43–52. <https://doi.org/10.1016/j.catena.2011.05.012>.
- Novara, A., Gristina, L., La Mantia, T., Rühl, J., 2013. Carbon dynamics of soil organic matter in bulk soil and aggregate fraction during secondary succession in a Mediterranean environment. *Geoderma* 193–194, 213–221. <https://doi.org/10.1016/j.geoderma.2012.08.036>.
- Novara, A., La Mantia, T., Rühl, J., Badalucco, L., Kuzyakov, Y., Gristina, L., Laudicina, V.A., 2014. Dynamics of soil organic carbon pools after agricultural abandonment. *Geoderma* 235–236, 191–198. <https://doi.org/10.1016/j.geoderma.2014.07.015>.
- Novara, A., Gristina, L., Sala, G., Galati, A., Crescimanno, M., Cerdà, A., Badalamenti, E., La Mantia, T., 2017. Agricultural land abandonment in Mediterranean environment provides ecosystem services via soil carbon sequestration. *Sci. Total Environ.* 576, 420–429. <https://doi.org/10.1016/j.scitotenv.2016.10.123>.
- OECD, 2021. *Effective Carbon Rates 2021: Pricing Carbon Emissions through Taxes and Emissions Trading*. OECD Publishing, Paris, OECD Series on Carbon Pricing and Energy Taxation.
- Pardini, G., Dunjó, G., Gispert, M., Barrena, R., Guidi, G.V., 2002. Land use effects on soil response to runoff generation and sediment yield in the Serra de Rodés catchment, Alt Empordà, NE Spain. In: *Man and Soil at the Third Millennium. Proceedings International Congress of the European Society for Soil Conservation, Valencia, Spain, 28 March-1 April, 2000*, Vol. 2, pp. 1323–1339.
- Pellis, G., Chiti, T., Rey, A., Curiel Yuste, J., Trotta, C., Papale, D., 2019. The ecosystem carbon sink implications of mountain forest expansion into abandoned grazing land: the role of subsoil and climatic factors. *Sci. Total Environ.* 672, 106–120. <https://doi.org/10.1016/j.scitotenv.2019.03.329>.
- Perpiña Castillo, C., Kavalov, B., Ribeiro, B.R., Diogo, V., Jacobs, C., Batista, E.S.F., Baranzelli, C., Lavalle, C., 2018. Territorial facts and trends in the EU rural areas within 2015–2030. *JRC Pub. Reposit.* <https://doi.org/10.2760/525571>.
- Perpiña Castillo, C., Coll Aliaga, E., Lavalle, C., Martínez Llario, J.C., 2020. An assessment and spatial modelling of agricultural land abandonment in Spain (2015–2030). *Sustainability* 12 (2), 560. <https://doi.org/10.3390/su12020560>.
- Petanidou, T., Kizos, T., Soulakellis, N., 2008. Socioeconomic dimensions of changes in the agricultural landscape of the Mediterranean Basin: A case study of the abandonment of cultivation terraces on Nisyros Island. *Greece Environ. Manag.* 41 (2), 250–266. <https://doi.org/10.1007/s00267-007-9054-6>.
- Piché, N., Kelting, D.L., 2015. Recovery of soil productivity with forest succession on abandoned agricultural land. *Restor. Ecol.* 23 (5), 645–654. <https://doi.org/10.1111/rec.12241>.
- Raiesi, F., 2012. Soil properties and C dynamics in abandoned and cultivated farmlands in a semi-arid ecosystem: land abandonment and C dynamics. *Plant Soil* 351 (1–2), 161–175. <https://doi.org/10.1007/s11104-011-0941-5>.
- Robledano-Aymerich, F., Romero-Díaz, A., Belmonte-Serrato, F., Zapata-Pérez, V.M., Martínez-Hernández, C., Martínez-López, V., 2014. Ecogeomorphological consequences of land abandonment in semi-arid Mediterranean areas: integrated assessment of physical evolution and biodiversity. *Agric. Ecosyst. Environ.* 197, 222–242. <https://doi.org/10.1016/j.agee.2014.08.006>.
- Rocci, K.S., Lavallee, J.M., Stewart, C.E., Cotrufo, M.F., 2021. Soil organic carbon response to global environmental change depends on its distribution between mineral-associated and particulate organic matter: A meta-analysis. *Sci. Total Environ.* 793, 148569. <https://doi.org/10.1016/j.scitotenv.2021.148569>.
- Romero-Díaz, A., Ruiz-Sinoga, J.D., Robledano-Aymerich, F., Brevik, E.C., Cerdà, A., 2017. Ecosystem responses to land abandonment in Western Mediterranean Mountains. *CATENA* 149, 824–835. <https://doi.org/10.1016/j.catena.2016.08.013>.
- Savitzky, A., Golay, M.J.E., 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 36 (8), 1627–1639. <https://doi.org/10.1021/ac60214a047>.
- Serrano, J., Shahidian, S., Marques Da Silva, J., Paixão, L., De Carvalho, M., Moral, F., Nogales-Bueno, J., Teixeira, R.F.M., Jongen, M., Domingos, T., Rato, A.E., 2021. Evaluation of near infrared spectroscopy (NIRS) for estimating soil organic matter and phosphorus in Mediterranean Montado ecosystem. *Sustainability* 13 (5), 2734. <https://doi.org/10.3390/su13052734>.
- Spohn, M., Novák, T.J., Incze, J., Giani, L., 2016. Dynamics of soil carbon, nitrogen, and phosphorus in calcareous soils after land-use abandonment – A chronosequence study. *Plant Soil* 401 (1–2), 185–196. <https://doi.org/10.1007/s11104-015-2513-6>.
- Strijker, D., 2005. Marginal lands in Europe—causes of decline. *Basic Appl. Ecol.* 6 (2), 99–106. <https://doi.org/10.1016/j.baec.2005.01.001>.
- Thornes, J.B., 1985. *The ecology of erosion*. *Geography* 70 (3), 222–235.
- Tommaso, C., Emanuele, B., Guido, P., Lucia, P., Vincenza, C.M., Riccardo, V., 2018. Soil organic carbon pool's contribution to climate change mitigation on marginal land of a Mediterranean montane area in Italy. *J. Environ. Manag.* 218, 593–601. <https://doi.org/10.1016/j.jenvman.2018.04.093>.
- Trigalet, S., Gabarrón-Galeote, M.A., Van Oost, K., Van Wesemael, B., 2016. Changes in soil organic carbon pools along a chronosequence of land abandonment in southern Spain. *Geoderma* 268, 14–21. <https://doi.org/10.1016/j.geoderma.2016.01.014>.
- Van Leeuwen, C.C.E., Cammeraat, E.L.H., De Vente, J., Boix-Fayos, C., 2019. The evolution of soil conservation policies targeting land abandonment and soil erosion in Spain: A review. *Land Use Policy* 83, 174–186. <https://doi.org/10.1016/j.landusepol.2019.01.018>.
- Vasques, G.M., Grunwald, S., Sickman, J.O., 2008. Comparison of multivariate methods for inferential modeling of soil carbon using visible/near-infrared spectra. *Geoderma* 146 (1–2), 14–25. <https://doi.org/10.1016/j.geoderma.2008.04.007>.
- Webster, R., Lark, M., 2012. *Field Sampling for Environmental Science and Management*. Routledge.
- Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *Am. J. Altern. Agric.* 18 (1), 3–17.



- Wertebach, T., Hölzel, N., Kämpf, I., Yurtaev, A., Tupitsin, S., Kiehl, K., Kamp, J., Kleinebecker, T., 2017. Soil carbon sequestration due to post-soviet cropland abandonment: estimates from a large-scale soil organic carbon field inventory. *Glob. Chang. Biol.* 23 (9), 3729–3741. <https://doi.org/10.1111/gcb.13650>.
- Wetterlind, J., Stenberg, B., Söderström, M., 2010. Increased sample point density in farm soil mapping by local calibration of visible and near infrared prediction models. *Geoderma* 156 (3–4), 152–160. <https://doi.org/10.1016/j.geoderma.2010.02.012>.
- Wold, S., Martens, H., Wold, H., 1983. The multivariate calibration problem in chemistry solved by the PLS method. In: Kågström, B., Ruhe, A. (Eds.), *Matrix Pencils*, vol. 973. Springer, Berlin Heidelberg, pp. 286–293. <https://doi.org/10.1007/BFb0062108>.
- Zhao, Y.-G., Liu, X.-F., Wang, Z.-L., Zhao, S.-W., 2015. Soil organic carbon fractions and sequestration across a 150-yr secondary forest chronosequence on the loess plateau, China. *CATENA* 133, 303–308. <https://doi.org/10.1016/j.catena.2015.05.028>.
- Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V., Mataix-Beneyto, J., 2008. Near infrared spectroscopy for determination of various physical, chemical and biochemical properties in Mediterranean soils. *Soil Biol. Biochem.* 40 (7), 1923–1930. <https://doi.org/10.1016/j.soilbio.2008.04.003>.

## 5. Bibliography

- Angelopoulou, T., Balafoutis, A., Zalidis, G., and Bochtis, D., 2020, From Laboratory to Proximal Sensing Spectroscopy for Soil Organic Carbon Estimation - A Review: Sustainability-Basel, v. 12, no. 2. <https://doi.org/10.3390/su12020443>.
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., and Smith, D. L., 2018, Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture: Front. Plant Sci., v. 9, p. 1473. <https://doi.org/10.3389/fpls.2018.01473>.
- Bell, S. M., Terror, C., Barriocanal, C., Jackson, R. B., and Rosell-Mele, A., 2021, Soil organic carbon accumulation rates on Mediterranean abandoned agricultural lands: Sci. Total Environ., v. 759. <https://doi.org/10.1016/j.scitotenv.2020.143535>.
- Bellon-Maurel, V., Fernandez-Ahumada, E., Palagos, B., Roger, J. M., and McBratney, A., 2010, Critical review of chemometric indicators commonly used for assessing the quality of the prediction of soil attributes by NIR spectroscopy: Trac-Trend. Anal. Chem., v. 29, no. 9, p. 1073-1081. <https://doi.org/10.1016/j.trac.2010.05.006>.
- BioSuisse, 2022, BioSuisse, Richtlinien für die Erzeugung, Verarbeitung und den Handel von Knospe-Produkten. <https://knospe.bio-suisse.ch/verarbeitung-handel/richtlinien-merkblaetter.html>.
- Blanco-Canqui, H., 2022, Cover crops and carbon sequestration: Lessons from US studies: Soil Sci. Soc. Am. J., v. 86, no. 3, p. 501-519. <https://doi.org/10.1002/saj2.20378>.
- Blume, H.-P., Scheffer, F., and Schachtschabel, P., 2016, Scheffer/Schachtschabel soil science, Berlin, Springer-Verlag, Soil science.
- Breure, T. S., Prout, J. M., Haefele, S. M., Milne, A. E., Hannam, J. A., Moreno-Rojas, S., and Corstanje, R., 2022, Comparing the effect of different sample conditions and spectral libraries on the prediction accuracy of soil properties from near- and mid-infrared spectra at the field-scale: Soil Till. Res., v. 215. <https://doi.org/10.1016/j.still.2021.105196>.
- Brito, L. F., Galvao, J. C. C., Giehl, J., Coelho, S. P., Campos, S. D., Barrella, T. P., dos Santos, T. R., Mendonca, B. F., and de Jesus, E. V., 2019, Decomposition of cover crop mulch and weed control under a no-till system for organic maize: Bioscience Journal, v. 35, no. 5, p. 1339-1348. <https://doi.org/10.14393/BJ-v35n5a2019-41836>.
- Brown, D. J., Shepherd, K. D., Walsh, M. G., Mays, M. D., and Reinsch, T. G., 2006, Global soil characterization with VNIR diffuse reflectance spectroscopy: Geoderma, v. 132, no. 3-4, p. 273-290. <https://doi.org/10.1016/j.geoderma.2005.04.025>.
- Bundesamt für Landwirtschaft, 2022, Agrarbericht 2022. <https://www.agrarbericht.ch/de/produktion/pflanzliche-produktion/ackerkulturen>.
- Bünemann, E. K., Bongiorno, G., Bai, Z. G., Creamer, R. E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T. W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J. W., and Brussaard, L., 2018, Soil quality - A critical review: Soil Biol. Biochem., v. 120, p. 105-125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.
- Cambardella, C. A., Moorman, T. B., and Singer, J. W., 2010, Soil nitrogen response to coupling cover crops with manure injection: Nutr. Cycl. Agroecosyst., v. 87, no. 3, p. 383-393. <https://doi.org/10.1007/s10705-010-9345-9>.
- Chahal, I., Vyn, R. J., Mayers, D., and Van Eerd, L. L., 2020, Cumulative impact of cover crops on soil carbon sequestration and profitability in a temperate humid climate: Sci. Rep., v. 10, no. 1. <https://doi.org/10.1038/s41598-020-70224-6>.
- Cheik, S., and Jouquet, P., 2020, Integrating local knowledge into soil science to improve soil fertility: Soil Use Manage., v. 36, no. 4, p. 561-564. <https://doi.org/10.1111/sum.12656>.
- Chenu, C., Angers, D. A., Barré, P., Derrien, D., Arrouays, D., and Balesdent, J., 2019, Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations: Soil Till. Res., v. 188, p. 41-52. <https://doi.org/10.1016/j.still.2018.04.011>.
- Clairotte, M., Grinand, C., Kouakoua, E., Thebault, A., Saby, N. P. A., Bernoux, M., and Barthes, B. G., 2016, National calibration of soil organic carbon concentration using diffuse infrared

- 
- reflectance spectroscopy: *Geoderma*, v. 276, p. 41-52. <https://doi.org/10.1016/j.geoderma.2016.04.021>.
- Daryanto, S., Fu, B. J., Wang, L. X., Jacinthe, P. A., and Zhao, W. W., 2018, Quantitative synthesis on the ecosystem services of cover crops: *Earth-Sci. Rev.*, v. 185, p. 357-373. <https://doi.org/10.1016/j.earscirev.2018.06.013>.
- Dhakal, M., Singh, G., Cook, R. L., and Sievers, T., 2020, Modeling Hairy Vetch and Cereal Rye Cover Crop Decomposition and Nitrogen Release: *Agronomy-Basel*, v. 10, no. 5. <https://doi.org/10.3390/agronomy10050701>.
- Durner, W., and Iden, S. C., 2021, The improved integral suspension pressure method (ISP plus ) for precise particle size analysis of soil and sedimentary materials: *Soil Till. Res.*, v. 213, p. 105086. <https://doi.org/10.1016/j.still.2021.105086>.
- EM Schweiz (2023). "Rottelenker." Retrieved 10.05.2023, 2023, from <https://www.em-schweiz.ch/rottelenker-25-l>.
- Everett, L. A., Wilson, M. L., Pepin, R. J., and Coulter, J. A., 2019, Winter Rye Cover Crop with Liquid Manure Injection Reduces Spring Soil Nitrate but Not Maize Yield: *Agronomy-Basel*, v. 9, no. 12. <https://doi.org/10.3390/agronomy9120852>.
- FAO, 2022, *Soils for nutrition: state of the art*, Rome, FAO.
- Gentsch, N., Boy, J., Batalla, J. D. K., Heuermann, D., von Wiren, N., Schweneker, D., Feuerstein, U., Gross, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Cespedes, F. C., and Guggenberger, G., 2020, Catch crop diversity increases rhizosphere carbon input and soil microbial biomass: *Biol. Fert. Soils*, v. 56, no. 7, p. 943-957. <https://doi.org/10.1007/s00374-020-01475-8>.
- Gouda, S., Kerry, R. G., Das, G., Paramithiotis, S., Shin, H. S., and Patra, J. K., 2018, Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture: *Microbiol. Res.*, v. 206, p. 131-140. <https://doi.org/10.1016/j.micres.2017.08.016>.
- Grüne Brücke (2022). "Büro für Bodenfruchtbarkeit." Retrieved 10.10.2022, from <http://www.gruenebruecke.de/>.
- Hacker, N., Ebeling, A., Gessler, A., Gleixner, G., Mace, O. G., de Kroon, H., Lange, M., Mommer, L., Eisenhauer, N., Ravenek, J., Scheu, S., Weigelt, A., Wagg, C., Wilcke, W., and Oelmann, Y., 2015, Plant diversity shapes microbe-rhizosphere effects on P mobilisation from organic matter in soil: *Ecol. Lett.*, v. 18, no. 12, p. 1356-1365. <https://doi.org/10.1111/ele.12530>.
- Harris, D. C., Werner, G., and Werner, T., 2014, *Lehrbuch der Quantitativen Analyse*, Berlin, Heidelberg, Springer Berlin Heidelberg.
- Higa, T., 1991, Effective microorganisms: a biotechnology for mankind, *in* Parr, J. F., Hornick, S. B., and Whitman, C. E., eds., *First International Conference on Kyusei Nature Farming*: Washington DC, USA, p. 8-14.
- Higa, T., 2003, *Kyusei nature farming and environmental management through effective microorganisms—the past, present and future*, Seventh International Conference on Kyusei Nature Farming: Christchurch, New Zealand.
- Hoffland, E., Kuyper, T. W., Comans, R. N. J., and Creamer, R. E., 2020, Eco-functionality of organic matter in soils: *Plant Soil*, v. 455, no. 1-2, p. 1-22. <https://doi.org/10.1007/s11104-020-04651-9>.
- Hoffmann, M., Jurisch, N., Alba, J. G., Borraz, E. A., Schmidt, M., Huth, V., Rogasik, H., Rieckh, H., Verch, G., Sommer, M., and Augustin, J., 2017, Detecting small-scale spatial heterogeneity and temporal dynamics of soil organic carbon (SOC) stocks: a comparison between automatic chamber-derived C budgets and repeated soil inventories: *Biogeosciences*, v. 14, no. 4, p. 1003-1019. <https://doi.org/10.5194/bg-14-1003-2017>.
- Hu, C., 2018, Soil carbon and nitrogen sequestration and crop growth as influenced by long-term application of effective microorganism compost: *Chil. J. Agric. Res.*, v. 78, no. 1, p. 13-22.
- Hu, C., and Qi, Y. C., 2013, Long-term effective microorganisms application promote growth and increase yields and nutrition of wheat in China: *Eur. J. Agron.*, v. 46, p. 63-67. <https://doi.org/10.1016/j.eja.2012.12.003>.
- Humusfarming (2022). "Wenz Academy." Retrieved 10.10.2022, from <http://www.humusfarming.de>.

- Hutengs, C., Seidel, M., Oertel, F., Ludwig, B., and Vohland, M., 2019, In situ and laboratory soil spectroscopy with portable visible-to-near-infrared and mid-infrared instruments for the assessment of organic carbon in soils: *Geoderma*, v. 355. <https://doi.org/10.1016/j.geoderma.2019.113900>.
- Janzen, H. H., 2006, The soil carbon dilemma: Shall we hoard it or use it?: *Soil Biol. Biochem.*, v. 38, no. 3, p. 419-424. <https://doi.org/10.1016/j.soilbio.2005.10.008>.
- Javaid, A., 2011, Effects of Biofertilizers Combined with Different Soil Amendments on Potted Rice Plants: *Chil. J. Agric. Res.*, v. 71, p. 157-163.
- Ji, W., Rossel, R. A. V., and Shi, Z., 2015, Accounting for the effects of water and the environment on proximally sensed vis-NIR soil spectra and their calibrations: *Eur. J. Soil Sci.*, v. 66, no. 3, p. 555-565. <https://doi.org/10.1111/ejss.12239>.
- Jian, J. S., Du, X., Reiter, M. S., and Stewart, R. D., 2020, A meta-analysis of global cropland soil carbon changes due to cover cropping: *Soil Biol. Biochem.*, v. 143. <https://doi.org/10.1016/j.soilbio.2020.107735>.
- Johannes, A., Weisskopf, P., Schulin, R., and Boivin, P., 2017, To what extent do physical measurements match with visual evaluation of soil structure?: *Soil Till. Res.*, v. 173, p. 24-32. <https://doi.org/10.1016/j.still.2016.06.001>.
- Khaliq, A., Abbasi, M. K., and Hussain, T., 2006, Effects of integrated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan: *Bioresour. Technol.*, v. 97, no. 8, p. 967-972. <https://doi.org/10.1016/j.biortech.2005.05.002>.
- Kim, N., Zabaloy, M. C., Guan, K. Y., and Villamil, M. B., 2020, Do cover crops benefit soil microbiome? A meta-analysis of current research: *Soil Biol. Biochem.*, v. 142. <https://doi.org/10.1016/j.soilbio.2019.107701>.
- Knox, N. M., Grunwald, S., McDowell, M. L., Bruland, G. L., Myers, D. B., and Harris, W. G., 2015, Modelling soil carbon fractions with visible near-infrared (VNIR) and mid-infrared (MIR) spectroscopy: *Geoderma*, v. 239, p. 229-239. <https://doi.org/10.1016/j.geoderma.2014.10.019>.
- Kuang, B., and Mouazen, A. M., 2011, Calibration of visible and near infrared spectroscopy for soil analysis at the field scale on three European farms: *Eur. J. Soil Sci.*, v. 62, no. 4, p. 629-636. <https://doi.org/10.1111/j.1365-2389.2011.01358.x>.
- Lal, R., 2015, Restoring Soil Quality to Mitigate Soil Degradation: *Sustainability-Basel*, v. 7, no. 5, p. 5875-5895. <https://doi.org/10.3390/su7055875>.
- Lee, J., Hopmans, J. W., Rolston, D. E., Baer, S. G., and Six, J., 2009, Determining soil carbon stock changes: Simple bulk density corrections fail: *Agr. Ecosyst. Environ.*, v. 134, no. 3-4, p. 251-256. <https://doi.org/10.1016/j.agee.2009.07.006>.
- Lehmann, J., and Kleber, M., 2015, The contentious nature of soil organic matter: *Nature*, v. 528, no. 7580, p. 60-68. <https://doi.org/10.1038/nature16069>.
- Leifeld, J., 2023, Carbon farming: Climate change mitigation via non-permanent carbon sinks: *J. Environ. Manage.*, v. 339. <https://doi.org/10.1016/j.jenvman.2023.117893>.
- Liu, X., Herbert, S. J., Hashemi, A. M., Zhang, X., and Ding, G., 2006, Effects of agricultural management on soil organic matter and carbon transformation - a review: *Plant Soil Environ.*, v. 52, no. 12, p. 531-543. <https://doi.org/10.17221/3544-pse>.
- Mayer, J., Scheid, S., Widmer, F., Fliessbach, A., and Oberholzer, H. R., 2010, How effective are 'Effective microorganisms (R) (EM)'? Results from a field study in temperate climate: *Appl. Soil Ecol.*, v. 46, no. 2, p. 230-239. <https://doi.org/10.1016/j.apsoil.2010.08.007>.
- Mayer, M., Krause, H. M., Fliessbach, A., Mader, P., and Steffens, M., 2022, Fertilizer quality and labile soil organic matter fractions are vital for organic carbon sequestration in temperate arable soils within a long-term trial in Switzerland: *Geoderma*, v. 426. <https://doi.org/10.1016/j.geoderma.2022.116080>.
- McBride, M. B., 2022, Estimating soil chemical properties by diffuse reflectance spectroscopy: Promise versus reality: *Eur. J. Soil Sci.*, v. 73, no. 1. <https://doi.org/10.1111/ejss.13192>.

- 
- McClelland, S. C., Paustian, K., and Schipanski, M. E., 2021, Management of cover crops in temperate climates influences soil organic carbon stocks: a meta-analysis: *Ecol. Appl.*, v. 31, no. 3. <https://doi.org/10.1002/eap.2278>.
- McDaniel, M. D., Tiemann, L. K., and Grandy, A. S., 2014, Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis: *Ecol. Appl.*, v. 24, no. 3, p. 560-570. <https://doi.org/10.1890/13-0616.1>.
- Metzger, K., Liebisch, F., Herrera, J. M., Guillaume, T., Walder, F., and Bragazza, L., 2024, The use of visible and near-infrared spectroscopy for in-situ characterization of agricultural soil fertility: A proposition of best practice by comparing scanning positions and spectrometers: *Soil Use Manage.*, v. 40, no. 1. <https://doi.org/10.1111/sum.12952>.
- Munnaf, M. A., and Mouazen, A. M., 2021, Development of a soil fertility index using on-line Vis-NIR spectroscopy: *Comput. Electron. Agric.*, v. 188. <https://doi.org/10.1016/j.compag.2021.106341>.
- Näser, D., 2021, *Regenerative Landwirtschaft*, Stuttgart, Eugen Ulmer KG.
- Ng, W., Minasny, B., Jones, E., and McBratney, A., 2022, To spike or to localize? Strategies to improve the prediction of local soil properties using regional spectral library: *Geoderma*, v. 406. <https://doi.org/10.1016/j.geoderma.2021.115501>.
- Nocita, M., Stevens, A., van Wesemael, B., Aitkenhead, M., Bachmann, M., Barthes, B., Ben Dor, E., Brown, D. J., Clairotte, M., Csorba, A., Dardenne, P., Dematte, J. A. M., Genot, V., Guerrero, C., Knadel, M., Montanarella, L., Noon, C., Ramirez-Lopez, L., Robertson, J., Sakai, H., Soriano-Disla, J. M., Shepherd, K. D., Stenberg, B., Towett, E. K., Vargas, R., and Wetterlind, J., 2015, Soil Spectroscopy: An Alternative to Wet Chemistry for Soil Monitoring: *Adv. Agron.*, v. 132, p. 139-159. <https://doi.org/10.1016/bs.agron.2015.02.002>.
- Olle, M., 2021, Review: Bokashi technology as a promising technology for crop production in Europe: *J. Hortic. Sci. Biotechnol.*, v. 96, no. 2, p. 145-152. <https://doi.org/10.1080/14620316.2020.1810140>.
- Patzel, N., Sticher, H., and Karlen, D. L., 2000, Soil fertility - Phenomenon and concept: *J. Plant Nutr. Soil Sci.*, v. 163, no. 2, p. 129-142. [https://doi.org/10.1002/\(sici\)1522-2624\(200004\)163:2<129::Aid-jpln129>3.0.Co;2-d](https://doi.org/10.1002/(sici)1522-2624(200004)163:2<129::Aid-jpln129>3.0.Co;2-d).
- Perez, C., Roncoli, C., Neely, C., and Steiner, J. L., 2007, Can carbon sequestration markets benefit low-income producers in semi-arid Africa? Potentials and challenges: *Agric. Syst.*, v. 94, no. 1, p. 2-12. <https://doi.org/10.1016/j.agsy.2005.09.009>.
- Pranagal, J., Ligeza, S., and Smal, H., 2020, Impact of Effective Microorganisms (EM) Application on the Physical Condition of Haplic Luvisol: *Agronomy-Basel*, v. 10, no. 7, p. 1049. <https://doi.org/10.3390/agronomy10071049>.
- Rhodes, C. J., 2017, The imperative for regenerative agriculture: *Sci. Prog.*, v. 100, no. 1, p. 80-129. <https://doi.org/10.3184/003685017x14876775256165>.
- Rietra, R., Heinen, M., and Oenema, O., 2022, A Review of Crop Husbandry and Soil Management Practices Using Meta-Analysis Studies: Towards Soil-Improving Cropping Systems: *Land*, v. 11, no. 2. <https://doi.org/10.3390/land11020255>.
- Ruiz, I., Almagro, M., de Jalon, S. G., Sola, M. D., and Sanz, M. J., 2020, Assessment of sustainable land management practices in Mediterranean rural regions: *J. Environ. Manage.*, v. 276. <https://doi.org/10.1016/j.jenvman.2020.111293>.
- Scavo, A., Fontanazza, S., Restuccia, A., Pesce, G. R., Abbate, C., and Mauromicale, G., 2022, The role of cover crops in improving soil fertility and plant nutritional status in temperate climates. A review: *Agron. Sustain. Dev.*, v. 42, no. 5. <https://doi.org/10.1007/s13593-022-00825-0>.
- Schenck zu Schweinsberg-Mickan, M., and Müller, T., 2009, Impact of effective microorganisms and other biofertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth: *J. Plant Nutr. Soil Sci.*, v. 172, no. 5, p. 704-712. <https://doi.org/10.1002/jpln.200800021>.

- Schrumpf, M., Schulze, E. D., Kaiser, K., and Schumacher, J., 2011, How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories?: *Biogeosciences*, v. 8, no. 5, p. 1193-1212. <https://doi.org/10.5194/bg-8-1193-2011>.
- Schwab, P., Dietrich, M., and Gubler, A., 2017, Messung des Eindringwiderstands und des Bodenwasserzustandes - Methodenvergleich verschiedener Geräte und Verfahren: Nationale Bodenbeobachtung (NABO), Agroscope, Zürich-Reckenholz
- Singh, K., Aitkenhead, M., Fidelis, C., Yinil, D., Sanderson, T., Snoeck, D., and Field, D. J., 2022, Optimization of spectral pre-processing for estimating soil condition on small farms: *Soil Use Manage.*, v. 38, no. 1, p. 150-163. <https://doi.org/10.1111/sum.12684>.
- Soriano-Disla, J. M., Janik, L. J., Viscarra Rossel, R. A., Macdonald, L. M., and McLaughlin, M. J., 2014, The Performance of Visible, Near-, and Mid-Infrared Reflectance Spectroscopy for Prediction of Soil Physical, Chemical, and Biological Properties: *Appl. spectrosc. Rev.*, v. 49, no. 2, p. 139-186. <https://doi.org/10.1080/05704928.2013.811081>.
- Swiss Ordinance 910.13, 2013, Direktzahlungsverordnung, Stand 2022. <https://www.blw.admin.ch/blw/de/home/instrumente/direktzahlungen.html>.
- Taubner, H., Roth, B., and Tippkotter, R., 2009, Determination of soil texture: Comparison of the sedimentation method and the laser-diffraction analysis: *J. Plant Nutr. Soil Sci.*, v. 172, no. 2, p. 161-171. <https://doi.org/10.1002/jpln.200800085>.
- Thapa, R., Tully, K. L., Cabrera, M., Dann, C., Schomberg, H. H., Timlin, D., Gaskin, J., Reberg-Horton, C., and Mirsky, S. B., 2021, Cover crop residue moisture content controls diurnal variations in surface residue decomposition: *Agric. For. Meteorol.*, v. 308. <https://doi.org/10.1016/j.agrformet.2021.108537>.
- Thorup-Kristensen, K., Magid, J., and Jensen, L. S., 2003, Catch crops and green manures as biological tools in nitrogen management in temperate zones, *in* Sparks, D. L., ed., *Adv. Agron.*, Volume 79, p. 227-302. [https://doi.org/10.1016/s0065-2113\(02\)79005-6](https://doi.org/10.1016/s0065-2113(02)79005-6).
- Tiemann, L. K., 2015, Crop rotational diversity enhances belowground communities and functions in an agroecosystem: *Ecol. Lett.*, v. 18, no. 8, p. 761-771.
- Tittonell, P., Vanlauwe, B., Corbeels, M., and Giller, K. E., 2008, Yield gaps, nutrient use efficiencies and response to fertilisers by maize across heterogeneous smallholder farms of western Kenya: *Plant Soil*, v. 313, no. 1-2, p. 19-37. <https://doi.org/10.1007/s11104-008-9676-3>.
- Tziolas, N., Tsakiridis, N., Ben-Dor, E., Theocharis, J., and Zalidis, G., 2019, A memory-based learning approach utilizing combined spectral sources and geographical proximity for improved VIS-NIR-SWIR soil properties estimation: *Geoderma*, v. 340, p. 11-24. <https://doi.org/10.1016/j.geoderma.2018.12.044>.
- Van Fan, Y., Lee, C. T., Klemes, J. J., Chua, L. S., Sarmidi, M. R., and Leow, C. W., 2018, Evaluation of Effective Microorganisms on home scale organic waste composting: *J. Environ. Manage.*, v. 216, p. 41-48. <https://doi.org/10.1016/j.jenvman.2017.04.019>.
- Varela, M. F., Barraco, M., Gili, A., Taboada, M. A., and Rubio, G., 2017, Biomass Decomposition and Phosphorus Release from Residues of Cover Crops under No-Tillage: *Agron. J.*, v. 109, no. 1, p. 317-326. <https://doi.org/10.2134/agronj2016.03.0168>.
- Viscarra Rossel, R. A., Behrens, T., Ben-Dor, E., Chabrilat, S., Dematte, J. A. M., Ge, Y. F., Gomez, C., Guerrero, C., Peng, Y., Ramirez-Lopez, L., Shi, Z., Stenberg, B., Webster, R., Winowiecki, L., and Shen, Z. F., 2022, Diffuse reflectance spectroscopy for estimating soil properties: A technology for the 21st century: *Eur. J. Soil Sci.*, v. 73, no. 4. <https://doi.org/10.1111/ejss.13271>.
- Wuest, S., 2014, Seasonal Variation in Soil Organic Carbon: *Soil Sci. Soc. Am. J.*, v. 78, no. 4, p. 1442-1447. <https://doi.org/10.2136/sssaj2013.10.0447>.
- Zhou, Y. X., Roosendaal, L., and Van Eerd, L. L., 2020, Increased nitrogen retention by cover crops: implications of planting date on soil and plant nitrogen dynamics: *Renew. Agric. Food Syst.*, v. 35, no. 6, p. 720-729. <https://doi.org/10.1017/s1742170519000383>.

## Part IV Appendices

### Appendix I: Reflections

#### 6. Data that were not used in publications and reasons for it

##### 6.1. How to compare changes in soil organic carbon

There are different methods to compare SOC measurements over time. They can be assessed as concentrations, as stocks calculated with a fixed depth (FD) or as stocks calculated with an equivalent soil mass (ESM) approach (see Paper 1 as well as Lee et al. (2009)). The comparison of SOC based on a fixed depth approach is not so often chosen because changes in bulk density and changes in SOC carbon cannot be distinguished. Nevertheless, changes in SOC stocks based on a fixed depth approach are still published in research (Bell et al., 2021). Most SOC changes in literature are compared on a concentration basis because the assessment of soil bulk density is quite arduous. Though, also for concentrations, the bulk density in the field has an influence because the bulk density is the factor that determines which soil layers are reached when a soil auger is pushed into the soil. Coring soil at a fixed depth brings, depending on the bulk density more or less soil from the lowest layer into the soil sample which influences in the end the mean concentration in the sample.

In this section I show the results from Paper 1 for SOC (Figure 3) and POXC (Figure 4) based on the three different possibilities to report changes of SOC over time. The concentration and the ESM stocks approach are relatively similar while the FD stocks would have drawn a completely different conclusion. With a full depth approach, the FD approach results in a significantly higher increase in the DCC compared to the PSC treatment on fields A and B. Field D shows significantly lower changes in FD stocks for SOC and POXC in the DCC compared to the PSC treatment. These results based on FD stocks could be interpreted by the higher cover crop aboveground biomass input in the DCC plot on fields A and B compared to the other fields. However, examining the changes based on concentrations or ESM stock we see that the changes with the FD approach were mainly bulk density driven. The results (significant differences) between the concentration and the ESM approach were only different on field B for SOC and field C for POXC. On all other fields the concentration and the ESM approach resulted in the same significant differences between treatments. One could argue on the one hand, that the ESM approach is superior to the concentration approach because bulk density is considered but on the other hand bulk density is a relatively rough measurement with a high variation and therefore it might also be a correction that brings additional error sources. Thus, the ESM approach is superior when the focus lies on stock changes because with a concentration approach one has to estimate a bulk density or take the bulk density of one

sampling time which probably introduces a bigger error than with the ESM approach. For significant differences between treatments or over time, both the ESM as well as the concentration approach, deliver reliable results even though they might have a slightly different statistical outcome.



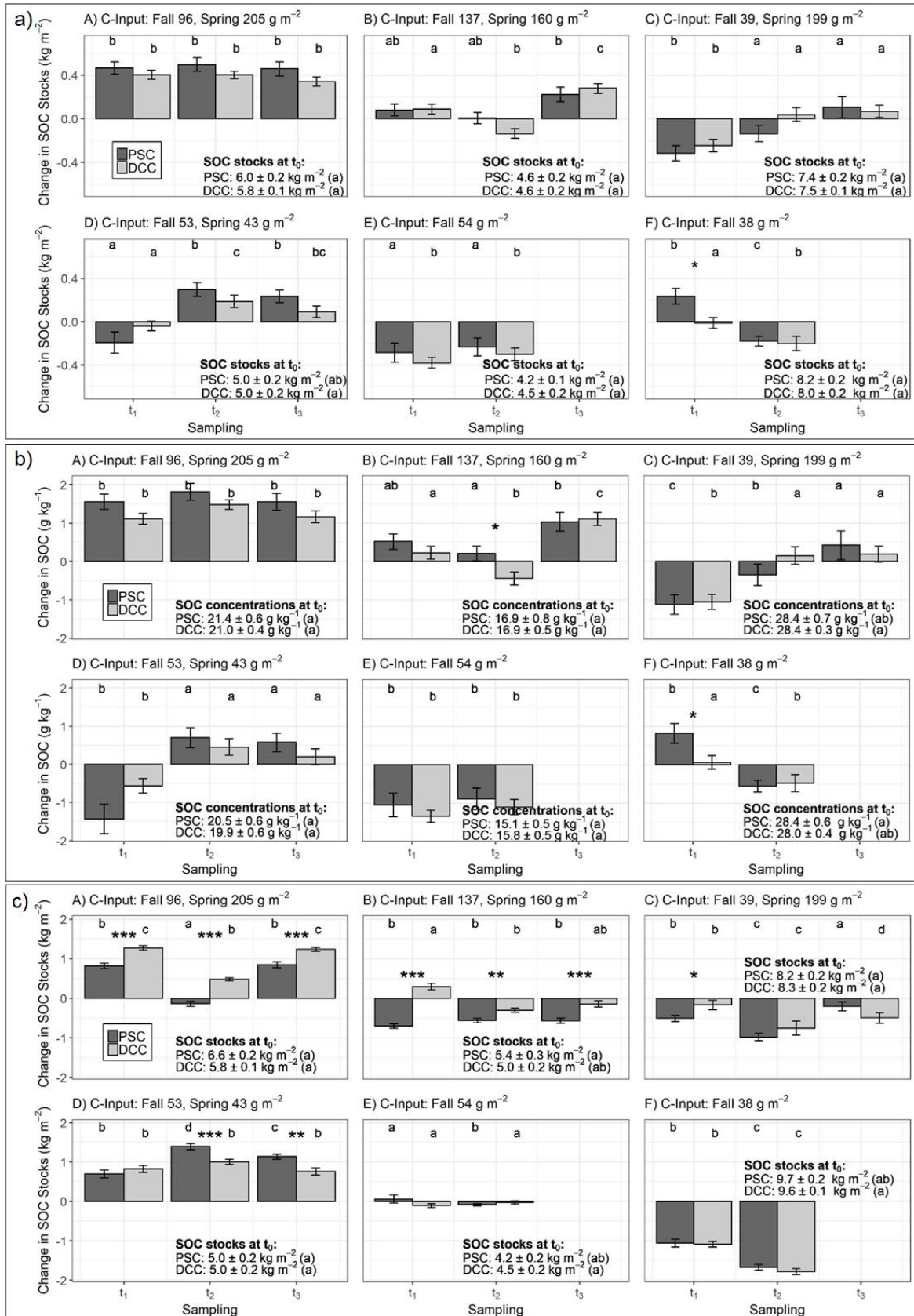


Figure 3: Changes in soil organic C (SOC) in 0-20 cm soil depth expressed as stocks calculated with a minimum equivalent soil mass (a), as concentrations (b) and as stocks calculated with fixed depth (c) over time relative to sampling t<sub>0</sub>. For every field A-F the aboveground cover crop C input in the double cover cropping (DCC) treatment is given in the title. Significant differences between treatments were tested with a t-test and are indicated with the codes: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. Significant changes within each treatment over time are indicated with letters for both treatments separately and were tested with a paired t-test. Error bars represent standard errors.

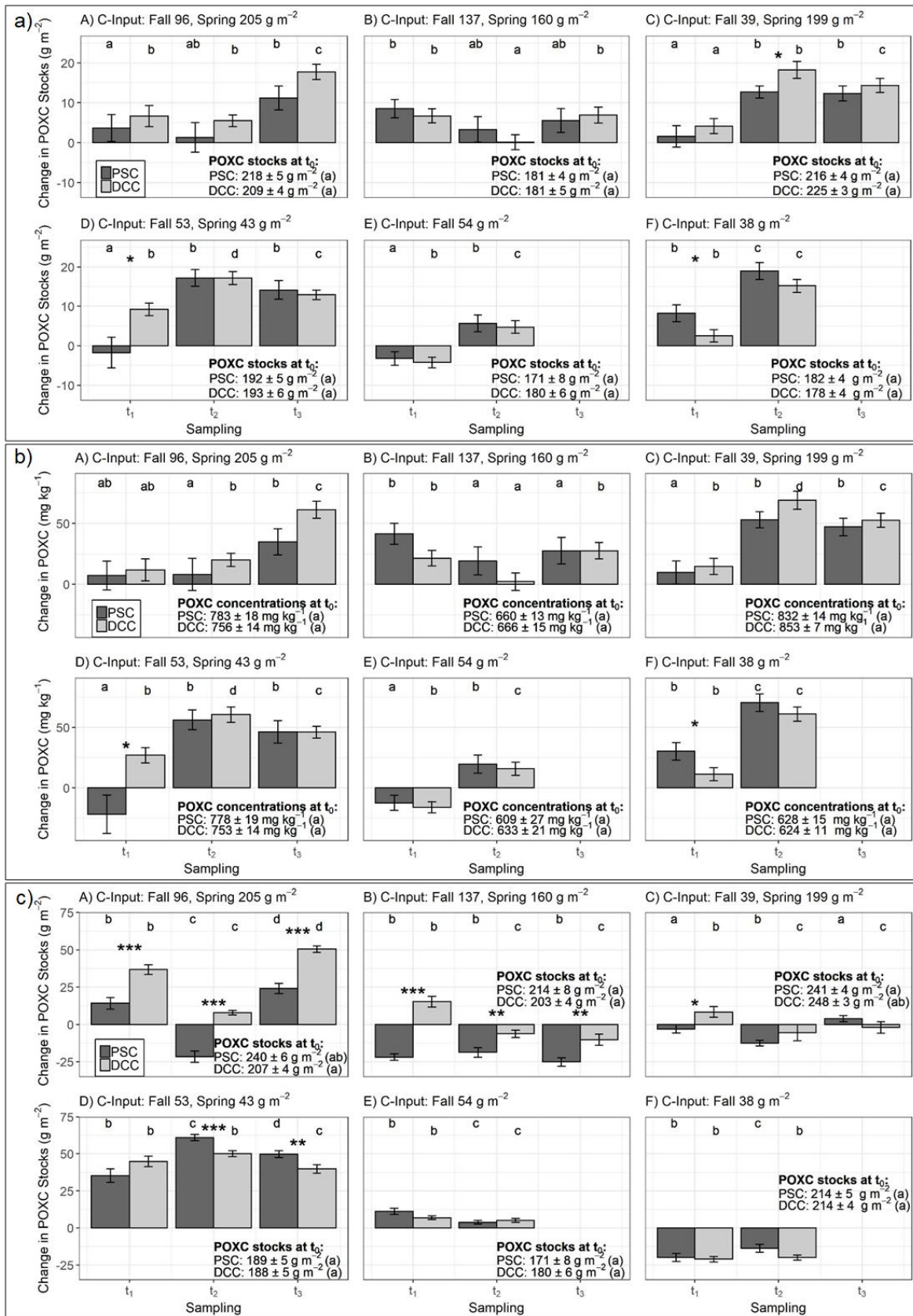


Figure 4: Changes in permanganate oxidizable C (POXC) in 0-20 cm soil depth expressed as stocks calculated with a minimum equivalent soil mass (a), concentrations (b) and stocks calculated with fixed depth (c) over time relative to sampling t<sub>0</sub>. For every field A-F the aboveground cover crop C input in the double cover cropping (DCC) treatment is given in the title. Significant differences between treatments were tested with a t-test and are indicated with the codes: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05. Significant changes within each treatment over time are indicated with letters for both treatments separately and were tested with a paired t-test. Error bars represent standard errors.

## 6.2. Prediction of soil texture with vis–NIR spectroscopy

The cover crop paper and the spectroscopy paper only include an average soil texture per field in the description of the dataset. These data were gained with the improved integral suspension pressure method (ISP+), which is in very good agreement with the standard pipette method (Durner and Iden, 2021). Nevertheless, at every second dGPS-referenced point of the first soil sampling, grain size was measured with the samples of 10–20 cm soil depth with the laser diffraction analysis (LDA) on a Mastersizer 2000. Although, LDA is the standard method in the laboratory of the Institute of Geography at the University of Bern, its results differ normally from the standard pipette method (Taubner et al., 2009). I also made this observation and concluded that the LDA substantially overestimated the silt fraction and underestimated the clay fractions (Table 2). I used the LDA measurement to estimate the variability of soil texture in the field (see Figure 1 and Table S2 (supplementary) in Paper 2) but did not present spectral models predicting soil texture.

Table 2: Mean percentages of sand, silt and clay measured with the integral suspension pressure method (ISP+) and laser diffraction method (LDA). For the ISP+ method one sample was analyzed per field while with LDA 20 d-GPS referenced samples were analyzed per field and the minimum and maximum values are presented in brackets.

Field	Sand (%)		Silt (%)		Clay (%)	
	ISP+	LDA (min, max)	ISP+	LDA (min, max)	ISP+	LDA (min, max)
A	50	44 (35, 53)	29	47 (39, 54)	21	10 (8, 11)
B	44	43 (38, 50)	35	48 (42, 52)	20	9 (7, 11)
C	27	29 (23, 39)	35	57 (49, 61)	38	14 (12, 17)
D	28	26 (21, 34)	44	62 (55, 66)	28	12 (10,14)
E	30	25 (13, 29)	48	65 (60, 74)	23	11 (10, 13)
F	39	37 (25, 51)	43	54 (42, 63)	18	9 (7, 12)

I built spectral general models for sand, silt and clay with 20 reference samples per field and a total of 120 samples. The preprocessing and the model metrics of the final chosen models for sand, silt and clay can be seen in Table 3. I evaluated the models in five times repeated 10-fold cross-validation. Very similar preprocessing approaches (gap segment derivative) resulted in the best model performance which makes sense because the three grain size classes depend strongly on each other.

Table 3: Description of applied pre-processing and model performance of the final chosen models for sand silt and clay. All models were developed with partial least square regression and evaluated with 5 times repeated 10-fold cross-validation. RMSE = Root mean standard error, RPD = ratio of RMSE to standard deviation, Refl. = Reflectance, GSD = Gap Segment Derivative (m = derivative, w = window width, s = segment size)

Field	Property	Range of wavelength / interval	Pre-processing	Latent variables	n	Calibration			Cross-validation			Model performance
						R <sup>2</sup>	RMSE [%]	RPD	R <sup>2</sup>	RMSE [%]	RPD	
A	Sand	370-2500 / 1	Refl., GSD (m = 2, w =21, s = 21)	6	120	0.82	3.93	2.35	0.77	4.45	2.05	accurate
	Silt	350-2500 / 1	Refl., GSD (m = 2, w =21, s = 21)	6	120	0.84	3.16	2.48	0.79	3.61	2.18	accurate
	Clay	350-3500 / 2	Refl., GSD (m = 2, w =21, s = 21)	6	120	0.78	0.95	2.13	0.74	1.03	1.99	approximate

The results show that soil texture can be well predicted across sites (see Table 3 and Figure 5). Based on RPD, all three models showed an accurate (sand and silt) or approximate (clay) model performance. However, the plotted models (Figure 5) show, that the models mainly differentiate the variability between fields but not really the variability within one field. I did not do field-specific models for soil texture because 20 samples per field was too little for an adequate model procedure.

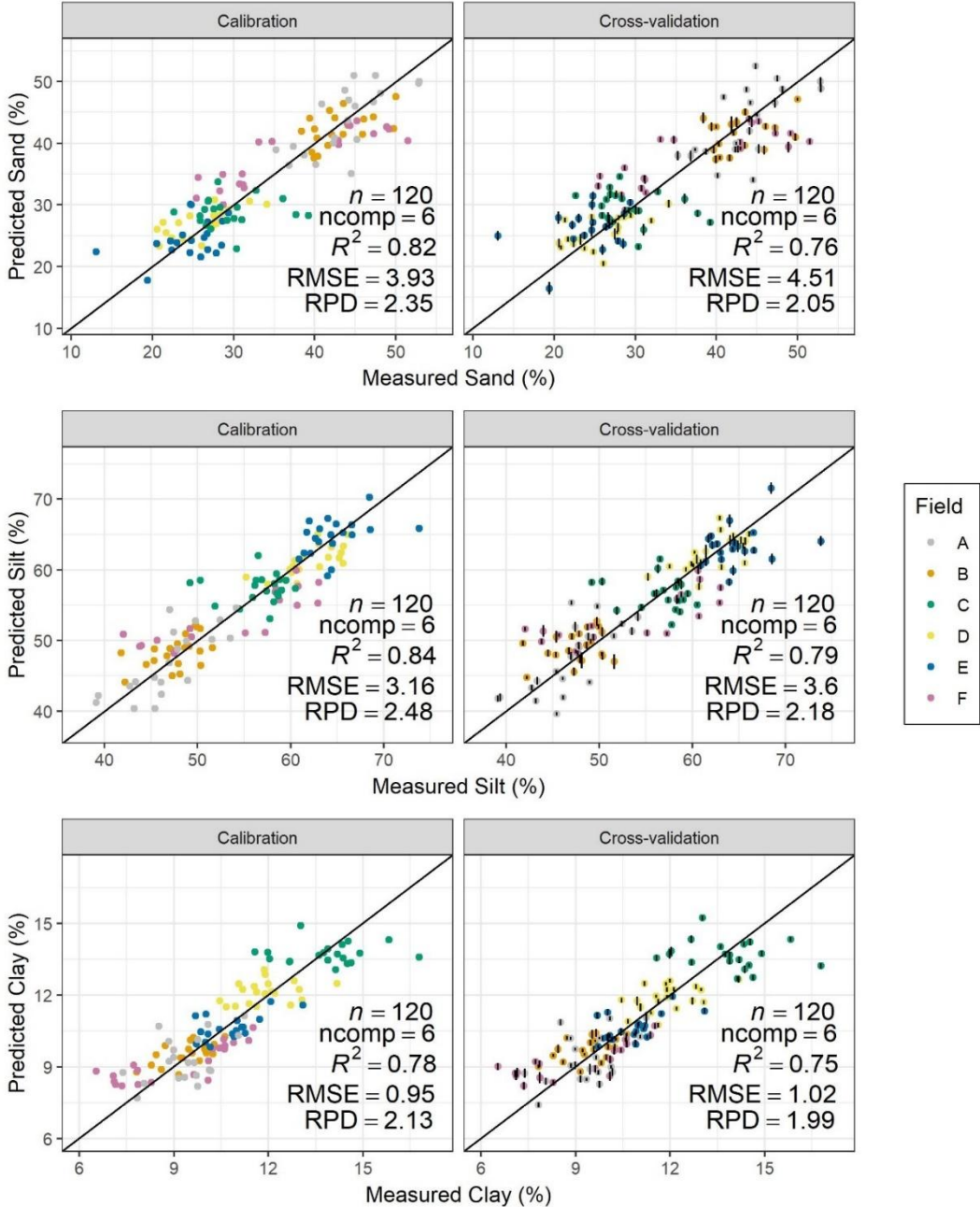


Figure 5: Spectral models for sand, silt and clay with 20 samples per field (total 120). Preprocessing was for all models a gap segment derivative. Partial least square regression was used as a modeling approach and all models were evaluated with five times repeated 10-fold cross-validation for the selection of the optimal number of latent variables (ncomp).

### 6.3. Penetrometer Resistance

The original idea to measure penetrometer resistance was to have a fast and cheap indicator for soil structure that could be applied in a high spatial and temporal resolution. At every d-GPS referenced sampling point and at all sampling times penetrometer resistance (PR) was measured in three replicates per point. A hand-held electronic penetrometer (Penetrologger 06.15.SA, Royal Eijkelkamp Company, Netherland) equipped with a 60° cone of 1 cm<sup>2</sup> basal area was pushed into the soil at an approximate speed of 2 cm s<sup>-1</sup>. The device provides a penetrometer resistance per cm of soil depth. To smooth the data, depth segments of 5 cm were represented by the median of the five measurements in it. The median instead of the mean was taken to exclude the extreme values that were reached when the cone grazed a stone. The mean of the medians per 5 cm segments in the top 20 cm (Figure 6) showed only very limited effects of the two treatments. On Field A the PR was over all sampling times consistently higher in the PSC compared to the DCC plot which was probably mainly an effect of the plot characteristics than the treatment itself. However, the big challenge was to compare the measurements over time because PR is strongly and nonlinearly related with the water content which remains an unsolved problem (Schwab et al., 2017). The little influence of the treatments as well as the difficulty to compare different timepoints were the reasons why these data were not further analyzed.

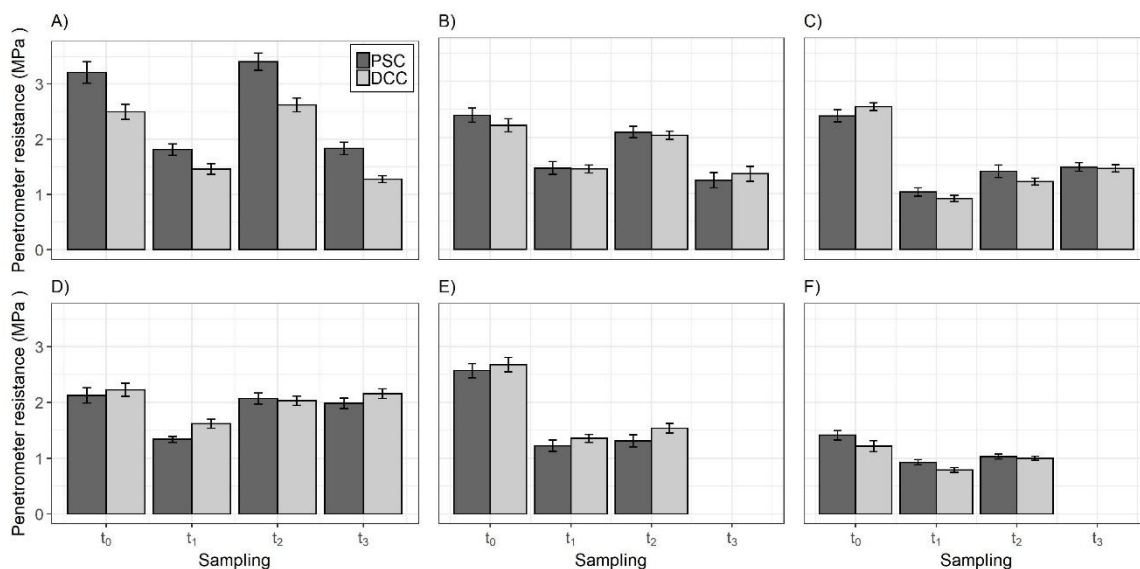


Figure 6: Mean penetrometer resistance in 0-20 cm soil depth. The original data with a penetrometer resistance per cm of soil depth were aggregated with mean of the medians in 0-5, 5-10, 10-15 and 15-20 cm. Error bars represent the standard error of the 13 measurements in the PSC and the 26 measurements in the DCC plot

## Appendix II: Declaration of consent

### Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

Name/First Name: Oberholzer Simon

Registration Number: 11-917-721

Study program: Geography and Sustainability

Bachelor  Master  Dissertation

Title of the thesis: Cover cropping in organic reduced tillage systems – Soil fertility effects and measurement methodologies

Supervisor: Prof. Dr. Chinwe Ifejika Speranza  
Dr. Markus Steffens

I declare herewith that this thesis is my own work and that I have not used any sources other than those stated. I have indicated the adoption of quotations as well as thoughts taken from other authors as such in the thesis. I am aware that the Senate pursuant to Article 36 paragraph 1 litera r of the University Act of September 5th, 1996 and Article 69 of the University Statute of June 7th, 2011 is authorized to revoke the doctoral degree awarded on the basis of this thesis.

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Place/Date

Bern 22.10.2024

Signature *S. Oberholzer*