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Ecosystem services driven by soil biota in agricultural landscapes - Effects of farm-based soil management practices

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submitted by
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"Imagination is more important than knowledge.

Knowledge is limited. Imagination encircles the world."

\- Albert Einstein

Preface

This thesis was prepared within the European *Biodiversa* project "SoilMan – Ecosystem services of soil biota in agriculture". Chapter 1 & 2 contain a summary of this thesis (English and German version). In Chapter 3, a general introduction of the topic, reflecting all parts of this work, is given. The cumulative dissertation consists of three papers as first author which are published in international, peer reviewed journals. These papers are given in Chapter 4, 5 and 6. A general discussion and conclusion covering all three papers is presented in Chapter 7 and 8.

Study 1 (Chapter 4)

ENGELL, I., LINSLER, D., SANDOR, M., JOERGENSEN, J, MEINEN, C., POTTHOFF, M. (2022): The effects of conservation tillage on chemical and microbial soil parameters at four sites across Europe. *Plants* 11 (13), 1747. https://doi.org/10.3390/plants11131747

Study 2 (Chapter 5)

HANISCH, J. & ENGELL, I., LINSLER, D., SCHEU, S., POTTHOFF, M. (2022): The role of Collembola in decomposition processes of crop residues under minimum and conventional tillage. *Journal of Plant Nutrition and Soil Science* 185 (4), 529-538. https://doi.org/10.1002/jpln.202200077

Study 3 (Chapter 6)

ENGELL, I., LINSLER, D., SCHRADER, S., TAYLOR, A., LUDWIG, B., POTTHOFF, M. (2021): Crop residue displacement by soil inversion: Annelid responses and their impact on carbon and nitrogen dynamics in a lab-based mesocosm study. *Applied Soil Ecology* (167) 104151. https://doi.org/10.1016/j.apsoil.2021.104151

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Authorship contribution statement

All three research papers were prepared within research groups. This section highlights the contribution to the individual papers as first author.

Study 1 (Chapter 4): As first author Ilka Engell was involved in the conceptualization of the study. The original draft was written by Ilka Engell. Information for site descriptions were provided by the project coordinator. The laboratory work and data analysis was carried out by Ilka Engell. In addition, one co-author offered raw data for further calculations and contributed to the methodology part of the paper. Visualization of results was done by Ilka Engell. All co-authors contributed to the review and editing of the paper. Ilka Engell is the corresponding author and responsible for the submission.

Study 2 (Chapter 5): The second study is based on a collaboration between two research groups. The conceptualization of the study was developed by both groups. The work is divided into two parts: soil fauna ecology and litter breakdown. Data on litter breakdown was analysed by Ilka Engell and soil fauna data were evaluated by the other first author. Ilka Engell carried out the quantification of carbon and nitrogen contents as well as litter losses of litterbag material in the laboratory and prepared data for a statistical evaluation. In addition, isotopic analysis of maize leaves and soil samples was carried out by Ilka Engell. Statistical analyses was carried out by the other first author in consultation with Ilka Engell. Work on the field site was done jointly by the first three authors. Both first authors contributed to the writing on the original draft in equal parts for their respective topics. The introduction and discussion part was written together. Visualization of results was done by the respective first author, depending on the data. All co-authors were involved in reviewing and editing the paper prior to submission. Ilka Engell is the corresponding author in the submission progress.

Study 3 (Chapter 6): For the third paper the conceptualization of the study was developed by the research group from the University of Göttingen, including the first author. Ilka Engell was responsible for the laboratory work and methodology part in consultation with the project coordinator. The methodology part was written by Ilka Engell in accord with part of the co-authors. Analysis and validation of data was carried out by Ilka Engell in consultation with her supervisors. Statistical analysis was done by Ilka Engell in consultation with one co-author. Writing the original draft, including visualization was done by Ilka Engell. All co-authors participated in reviewing and editing the paper. Ilka Engell is the corresponding author and was responsible for the submission progress.

List of abbreviations

BD	bulk density
C_{mic}	soil microbial biomass carbon
C_{org}	soil organic carbon
CON	control columns
CT	conventional tillage
DS	Direktsaat
ENCH	enchytraeids
EW_{anecic}	anecic earthworms
$EW_{endo.}$	endogeic earthworms
GHG	greenhouse gas
MAP	mean annual precipitation
MAT	mean annual temperature
MB	Minimalbodenbearbeitung
MBC	microbial biomass carbon, Mikrobielle Biomasse Kohlenstoff
MT	minimum tillage
NI	non-inversion
NT	no-tillage
N_{total}	total nitrogen
P	Pflügen (konventionelle Bodenbearbeitung)
qCO_2	metabolic quotient
SI	soil inversion
SOC	soil organic carbon
SOM	soil organic matter

PART I

Chapter 1

Summary

Diversity, abundance, and biomass of soil biota depend on the biotic and abiotic characteristics of the soil environment and are essential for the provision of important ecosystem services. Agricultural systems are shaped by the long-term history of farming practices and the recent management decisions of the farmer. The present thesis reports on the effects of different tillage practices on soil organisms, their functions and performances. Different approaches were applied: (i) first, investigations on microbial and chemical soil parameters were carried out under different climatic and soil conditions across Europe, (ii) second, an experiment on litter breakdown driven by the soil mesofauna was conducted at a long-term experimental field site and (iii) third, detailed insights on the effect of soil inversion and crop residue placement on soil annelids and their functional feedback were gained under laboratory conditions.

Study 1 investigates the effects of reduced tillage applications compared with conventional tillage on microbial and chemical soil parameters at four different long-term experimental field sites at Germany, Romania, Spain and Sweden. Reduced tillage meant either no-tillage (NT) as direct seeding or minimum tillage (MT) that had a working depth between 5 and 12 cm (3 sites) or down to 25-30 cm (1 site) with non-inversive machinery. Conventional tillage (CT) was carried out as mouldboard ploughing at all sites, inverting the soil down to a depth of 23-30 cm. At the German site, MT increased concentrations of organic carbon (C), total nitrogen (N) and microbial biomass carbon (MBC) in the top 10 cm. In contrast, CT increased MBC contents and bulk densities between 20 and 30 cm soil depth. In Sweden, NT and MT on a soil with an acidic pH had positive effects on concentrations of C, N and MBC in the upper 20 cm, compared with CT. At the Romanian site, a clay-rich soil, soil parameters showed no differences between inversion tillage (CT) and non-inversion tillage (MT), both of which had a working depth of 25 to 30 cm. At the Spanish site the use of NT increased the concentrations as well as stocks of C, N and MBC significantly, compared with CT. To sum up, reduced tillage enhanced soil microbial properties in most cases. However, the effectiveness of tillage reduction seems to highly depend on site conditions such as pH level, soil texture and climatic conditions.

Study 2 focuses on the effects of soil mesofauna and reduced tillage on decomposition of crop residues under field conditions. In a long-term field experiment near Göttingen (Germany), a litterbag experiment was carried out to compare decomposition rates at in plots under minimum tillage and ploughing. The litterbags were filled with 5 g of chopped maize leaves (*Zea mays* L.). Two different mesh sizes were used to quantify the contribution of mesofauna to decomposition processes. The coarse mesh size (2 mm) allowed mesofauna and microorganisms to access the litterbags, while the fine mesh (48 µm) only allowed the colonisation by microorganisms. In October 2017, litterbags were placed at a depth of 5-8 cm in minimum tillage and at a soil depth of 25 cm in the ploughed plots and were removed after 2, 5 and 7 months. In addition to C and N contents of litter material and the overall litter loss, the abundance and species composition of

Collembola, as an important representative group of the mesofauna in agricultural systems, were measured. Stable isotopes analysis ($\delta^{13}C$ and $\delta^{15}N$) was used to quantify the uptake of the maize-derived C by Collembola. Mesofauna access improved litter quality (indicated by low C/N ratios). This effect is presumably more indirect by stimulating microbial activity and increasing the supply of N. Overall, Collembola abundances were increased in ploughed plots compared with the minimum tilled plots. Collembola species composition was not significantly changed by tillage, although euedaphic species occurred mainly in ploughed plots and epedaphic species under minimum tillage. In deeper soil depths crop residues seem to be important as a habitat and a food source for Collembola, because other carbon sources are difficult to access. The results suggest that soil mesofauna greatly contributes to the transformation of organic matter and the availability of nutrients.

Study 3 is a mesocosm laboratory experiment on the effect of soil inversion with residue displacement on the activity and functions of microorganisms, enchytraeids and earthworms. Undisturbed soil columns (30 cm high, 15 cm Ø) from a long-term experimental field site near Göttingen (Germany) were used. The experiment was shaped to investigate the two factors 'tillage' and 'soil fauna'. Tillage had the factor levels (1) simulated soil inversion (SI): soil columns were 'ploughed' manually with residues (5 g leaves of *Zea mays* L.) placed at a depth of 15 cm and (2) non-inversion (NI): undisturbed soil columns with residues placed on the surface. Soil fauna had four factor levels (addition of different soil organisms to the soil columns): (1) anecic earthworms (*Lumbricus terrestris*), (2) endogeic earthworms (*Octolasion cyaneum*), (3) enchytraeids (*Enchytraeus crypticus and Enchytraeus christenseni*) and (4) control columns without soil fauna. All different combinations of factor levels were replicated four times (n = 4). The experiment ran for 114 days in a dark climate chamber at a temperature of 10 °C. The gas fluxes (CO_2, N_2O) were measured automatically every 4.5 hours using a gas chromatograph. The concentrations of microbial biomass and ergosterol were quantified as soil biotic properties. The fate of crop residue-derived C was traced using stable isotopes analysis ($\delta^{13}C$ and $\delta^{15}N$). The results showed that the organisms reacted differently to the incorporation of the organic material. As expected, *L. terrestris* consumed more maize than *O. cyaneum* during the experiment. For endogeic earthworms, the incorporation of residues at a depth of 15 cm was advantageous, whereas tillage had no effect on the uptake of maize-derived C by anecic species. In addition, the number of enchytraeids was not affected by the crop residue placement. Anecic earthworms increased CO_2-C emissions by 22% (vs. control). Soil inversion enhanced N_2O-N emission by 188%. Overall, there was no interaction between the two factors. Microbial and chemical parameters were strongly affected by soil inversion. Results showed that incorporation of crop residues at deeper soil layers has neutral to even positive effects for soil annelids. Nevertheless, under field-conditions, the incorporation of residues into the soil may impair or endanger annelids mechanically.

In general, reduced tillage systems supported soil biota and thus were able to enhance the ecosystem services they provide. Ploughing can increase the abundance of individual species, however, a broad spectrum of species and thus functional diversity in the soil should be promoted. The incorporation of crop residues appeared to be positive for some functional groups of soil fauna. Even though earthworms increased gas emissions, this effect is considered to be offset by their contribution to carbon sequestration. Soil inversion, on the other hand, appears to make a large contribution to greenhouse gas emissions. The high variability of the results between different locations suggests that recommendations for reduced tillage practices can only be given at a local scale, related to the existing soil and climatic conditions. Overall, this thesis contributes to the development of sustainable management strategies with a focus on soil tillage and highlights the often underestimated importance of soil organisms for agriculture.

Chapter 2

Zusammenfassung

Vielfalt, Abundanz und Biomasse von Bodenlebewesen hängen von den biotischen und abiotischen Charakteristiken des Bodens ab und sind für die Bereitstellung wichtiger Ökosystemleistungen von wesentlicher Bedeutung. Landwirtschaftliche Systeme werden durch die langfristige Geschichte der landwirtschaftlichen Praktiken und die aktuellen Managemententscheidungen der Landwirte geprägt. In der vorliegenden Arbeit werden die Auswirkungen verschiedener Bodenbearbeitungsmethoden auf Bodenorganismen, ihre Funktionen und Leistungen untersucht. Es wurden verschiedene Ansätze angewandt: (i) Erstens, Untersuchungen zu mikrobiellen und chemischen Bodenparametern unter verschiedenen klimatischen und bodenkundlichen Bedingungen innerhalb Europas, (ii) zweitens, ein Experiment zum Streuabbau durch die Mesofauna des Bodens auf einem Langzeitversuchsfeld und (iii) drittens, ein Laborexperiment zu den Auswirkungen wendender Bodenbearbeitung und der Einarbeitung von Ernteresten für Anneliden und deren funktionelles Feedback.

In **Studie 1** wurden die Auswirkungen reduzierter Bodenbearbeitung im Vergleich zur konventionellen Bodenbearbeitung auf mikrobielle und chemische Bodenparameter an vier verschiedenen Langzeitversuchsstandorten in Europa untersucht. Bei der reduzierten Bodenbearbeitung handelte es sich entweder um Direktsaat ohne Bodenbearbeitung (DS) oder um minimale Bodenbearbeitung (MB), bei der der Boden bis zu einer Tiefe von 5 bis 12 cm (3 Standorte) oder bis zu 25-30 cm (1 Standort) mit nicht-wendenden Maschinen bearbeitet wurde. Die konventionelle Bodenbearbeitung (P) wurde an allen Standorten als Pflügen durchgeführt, wobei der Boden bis zu einer Tiefe von 23-30 cm bearbeitet wurde. Am deutschen Standort erhöhte MB die Konzentrationen von organischem Kohlenstoff (C), Gesamtstickstoff (N) und mikrobiellem Biomassekohlenstoff (MBC) in den oberen 10 cm. Im Gegensatz dazu erhöhte CT den MBC-Gehalt und die Lagerungsdichte zwischen 20 und 30 cm Bodentiefe. In Schweden hatten DS und MB auf einem Boden mit saurem pH-Wert positive Auswirkungen auf die Konzentrationen von C, N und MBC in den oberen 20 cm, verglichen mit P. Am rumänischen Standort, einem tonreichen Boden zeigten sich bei den Bodenparametern keine Unterschiede zwischen wendender (P) und nicht-wendender Bodenbearbeitung (MB), die beide eine Arbeitstiefe von 25-30 cm hatten. Auf dem spanischen Standort erhöhte der Einsatz von DS die Konzentrationen sowie die Vorräte von C, N und MBC im Vergleich zu P erheblich. Zusammenfassend lässt sich sagen, dass die reduzierte Bodenbearbeitung in den meisten Fällen die mikrobiellen Eigenschaften deutlich verbesserte. Die Wirksamkeit der reduzierten Bodenbearbeitung scheint jedoch in hohem Maße von den Standorteigenschaften, wie pH-Wert, Bodentextur und klimatischen Bedingungen abhängig zu sein.

Studie 2 befasst sich mit den Auswirkungen von Mesofauna und reduzierter Bodenbearbeitung auf die Zersetzung von Ernteresten unter Feldbedingungen. Auf einem Langzeitversuchsfeld in der Nähe von Göttingen wurde ein Litterbag-Experiment durchgeführt, um die Zersetzungsraten in Parzellen mit minimaler Bodenbearbeitung und gepflügten Parzellen zu vergleichen. Die Litterbags wurden mit 5 g gehäckselten Maisblättern (*Zea mays* L.) befüllt. Es wurden zwei verschiedene Maschenweiten verwendet, um den Beitrag der Mesofauna an den Zersetzungsprozessen zu quantifizieren. Die grobe Maschenweite (2 mm) ermöglichte Mesofauna und Mikroorganismen den Zugang zu den Litterbags, während die feine Maschenweite (48 µm) nur die Besiedlung durch Mikroorganismen zuließ. Im Oktober 2017 wurden die Litterbags in einer Tiefe von 5-8 cm bei minimaler Bodenbearbeitung und in einer Bodentiefe von 25 cm in den gepflügten Parzellen platziert und nach 2, 5 und 7 Monaten herausgeholt. Neben dem Gehalt von C und N des Streumaterials und dem Gesamtstreuverlust wurden auch die Häufigkeit und die Artenzusammensetzung von Collembolen, einer repräsentativen Gruppe der Mesofauna in landwirtschaftlichen Systemen, gemessen. Mit Hilfe der Analyse stabiler Isotope (δ^{13}C und δ^{15}N) wurde die Aufnahme von aus Mais gewonnenem C durch Collembolen quantifiziert. Der Zugang der Mesofauna verbesserte die Qualität der Streu (erkennbar am niedrigen C/N-Verhältnis). Diese Auswirkungen sind vermutlich indirekt, nämlich durch die Anregung der mikrobiellen Aktivität und der Erhöhung des Stickstoffangebots. Insgesamt war die Häufigkeit von Collembolen in gepflügten Parzellen im Vergleich zu den minimal bearbeiteten Parzellen erhöht. Die Zusammensetzung der Collembolen-Arten wurde durch die Bodenbearbeitung nicht signifikant beeinflusst, obwohl euedaphische Arten hauptsächlich in gepflügten Parzellen und epedaphische Arten bei minimaler Bodenbearbeitung vorkamen. Tiefer im Boden liegende Ernterückstände scheinen ein wertvoller Lebensraum und gleichzeitig eine wichtige Nahrungsquelle für Collembolen zu sein, da andere Kohlenstoffquellen schwer zugänglich sind. Die Ergebnisse deuten darauf hin, dass die Mesofauna im Boden einen wichtigen Beitrag zur Umwandlung von organischem Material und zur Nährstoffverfügbarkeit leistet.

Studie 3 ist ein Mesokosmos-Laborexperiment zur Auswirkung von Bodenbearbeitung mit der Einarbeitung von Ernteresten auf Mikroorganismen, Enchyträen und Regenwürmer. Es wurden ungestörte Bodensäulen (30 cm hoch, 15 cm Ø) aus einem Langzeitversuchsfeld in der

Nähe von Göttingen in Deutschland verwendet. Der Versuch war so angelegt, dass die zwei Faktoren (i) Bodenbearbeitung und (ii) Bodenfauna untersucht wurden. Die Bodenbearbeitungssimulation hatte die Faktorstufen (1) Wendend (W) - Bodensäulen, die manuell "gepflügt" wurden mit Ernteresten (5 g Blätter von *Zea mays* L.) in einer Tiefe von 15 cm und (2) Nicht-Wendend (NW) - Ungestörte Bodensäulen mit Ernteresten an der Oberfläche. Der Faktor Bodenfauna hatte vier Faktorstufen: (1) anektische Regenwürmer (*Lumbricus terrestris*), (2) endogäische Regenwürmer (*Octolasion cyaneum*), (3) Enchyträen (*Enchytraeus crypticus* und *Enchytraeus christenseni*) und (4) Kontrollsäulen ohne Bodenfauna. Alle verschiedenen Kombinationen von Faktoren wurden viermal wiederholt (n = 4). Der Versuch lief 114 Tage lang in einer dunklen Klimakammer bei einer Temperatur von 10 °C. Die Gasflüsse (CO_2, N_2O) wurden automatisch alle 4,5 Stunden mit einem Gaschromatographen gemessen. Die Konzentrationen der mikrobiellen Biomasse und der Ergosterolgehalt wurden als biotische Bodeneigenschaften quantifiziert. Der Verbleib des aus Ernteresten gewonnenen C wurde mit Hilfe der Analyse stabiler Isotope ($\delta^{13}C$ und $\delta^{15}N$) verfolgt. Die Ergebnisse zeigten, dass die Organismen unterschiedlich auf die Einarbeitung des organischen Materials reagierten. Erwartungsgemäß fraß *L. terrestris* während des Versuchs mehr Mais als *O. cyaneum.* Für endogäische Regenwürmer war die Einarbeitung von Ernteresten in einer Tiefe von 15 cm von Vorteil, während die Aufnahme von aus Mais gewonnenem C bei anektischen Regenwürmern durch die Bodenbearbeitung nicht verändert wurde. Auch die Enchyträen schienen durch die Einarbeitung der Erntereste weniger beeinflusst zu werden. Anektische Regenwürmer erhöhten die CO_2-C Emissionen um 22% (im Vergleich zur Kontrolle). Die Bodeninversion erhöhte die N_2O-N Emissionen um 188%. Insgesamt gab es keine Wechselwirkung zwischen den beiden Faktoren. Die mikrobiellen und chemischen Parameter wurden durch die Bodenbearbeitung stark beeinflusst. Die Ergebnisse zeigten, dass die Einarbeitung von Ernteresten für die Anneliden neutrale bis sogar positive Auswirkungen hatte. Dennoch kann die Einarbeitung, übertragen auf Feldbedingungen, Anneliden mechanisch beeinträchtigen oder gefährden.

Im Allgemeinen unterstützten Systeme mit reduzierter Bodenbearbeitung die Bodenorganismen und konnten somit die von ihnen erbrachten Ökosystemleistungen verbessern. Das Pflügen kann die Abundanz einzelner Arten erhöhen, es sollte jedoch ein breites Artenspektrum und damit die funktionelle Vielfalt im Boden gefördert werden. Die Einarbeitung von Ernteresten wirkte sich auf einige funktionelle Gruppen der Bodenfauna positiv aus. Auch wenn Regenwürmer die Gasemissionen erhöhten, wird dieser Effekt vermutlich durch ihren Beitrag zur Kohlenstoffbindung ausgeglichen. Die Bodeninversion hingegen scheint einen großen Beitrag zu den Treibhausgas-emissionen zu leisten. Die große Variabilität der Ergebnisse zwischen verschiedenen Standorten legt nahe, dass Empfehlungen für reduzierte Bodenbearbeitungsmethoden nur auf lokaler Ebene unter Berücksichtigung der vorhandenen Boden- und Klimabedingungen gegeben werden können. Insgesamt leistet diese Arbeit einen Beitrag zur Entwicklung nachhaltiger Bewirtschaftungsstrategien mit Schwerpunkt auf Bodenbearbeitung und verdeutlicht die oft unterschätzte Bedeutung von Bodenorganismen für die Landwirtschaft.

PART II

Chapter 3

General Introduction

The status of soils is directly linked to food security in a regional and global sense as well as to human health (Brevik and Sauer, 2015). Healthy soils provide benefits to humans such as food, water and clean air (Wall et al., 2015; Brevik et al., 2018). To ensure food security in the long-term, a global strategy for sustainable soil management is needed (Charles et al., 2010). Soils are at risk because they are a limited resource and poor management poses a major threat (Lal, 2009; Gomiero, 2016; FAO, 2021). Agricultural methods have to be adopted in order to avoid a shrinking production area in the future (Montgomery, 2007). In agricultural systems, the following four farm-based management practices have a large impact on soil conditions: i) crop rotation, (ii) fertilisation, (iii) tillage, (iv) residue management. In particular soil tillage affects biophysical and chemical modifications in the soil with consequences for soil biota (Roger-Estrade et al., 2010). These habitat characteristics for soil biota control their abundance, biomass and community structure (Kibblewhite et al., 2008; Roger-Estrade et al., 2010; Tsiafouli et al., 2015). One way of fostering soil biota as the basis of the soil food web and its key components is to adjust tillage and residue management of soils in a way that it promotes rather than damages soil organisms. This thesis reports on the effect of soil (non-) inversion and crop residue placement due to tillage applications in various forms on microbial, chemical and physical soil parameters and faunal responses. Therefore a field approach and a laboratory approach is used. Two well-studied methodologies in soil ecology are applied – the use of litterbags and a microcosm experiment. As key components of the soil food web i) microorganisms as primary decomposers and their activity are part of the thesis as well as ii) collembola and enchytraeids as important components of the mesofauna in agricultural systems and iii) earthworms, which are probably the most important component of the macrofauna in agricultural systems.

Variability of tillage systems

Deciding on a tillage system that promotes soil quality is still a challenge for farmers. Reduced tillage systems, in particular minimum tillage systems vary greatly in terms of machinery and working depth. Most commonly, a disc or rotary harrow with a working depth from 5 cm up to 20 cm is used (Table 1). The most popular device in conventional tillage is the mouldboard plough (Table 1). Whereas ploughing always leads to soil inversion, minimum tillage comprises both, non-inversion and inversion tillage. A typical German farming method is a combination of shallow tillage combined with chisel ploughing or a similar non-inversion tillage systems for seedbed preparation (Zikeli and Gruber, 2017). A cultivator is also widely used on stubble to loosen the soil, incorporate organic matter and is an important tool for weed control (Steinmann et al., 2012). No-till farming is usually applied as direct seeding into crop stubbles without any tillage operations and occasionally a narrow trench is opened before seeding (Table 1).

Table 1: Variability of tillage systems, classified as conventional tillage, minimum tillage and no-tillage.

Machinery	Working depth	References
Conventional Tillage		
Mouldboard plough	10-15 cm	(Chen et al., 2009)
Disc plough	20 cm	(Balota et al., 2004)
Mouldboard plough	23 cm	(Torppa and Taylor, 2022)
Disc plough	20-25 cm	(Babujia et al., 2010)
Mouldboard plough	25 cm	(Jacobs et al., 2010)
Mouldboard plough	25-30 cm	(Andruschkewitsch et al., 2014)
Mouldboard plough	30 cm	(Hernanz et al., 2002)
Mouldboard plough	30 cm	(Badagliacca et al., 2018)
Minimum Tillage		
Shallow tillage	5 cm	(Chen et al., 2009)
Rotary harrow	5-8 cm	(Jacobs et al., 2010)
Chisel ploughing	10 cm	(Fabrizzi et al., 2005)
Cultivator	10-12 cm	(Torppa and Taylor, 2022)
Cultivator or disc harrow	10-15 cm	(Andruschkewitsch et al., 2014)
Chisel ploughing	20 cm	(Hernanz et al., 2002)
No-Tillage		
Direct drilling	0 cm	(Hernanz et al., 2002; Fabrizzi et al., 2005; Badagliacca et al., 2018; Torppa and Taylor, 2022)
Narrow channel	1.5-4 cm	(Babujia et al., 2010)

Overall, conventional tillage is still the most common tillage practice in annual crops in European countries, e.g. in Sweden (Håkansson et al., 1998), Spain (Gonzalez-Sanchez et al., 2015) and Romania (Günal et al., 2015). This is also the case for Germany. For instance, in 2015/2016 only 14% of the farmers gave up ploughing completely and only 1% of the German arable land was no-tilled (Statistisches Bundesamt (Destatis), 2019). However, there is a trend towards implementing conservation tillage, particularly in arid areas where soil erosion is a major issue for agricultural land-use (García-Ruiz, 2010; Gonzalez-Sanchez et al., 2015). Arguments against no-tillage application include high costs for new machinery, difficulties of weed control and a potential for reduced yields. All these reasons raise concerns about the financial practicability amongst farmers (Schneider et al., 2010; Hernández Plaza et al., 2015).

Ecosystem services driven by soil fauna

Soil is a complex habitat for soil organisms characterised by great temporal and spatial heterogeneities (Roger-Estrade et al., 2010) and hosts around 23% of the world´s biodiversity (Decaëns et al., 2006). The soil fauna is highly diverse and can be grouped according to their body width: (i) microfauna (0.002 - 0.2 mm), (ii) mesofauna (0.02 - 20 mm) and (iii) macrofauna (20 - 200 mm) (Swift, 1997). Together, soil biota provide a wide range of ecosystem services (Brussaard, 1997; Barrios, 2007). The latter is defined as benefits that humans obtain from ecosystems and has been established by the Convention on Biological Diversity (CBD) and the Millenium Ecosystem Assessment (MA) in order to integrate the management of natural resources in a sustainable and impartial way (Millennium Ecosystem Assessment (Program), 2005).

Soil fauna contributes to the decomposition of organic matter (Jacobs et al., 2011; Murugan et al., 2014; Faust et al., 2019), the release and cycling of nutrients (Araujo et al., 2004; Whalen, 2014) and the suppression of plant diseases (Löbmann et al., 2016; Plaas et al., 2019; Meyer-Wolfarth et al., 2021). The decomposition of organic matter is one ecosystem service that is mainly driven by primary decomposers such as bacteria and fungi, but also affected by litter feeding (detritivores) soil meso- and macrofauna, including enchytraeids, collembola and earthworms (Bunning and Jiménez, 2003; van Eekeren et al., 2010). The soil meso- and macrofauna contribute to the cycling of organic matter as they break down plant detritus, and via (selective) feeding on microflora regulate the composition and activity of soil microbial communities (Artz et al., 2010). The regulation of nutrient uptake and availability is another service provided by soil- and litter-feeding invertebrates and microorganisms (Bunning and Jiménez, 2003; Blouin et al., 2013; Lavelle et al., 2006). The present thesis focuses on decomposition processes, nutrient cycles and gas fluxes.

By delivering ecosystem services, soil biodiversity has a value, which is difficult to measure economically even though its benefits are known (Mace et al., 2012; Pascual et al., 2015). In general, the ecology of soil biota is poorly understood and their functions are little appreciated by farmers (Hervé et al., 2020). Nevertheless, the provision of ecosystem services by soil organisms and their importance for human well-being has increasingly come into focus in recent years (Pascual et al., 2015; Plaas et al., 2019; Sijtsma et al., 2013; Farnsworth et al., 2015; Greiner et al., 2017).

The soil food web

Ecosystem services are delivered by the entirety of the soil food web, which includes all living organisms and their parts in the soil. The lowest trophic level in the soil food web are plants and other photosynthetic organisms. Further, the soil food web consists of microorganisms, which are preyed upon by protozoa and nematodes (microfauna), microarthropods (mesofauna), and other larger organisms such as earthworms (macrofauna). Selective feeding of soil fauna on microorganisms alters microbial communities and can lead to shifts towards fungi or bacteria rich communities (Cole, 2002; Dempsey et al., 2013; Hoeffner et al., 2018). There are also interactions between faunal groups, for example earthworms and collembolans feed on nematodes (Read et al., 2006; Tao et al., 2011). In addition to predator-prey relationships, soil organisms are in competition for resources and habitats (Scheu et al., 2005).

Besides the classification into taxonomic groups, there is also the possibility of distinguishing soil organisms, which have almost the same function in soils, into functional groups, (Brussaard, 1998; Scheu, 2002). The distinction between these functional groups is often used to study the response of soil organisms to changes in the soil, it usually says more about the impact on ecosystem functions than the taxonomy classification (Berg et al., 1998; Bossuyt et al., 2006; Wood et al., 2015). This is also part of the present thesis, investigating different functional groups of earthworms and collembolans and their response to tillage. The characteristics of soil organisms, i.e. their functional traits, affect the ecosystem functioning, e.g. by predation (Wood et al., 2015). Therefore, the entire soil food web that in its whole is responsible for the delivery of certain ecosystem services and needs to be considered in farm-based management decisions to avoid imbalances (Vries et al., 2013).

The problem, however, is that an agricultural system which purely aims at maximizing production, often results in the loss of biodiversity and thus the loss of ecosystem functions (Kibblewhite et al., 2008). These shifts are mainly driven by the four management factors mentioned above. For instance, the use of mineral fertilisation such as ammonium sulphate can drastically decrease earthworm abundances and biomass and leads to acidity (Ma et al., 1990). In general, low pH levels are normally connected with low abundances of earthworms as some species are sensitive to acidity, whereas others have a broader tolerance (Potthoff et al., 2008). Further, the use of broad-spectrum insecticides such as chlorpyrifos is known to decrease the abundance and taxonomic richness of Collembola (Frampton and van den Brink, 2007). In the context of crop rotation, it is known that diversification promotes microbial richness and diversity (Venter et al., 2016) as well as earthworm densities (Torppa and Taylor, 2022). At least, ploughing affects soil faunal groups and microorganisms with consequences for soil parameters, which is investigated in the present thesis.

Due to a the anthropogenic disturbances in agro-ecosystems, upcoming imbalances of nutrients and the presence of pathogens often follow (Steffan et al., 2018). Therefore, the preservation of soil biodiversity, in turn, improves not only the productivity, but also the stability and resilience of agricultural systems (Lemanceau et al., 2015; Doran and Zeiss, 2000). Stability is normally linked to the output, e.g. crop yield (Urruty et al., 2016). Resilience means that ecosystems have a greater capacity to recover after disturbances, which can be in the agricultural context: drought, floods, pests or plant diseases. Soil resilience is one approach to measure sustainability in soil management (Ludwig et al., 2018). Agricultural management that supports soil biodiversity can improve the resilience of agroecosystems, thereby contributing to sustainable management (Stratton et al., 2020). Preserving biodiversity and ensuring sustainability in agricultural landscapes is still a crucial topic in research (Bender and van der Heijden, 2015; Doran et al., 1994; Hurni et al., 2015; Lichtfouse 2010; Keesstra et al. 2016; Brussaard et al. 2007).

Microbial soil parameters

A healthy and active soil microbiome is crucial to ensure nutrient cycling and high crop yields in the long-term (Bender and van der Heijden, 2015). Stability of a soil system (resistance and resilience) bridges two domains: (i) physicochemical characteristics like concentrations of carbon and nitrogen as well as bulk density or water fluxes and (ii) the microbial community (Griffiths and Philippot, 2013). Microorganisms have a key function in the breakdown of organic matter and recycling of nutrients (Gleixner, 2013; Thiessen et al., 2013; Stenberg, 1999), they are interconnected with the carbon cycle (Wright et al., 2005) and reflect the nutrient status of soils (Halmi et al., 2018). Plants depend on the metabolic activity of soil microorganisms to gain access to soil-borne nutrients (Jacoby et al., 2017).

Soil microbial biomass carbon (MBC) and nitrogen (MBN) are commonly used as indicators to characterize the biological status of soils (Carter, 1986; Joergensen and Emmerling, 2006). Measures like the metabolic quotient (qCO_2) or the ratio of MBC to soil organic carbon (SOC) are useful tools for a deeper understanding of the soil microbial activity. The qCO_2 reflects the microbial carbon use efficiency and indicates how environmental conditions affect microbial turnover and growth (Anderson and Domsch, 1993). High turnover with small gain of biomass (high qCO_2) indicate conditions of reduced carbon use efficiency. The ratio of MBC to SOC is an indicator for the availability of soil organic matter for microorganisms (Anderson and Domsch, 1989). As microbes respond rapidly to the addition of easily decomposable nutrient sources, changes of the MBC/SOC ratio reflect organic matter inputs, carbon losses as well as stabilization of organic carbon (Sparling, 1992). Creamer et al. (2016) highlight that basal respiration, microbial biomass and fungal abundance are indicators for the ability of a system to store and cycle organic carbon over time. All of these parameters are used in the present thesis in order to identify tillage systems that promote microorganisms in soils and ergo soil quality.

The role of mesofauna in soils

The soil mesofauna includes arthropods such as collembolans, mites and enchytraeids. All of them contribute to the decomposition of organic matter (Artz et al., 2010), affect microorganisms via grazing (Gajda et al., 2017) and can increase nutrient availability due to the deposition of faeces (Kaneda and Kaneko, 2011). Mesofauna makes up a large part of the soil biome in agriculture. Enchytraeids and Collembola contribute to key functions in the soil like turnover of organic matter and soil structure (Schrader et al., 1997; Koutika et al., 2001; Pelosi and Römbke, 2016). Besides these effects on soil properties, mesofauna plays a key role as prey for organisms of higher trophic levels in order to preserve the entirety of soil biodiversity in agro-ecosystems (Bilde et al., 2000). In the present thesis, enchytraeids and collembolans and their role in agricultural soils are studied.

Enchytraeids (Annelida) are small and unpigmented annelids, also known as potworms (Coleman et al., 2004). Similar to earthworms, enchytraeids are able to directly influence the soil structure via the transport of mineral particles due to their burrowing activity (Didden, 1990). Their abundance and species composition can be altered by agricultural management. They react sensitively to tillage and their presence or absence indicate changes in agricultural practices (Pelosi and Römbke, 2016). Enchytraeids mainly feed on partially degraded plant fragments, bacteria, fungi, and microalgae, but their feeding behaviour can differ between species and within family (Gajda et al., 2017). Enchytraeids enhance carbon mineralization (Cole et al., 2000), influence the microbial community structure and nutrient dynamics (Cole, 2002; van Vliet et al., 2004). Most soil-dwelling microannelids belong taxonomically to the family of Enchytraeidae (Graefe et al., 2019). In arable land numbers of enchytraeids range from ca. 6 000 ind. m^{-2} (Jozefowska and Miechowka, 2015) up to approximately 25 000 ind. m^{-2}, depending on tillage regime and region (Graefe et al., 2019). Enchytraeids react sensitive to changes in management by both, abundance and species composition and can be considered as indicators of agricultural management practices (Pelosi and Römbke, 2016). Previous studies already showed that the abundance of enchytraeids is not constantly supported by a reduction in tillage. For instance, Severon et al. (2012) report 22 567 ind. m^{-2} under conventional tillage and 12 318 ind. m^{-2} under reduced tillage. Further, the review of van Capelle et al. (2012) showed most beneficial effects for enchytraeids under the intermediate tillage system (conservation tillage), compared with conventional tillage and no-tillage.

Collembolans (Arthropoda) make up a large part of the soil mesofauna and are an important prey group in agro-ecosystems (Bilde et al., 2000). More than 65 000 species of collembolans are known and their morphological characteristics differ widely (Rusek, 1998). Collembolans have a wide range of food sources, mainly consisting of decayed plants, root exudates, fungi and bacteria (Petersen, 2000; Berg et al., 2004). In the soil, they mainly contribute to the distribution of fungi, alter the microbial community via grazing, feed on roots and predate on nematodes (Filser, 2002) and contribute to soil aggregation (Siddiky et al., 2012). In soils, there are three life form types of Collembola: epedaphic (litter surface dweller), hemiedaphic (topsoil and litter dwellers) and euedaphic (soil horizon dwellers) (Hopkin, 2007). Former studies suggest that collembolans can be promoted by conservation tillage practices, whereas species richness seems to be less affected by tillage (Petersen, 2002; Brennan et al., 2006). Generally, the abundance of Collembola lie around 1 019 - 6 560 ind. m^{-2} (Abbas and Parwez, 2019) up to 12 520 ind. m^{-2}

(Hoeffner et al., 2021) in arable land. Dittmer and Schrader (2000) highlight that collembolan species have different preferences with regard to tillage and soil compaction. The effect of tillage on collembolan abundances varies. The review of van Capelle et al. (2012), including 9 studies on the effect of tillage intensity on collembolans, shows significant lower abundances of Collembola under no-tillage, compared with conservation and conventional tillage. In contrast, Rickerl et al. (1989) reports that 29% more Collembola were found under no-tillage, compared with conventional tillage.

Earthworms as ecosystem engineers

Earthworms (Annelida) are part of the soil macrofauna and species vary in body length between approximately 50 to 200 mm (Christian and Zicsi, 1999). The diet of earthworms mainly consists of decomposed organic material and also mineral soil fractions, predominantly they prefer organic-mineral mixtures (Doube et al., 1997). Microbiota, e.g. fungi, protozoa, algae and nematodes are also a significant part in the diet of earthworms (Curry and Schmidt, 2007). In general, three different ecological groups of earthworms can be differentiated: (i) anecic earthworms, (ii) endogeic earthworms, (iii) epigeic earthworms (Bouché, 1977). As epigeic earthworms mainly inhabit the litter layer of soils, they are less present in agricultural systems. Anecic earthworms, including *Lumbricus terrestris*, are often dark coloured on the dorsal side of the front half and unpigmented ventrally. In contrast, endogeic species such as *Octolasion cyaneum* are generally pale - light pigmented or unpigmented (Christian and Zicsi, 1999).

As geophages organisms, endogeic earthworms ingest preferentially soil and feed on partly decomposed organic material as well as microorganisms adherent to soil particles (Curry and Schmidt, 2007). Anecic earthworms are detrivores, they feed directly on plant litter and dung and prefer to feed on the soil surface. They burrow vertically, dragging the surface litter into their burrows, where it is colonized and decomposed by microbes. The small mounds of organic material e.g. leaves on the top of their burrows are so-called middens. Voids in the soil formed by plants and other soil organisms, are called biopores. Earthworm biopores can be rather durable, especially those of anecic earthworms and can be reused over years (Capowiez et al., 2014). Whereas anecic species create subvertical galleries in the soil, the burrows of endogeic species are directed more horizontally (Bouché, 1977). Those parts of the soil that are affected by earthworm secretions, burrowing, and casting (drilosphere) build microbial hotspots with a high microbial activity (Kuzyakov and Blagodatskaya, 2015; Banfield et al., 2017; Hoang et al., 2017; Hoeffner et al., 2018). For instance, Banfield (2018) highlights that a long-term activity in native earthworms pores (> 2.5 years) can increase carbon concentrations by 200%, compared with the surrounding bulk soil. The resulting higher carbon availability has a positive effect on microorganisms.

Earthworms are considered as "soil ecosystem engineers", which means they actively change the habitat they live in and the availability of resources for other soil food web components (Jones et al., 1994). This important role of earthworms is widely present in research (Lavelle et al., 1997; Jouquet et al., 2006; Eisenhauer, 2010; Blouin et al., 2013). They are often used as representative model organisms of soil fauna in laboratory studies (Fründ et al., 2010). Earthworms translocate soil particles due to feeding and egesting (Taylor and Taylor, 2014; Taylor et al., 2018) and are one of the most important soil organisms group for bioturbation in temperate ecosystems (Darwin, 1881; Meysman et al., 2006). Bioturbation means ´biological reworking´ of soil particles and sediments (Meysman et al., 2006). Further, earthworms are known to have a key function in soil carbon fluxes (Ruf et al., 2006; Speratti and Whalen, 2008), which is due to their burrowing and feeding and the resulting impact on microbial processes and litter breakdown. Their impact on nutrient cycling in the soil is an ongoing topic in research (Frouz et al., 2014; Knowles et al., 2016; Frazão et al., 2019).

In order to give recommendations for management decisions regarding tillage and crop residues that foster earthworms and other important soil fauna (e.g. mesofauna), a detailed investigation of their feeding ecology is needed. The analysis of stable isotope ratios ($^{13}C/^{12}C$) is a suitable method to investigate food preferences of soil fauna (Scheu and Falca, 2000; Schmidt et al., 2004; Curry and Schmidt, 2007; Potapov et al., 2018). With the help of this method, the fate of carbon (from crop residues, e.g. maize leaves as a C_4 plant, to soil organisms) can easily be quantified as the amount of residue-derived C by soil organisms on a soil that has been previously under C_3 vegetation. Different tillage applications vary regarding to crop residue placement in the soil, this may affect the feeding strategies of earthworms. Generally, the uptake of residue-derived C by soil fauna occurs in two ways: (i) directly by feeding on crop residues or (ii) indirectly via the uptake of microorganisms which pass carbon on to higher trophic levels (Kramer et al., 2012; Potapov et al., 2018).

Impacts of tillage on soil biota

A reduction in tillage is one suitable tool to reduce the impact of agricultural practices on soil and atmosphere (Busari et al., 2015). For instance, no-tillage can save even 82.7% of fuel, compared with conventional tillage (Filipovic et al., 2004). No-tillage (direct seeding) and other reduced tillage systems, grouped under the term “conservation tillage” are known to be beneficial for the activity of microorganisms and soil fauna groups. For instance, when cultivation abstains from ploughing microbial biomass and activity increases, in particular in the upper soil layer (Ahl et al., 1998; Babujia et al., 2010). Fungi profit from no-tillage due to the lack of disturbances, which enhances the fungal biomass, especially those of arbuscular mycorrhiza fungi (Zahangir, 2005). Further, the networks of fungal hyphae are known to be more stable in no-tilled soils (Wilkes et al., 2021). As a result of the positive effects on soil life by no-tillage systems, aggregate stability is also enhanced in no-tilled fields (Beare et al., 1992; Hoeffner et al., 2021). The latter is the most relevant indicator for soil vulnerability to erosion processes (Farres, 1987; Barthès and Roose, 2002).

The effect of tillage on soil mesofauna is not fully understood as these organisms respond, depending on their ecological performance, to a reduction in tillage. Previous studies indicate that often community composition rather than abundance is affected by tillage (Dittmer and Schrader, 2000; Brennan et al., 2006; van Capelle et al., 2012; Beylich et al., 2018). In the case of earthworms (macrofauna), the incorporation of crop residues by ploughing can promote one ecological group of earthworms, whereas others are generally harmed by such an intensive form of tillage (Ernst and Emmerling, 2009; Briones and Schmidt, 2017). For instance, ploughing destroys earthworms burrows and leads to a lower activity due to habitat destruction (Roger-Estrade et al., 2010). Further, without ploughing earthworms are not exposed to predators directly at the soil surface and are less harmed by the process itself (van Capelle et al., 2012; Briones and Schmidt, 2017). Overall, a reduction in tillage intensity usually promotes earthworms abundance, biomass and species composition (Chan, 2001; Ernst and Emmerling, 2009; Briones and Schmidt, 2017; Moos et al., 2017).

Litter decomposition in agricultural soils

The process of crop residue decomposition and the related supply of nutrients is essential for agricultural soils (Cattanio et al., 2008) and plays a crucial role in the transfer of organic matter and nutrients into the soil system (Casado-Murillo and Abril, 2013). The incorporation of crop residues through tillage brings litter into contact with the surrounding soil, which improves the initial accessibility of the litter for microorganisms (Potthoff et al., 2005; Faust et al., 2019) and accelerates the mineralization of soil carbon and nitrogen (Sainju et al., 2003). Aboveground litter derived from plants is one of the primary carbon sources in soil food webs besides soil organic matter and root exudates (Ruf et al., 2006). Carbon inputs through plants are transformed by soil organisms into structurally new carbon, which is found as organic matter in mineral soil. About 20-30% of the carbon assimilated by plants (cereals) is transferred into the soil, half of this amount goes in the roots, one third is evolved as CO_2 and the remaining part is translocated into soil microorganisms and soil organic matter (Kuzyakov and Domanski, 2000). There are three main drivers of litter decomposition: (i) the environment (physical and chemical parameters), (ii) the quality and structure of the litter and (iii) the decomposer community (Swift et al., 1979; Couteaux et al., 1995; Knacker et al., 2003).

Environmental conditions depend on the tillage regimes, ergo where in the soil profile litter or crop residues are placed due to the applied management practice. In no-tillage systems, residues are placed at the soil surface and are therefore directly exposed to changes of environmental conditions (Lupwayi et al., 2004). Thus, crop residues in no-tilled soils are usually exposed to higher fluctuations in soil temperature and moisture compared with residues ploughed down to 30 cm in conventionally tilled soils (Kladivko et al., 1986). This is of consequence for the decomposition process (Cortez, 1998). Especially in regions with low annual average temperatures, no-tillage systems with a high residue coverage can significantly decrease soil temperatures (Arvidsson et al., 2014; Shen et al., 2018). This might have direct effects on the microbial colonization of crop residues which plays an important role for decomposition (Potthoff et al., 2008). Low temperature levels do not only slow down microbial processes, but also the activity of mesofauna. However, the effects of no-tillage depend highly on the region. For instance, in arid or semi-arid areas, no-tillage often brings great advantages, e.g. protection against wind erosion and higher water retention in the soil (López-Fando et al., 2007; Wang et al., 2020).

Besides these abiotic factors, the quality of litter and the composition of the decomposer community also have major impacts on litter breakdown. In the process of litter decomposition, a wide range of soil organisms is involved: microorganisms (fungi and bacteria), microfauna (protozoa and nematodes), mesofauna (e.g. enchytraeids and collembola) and macrofauna (e.g. earthworms) (Brussaard, 1991). The relationship between soil animals and microorganisms also affects decomposition processes: detritivore soil animals indirectly stimulate the microbial activity via grazing (Parker et al., 1984; Seastedt, 1984; Wachendorf et al., 2014).

The litterbag method can be used to manipulate the composition of the decomposer community and thus to determine the contribution of different soil organisms to litter decomposition and litter decomposition rates (Karberg et al., 2008). Litter material is enclosed in mesh bags, placed in the field and collected in intervals in order to determine the weight loss over time. The use of different mesh sizes enables to include or exclude parts of soil fauna based on the body size (Bradford et al., 2002; Bokhorst and Wardle, 2013; Yang et al., 2017). The exclusion of one trophic group from the soil food web can lead to imbalances. This has been previously shown under experimental settings. For instance, in litterbags with a too small mesh size for macrofauna (including predators of mesofauna), the mesofauna overgrazed the microorganisms (Vreeken-Buijs and Brussaard, 1996). An resulting impoverishment of the decomposer community can have negative effects like a reduction of available soil nitrogen (Taylor et al., 2004). The litterbag method was applied in the present thesis in order to quantify the importance of parts of the soil mesofauna for decomposition processes under field-conditions (Vreeken-Buijs and Brussaard, 1996; Jacobs et al., 2011; Faust et al., 2019).

Carbon and nitrogen dynamics in soils

Soil organic matter is a key factor for the mitigation of climate change (Wolters, 2000) as a large quantity of carbon is stored within soil organic matter (SOM) (Scharlemann et al., 2014). Substances egested by microorganisms are able to act like binding agents for soil particles and contribute to aggregate formation (Artz et al., 2010). Thus, the stability of SOM can be greatly enhanced by microbial processes (Wolters, 2000). Generally, carbon sequestration contributes to the reduction of carbon losses, e.g. as greenhouse gas (GHG) emissions (Lavelle et al., 2006). In agricultural settings, carbon cycles are also affected by tillage. While ploughing transfers organic material deeper into the soil depths, conservation tillage is one possibility to increase SOC contents in the soil surface layer (Lal, 2014; Haddaway et al., 2017). This is particularly important because high SOC levels improve the soil structure, which in turn enhances the soils resilience to extreme weather conditions (Haddaway et al., 2017). In addition, the casts of meso- and macrofauna, in particular of earthworms, form aggregates including many microaggregates, which protect SOC physically from destabilization (Bossuyt et al., 2004; Bossuyt et al., 2005; Pulleman et al., 2005; Sánchez-de León et al., 2014). Frequent ploughing weakens the soil structure and soil aggregates in no-tilled fields were examined to be 13-16% more stable as under conventional tillage (Hevia et al., 2007). The aggregate-associated carbon can be enhanced in the upper 10 cm by the choice of no-tillage, compared with ploughing (Zheng et al., 2018). When SOC becomes available, it can leave the soil system through mineralization, for example in gaseous form (Bailey et al., 2019).

To develop a sustainable agriculture which can contribute to mitigate climate change, strategies are needed which increase the amount of carbon sequestered in soils. Therefore, it is important to assess the impact of tillage for carbon dynamics influenced by soil organisms. It is widely known that soil fauna directly and indirectly affects carbon and nitrogen dynamics (Laurén et al., 2012; Zhang et al., 2013). As an indirect effect, emissions of CO_2 from the soil are generally increased in the presence of soil fauna, e.g. earthworms, which mostly results from enhanced microbial respiration rates (Binet et al., 1998; Lubbers et al., 2015; Nieminen et al., 2015; Wu et al., 2015). Further, soil organisms can suppress, increase, delay or accelerate N_2O emissions (Kuiper et al., 2013) and influence the rate of carbon and nitrogen release in decomposition processes (Laurén et al., 2012). The interaction of detritivorous and predatory soil fauna can stimulate GHG emission processes (Kuiper et al., 2013; Thakur et al., 2014). In the present thesis, the effect of both, tillage and soil fauna on GHG emissions is assessed in order to contribute for future decision making in the mitigation of climate change.

Research objectives

All studies presented in this thesis were conducted within the framework of the **SoilMan** (Ecosystem services of soil biota in agriculture) project, which investigated the impact of different farm-based management practices on soil biota. The project received funding under the European ERA-Net BiodivErsA and ran from 2017-2020 with the expertise of researchers from six EU member states. The project aimed at transfer knowledge between socio-economy, ecology and policies and followed a simple idea, namely that soil biodiversity works for agriculture when agriculture (A) cares about soil biodiversity (B) – A for B = B for A.

The aim of this thesis is to identify tillage strategies that promote soil organisms and soil quality/conditions, taking into account region-specific conditions, e.g. soil and climate. Main focus are the processes driven by microorganisms, enchytraeids and collembolan (mesofauna) and earthworms (macrofauna) and their response to a reduction in tillage. The following research issues are divided into three chapters:

Study 1 (Chapter 4) applies a regional approach and investigates four long-term experimental field sites in different European countries with varying environmental conditions. This study focuses the comparison of reduced tillage systems and conventional ploughing on soil microorganisms and physicochemical soil parameters that are indicators of soil quality. The aim of this study was to find out which tillage system promotes soil properties and thus enhances soil quality.

Study 2 (Chapter 5) assesses soil functions and processes driven by soil mesofauna. In particular, the role of Collembola, a key mesofauna group, in the decomposition processes of crop residues was investigated, using a litterbags approach. The objective of this study was to determine which tillage system fosters soil mesofauna and thus litter decomposition under field conditions.

Study 3 (Chapter 6) is a mesocosm experiment under laboratory conditions on the role of micro-, meso- and macrofauna for carbon and nitrogen dynamics in soils. This study examines how earthworms and enchytraeids respond to the placement of organic material in the soil profile using stable isotope analysis. This study aimed to find out which tillage strategy and crop residue displacement promotes annelids and associated soil functions.

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PART III

Chapter 4

Study 1

The effects of conservation tillage on chemical and microbial soil parameters at four sites across Europe

plants

MDPI

Article

The Effects of Conservation Tillage on Chemical and Microbial Soil Parameters at Four Sites across Europe

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Abstract: Conservation tillage is often discussed as an effective tool to improve the soil quality in agriculture. Four sites across Europe (in Germany, Romania, Spain, and Sweden) were investigated as case studies for country-specific reductions in tillage intensity. Conventional tillage (CT) by mouldboard ploughing was compared with shallow and deep non-inversion minimum tillage (MT) and/or no-tillage (NT). In Sweden, NT and MT had positive effects on the concentrations of soil organic carbon (SOC), total nitrogen (N), and microbial biomass carbon (MBC) in the upper 20 cm compared with CT. At the German site, MT increased SOC, N, and MBC concentrations in the top 10 cm. In contrast, CT increased MBC contents and bulk density between 20 and 30 cm soil depth. At the Romanian site, soil parameters showed no differences between inverse tillage (CT) and non-inverse tillage (MT), both with a working depth of 25 to 30 cm. At the Spanish site, the use of NT significantly increased the concentrations as well as the stocks of C, N, and MBC compared to CT. In conclusion, reduced tillage improved soil microbial properties in most cases. However, the effectiveness of reduced tillage appears to be highly dependent on site conditions such as pH, soil texture, and climatic conditions.

Keywords: minimum tillage; no-tillage; mouldboard ploughing; soil quality; microbial biomass

1. Introduction

Conservation tillage has the potential to promote soil organisms [1], enhance the water infiltration capacity of soils, and reduce the risk of water erosion [2,3]. Further, the potential for carbon (C) sequestration is also a strong motivation for reducing tillage intensity in order to mitigate climate change [4,5]. Techniques of conservation tillage generally increase C and nitrogen (N) contents in the soil surface layer, thereby improving the soil structure and accelerating the soil's resilience to extreme weather conditions [6]. In addition, tillage systems with a mulch layer reduce the risk of wind erosion [7,8] and lessen evaporation processes [9]. Nevertheless, conventional tillage (CT) is still a common tillage system in most European countries [10–12]. Main arguments against no-tillage (NT) systems are yield loss due to weed competition [13] and problems with seed germination [14].

The effectiveness of conservation tillage to improve soil conditions is still difficult to predict as there are significant differences among the various techniques grouped under this term. For instance, NT can mean direct seeding without any tillage operations [15,16] or opening a narrow trench for sowing [17]. Minimum tillage (MT) can be applied with various machinery (e.g., a disc harrow or a rotary harrow) at 5 cm [18], down to 8 cm [19], between 10 and 15 cm [20,21] or even down to 20 cm [22]. In contrast, CT is defined more uniformly in Europe, using a mouldboard plough at a working depth of between 20 and 30 cm [10,15,23].

In order to assess the advantages of conservation tillage over ploughing, indicators that reflect the status of soils are needed. Soil microbial properties as well as physicochemical soil characteristics are the two most important factors for the stability of a soil system [24]. To promote sustainable soil management and to improve soil fertility, an increase in surface-near nutrients and microbial biomass is recommended. The measurement of microbial biomass carbon (MBC) is a common tool to investigate the relationship between plant input, soil organic C (SOC) storage, nutrient mobilization and immobilisation processes [25,26]. MBC is often used in combination with measuring basal respiration, the mineralisation of SOC in the absence of fresh plant substrates [27,28]. In addition, the metabolic quotient $q\mathrm{CO}_2$ calculated as respiration-to-biomass ratio reflects the microbial demand for maintenance energy [29], whereas the ratio of MBC to SOC expresses the C availability for microorganisms [30]. Previous studies showed that conservation tillage, compared with ploughing, has the potential to increase MBC stocks and enhance microbial indicators [31–34]. In contrast, only moderate or even no increases in SOC stocks were found for non-inversion tillage treatments compared with mouldboard ploughing [21,35–38]. However, the effects seem to vary between sites and tillage regimes.

Agricultural soils are influenced by the long-term history of cultivation practices and current management decisions of the farmer. Therefore, the present study follows a regional approach and investigates four sites, where tillage intensity was reduced in a country-specific way to match the demand of local management (Table 1). The effects of conservation tillage systems on microbial, chemical, and physical soil parameters are compared with those of ploughing. The aim of this study was to find out which tillage system improves soil properties and thus enhances soil quality the most, taking into account site-specific conditions. The four sites form a climate gradient from Northern Europe (Sweden), via Central Europe (Germany) to South-Eastern Europe (Romania) and South-Western Europe (Spain), representing large areas of arable land in each country. The following four hypotheses were examined: (1) The positive effects of reducing tillage intensity on MBC are most visible in the upper soil and decrease with soil depth. (2) SOC stocks are not significantly different between conventional mouldboard ploughing and conservation tillage when regarding the whole sampled soil profile, whereas (3) MBC stocks are increased by a tillage reduction. (4) However, the effectiveness of conservation tillage regarding MBC dynamics depends on site-specific conditions such as soil texture, pH, and climatic conditions.

2. Results and Discussion

2.1. *Säby, Sweden*

In Sweden, SOC contents varied around 25.9 mg g^{-1} soil and declined by 34% from the top to the bottom layer under MT and by 50% under NT. In contrast, SOC concentrations at 0–20 cm under CT were nearly equally distributed with highest amounts in the deepest soil layer. At 20–30 cm, SOC contents were significantly affected by tillage treatments ($F = 16.99$; $p < 0.05$); contents of SOC were significantly higher ($p < 0.05$) at CT than at NT or MT plots (Table 2). Total N contents were in a range from 1.6 to 2.7 mg g^{-1} soil and were significantly influenced by tillage ($F = 7.60$; $p < 0.05$) at 10–20 cm. Soil samples at 10–20 cm soil depth from CT plots had greater total N contents ($p = 0.05$) than those from NT (Table 2). MBC contents were in a range from 61 to 333 µg g^{-1} and differed significantly ($F = 52.77$; $p < 0.01$) between tillage treatments at 0–10 cm soil depth; MT ($p < 0.01$) and NT ($p < 0.01$) enhanced MBC contents strongly compared with CT (Figure 1).

Summing up, the 11 years of MT and NT increased the contents of SOC, total N, and MBC in the top layers, accompanied by a strong depth decline in the bottom 20–30 cm layer. Similar depth declines have been repeatedly observed in Sweden [31,39]. The increase at 0–10 cm and the depth decline were most pronounced for MBC, indicating a closer relationship to the actual C input than SOC and N already stored in soil [1,40]. This effect was intensified by the strong bulk density (BD) increase from the 0–10 cm to the 10–20 cm layer. This increase occurred under all three tillage systems but especially under MT and NT (+40%) vs. CT (+30%).

The BD varied around 1.02 g cm^{-3} at 0–10 cm and around 1.38 g cm^{-3} at 10–30 cm, without tillage effects at any depth (Table 2). The increased BD below followed from cultivation might form a barrier, which can reduce the C input by crop roots and, thus, MBC in the long-term [39,41,42]. The soil at this site, an acidic Eutric Cambisol, was characterised by high SOC stocks and low MBC stocks. Differences in SOC stocks were relatively strong between tillage treatments ($F = 5.51$; $p = 0.07$) whereas MBC stocks showed quite smaller variations (Table 3). The MBC/SOC ratio was around 0.7% among tillage treatments, which is most common in strongly acidic forest soils [29,43] and has rarely been measured in arable Cambisols [44], especially not in those with a relatively high clay content [25]. Acidification usually also increases the microbial demand for maintenance energy [29,45], which was reflected by high qCO_2 values with a mean of 134 mg CO_2-C g^{-1} MBC d^{-1} (0–30 cm soil depth). A high demand for maintenance energy lowers the MBC contents of a soil in the long-term [27,28]. However, low mean annual temperatures (MAT) and high mean annual precipitation (MAP) might also have reduced microbial decomposition of the annual C input, as indicated by the high SOC/total N ratio of 11.9. Overall, the study site at Säby is characterised by acidic conditions and low annual temperatures, high SOC contents and stocks. The low microbial availability of resources was reflected by low MBC/SOC ratios and quite small effects of tillage reduction on MBC contents.

Table 1. Overview of the different tillage treatments at the four field sites; CT = conventional tillage, MT = minimum tillage, NT = no-tillage.

Site	Tillage	Machinery Used (Working Depth)
Säby	CT	Mouldboard plough (23 cm)
	MT	Cultivator (10–12 cm)
	NT	Direct seeding without any tillage operations
Garte Süd	CT	Mouldboard plough (25–30 cm), followed by a rotary harrow
	MT	Rotary harrow (5–8 cm)
Turda	CT	Mouldboard plough (25–30 cm), seedbed preparation by a rotary harrow
	MT	Chisel processing (25–30 cm) after maize and wheat followed by disk harrow while direct seeding was applied after soybean
La Hampa	CT	Mouldboard plough (25–30 cm) plus cultivator (15–20 cm) and a disc harrow (15 cm)
	NT	Direct seeding without any tillage operations

Table 2. Mean (standard deviation) of bulk density, soil organic carbon (SOC) and total nitrogen (N) contents at the field sites Säby (*n* = 3), Garte Süd (*n* = 4), Turda (*n* = 3), and La Hampa (*n* = 3) under different tillage treatments (CT = conventional tillage, MT = minimum tillage, NT = no-tillage) at three soil depths (0–10 cm, 10–20 cm, 20–30 cm).

Site	**Soil Depth**	**Bulk Density (g cm^{-3})**			**SOC (mg g^{-1} Soil)**			**Total N (mg g^{-1} Soil)**		
	(cm)	**CT**	**MT**	**NT**	**CT**	**MT**	**NT**	**CT**	**MT**	**NT**
Säby	0–10	0.97 (0.10)	1.01 (0.14)	1.08 (0.16)	26.67 (1.51)	30.63 (1.55)	34.90 (4.19)	2.33 (0.05)	2.53 (0.09)	2.73 (0.19)
	10–20	1.25 (0.11)	1.41 (0.12)	1.50 (0.07)	27.00 (1.61)	26.13 (2.76)	23.53 (1.27)	2.30 (0.08) a	2.17 (0.12) ab	1.97 (0.05) b
	20–30	1.33 (0.18)	1.43 (0.07)	1.38 (0.13)	26.73 (2.40) a	20.17 (3.84) b	17.67 (3.73) b	2.20 (0.14)	1.77 (0.17)	1.63 (0.21)
Garte Süd	0–10	1.67 (0.12)	1.66 (0.10)		14.38 (1.48) b	18.28 (0.75) a		1.43 (0.04) b	1.75 (0.05) a	
	10–20	1.63 (0.05)	1.67 (0.09)		14.60 (0.99)	14.80 (0.94)		1.50 (0.00)	1.48 (0.04)	
	20–30	1.65 (0.05) b	1.83 (0.03) a		14.60 (2.42)	13.13 (1.61)		1.40 (0.07)	1.30 (0.00)	
Turda	0–10	0.89 (0.00)	0.85 (0.01)		22.13 (0.39)	20.67 (0.60)		2.13 (0.10)	2.00 (0.00)	
	10–20	0.98 (0.02)	0.88 (0.02)		22.53 (0.09)	20.87 (1.53)		2.13 (0.10)	2.00 (0.10)	
	20–30	1.01 (0.04)	0.90 (0.03)		22.43 (0.33)	19.53 (0.90)		2.13 (0.10)	1.90 (0.00)	
La Hampa	0–10	1.18 (0.29)		1.46 (0.19)	9.03 (0.48) b		10.30 (0.62) a	1.07 (0.05)		1.20 (0.08)
	10–20	1.29 (0.25)		1.26 (0.03)	8.23 (0.54)		9.13 (0.38)	0.90 (0.08)		1.07 (0.05)
	20–30	1.38 (0.05)		1.27 (0.16)	7.77 (0.05)		7.90 (0.65)	0.97 (0.05)		1.00 (0.08)

Different letters (a, b, ab) indicate a depth and site-specific significant difference between the tillage treatments ($p < 0.05$).

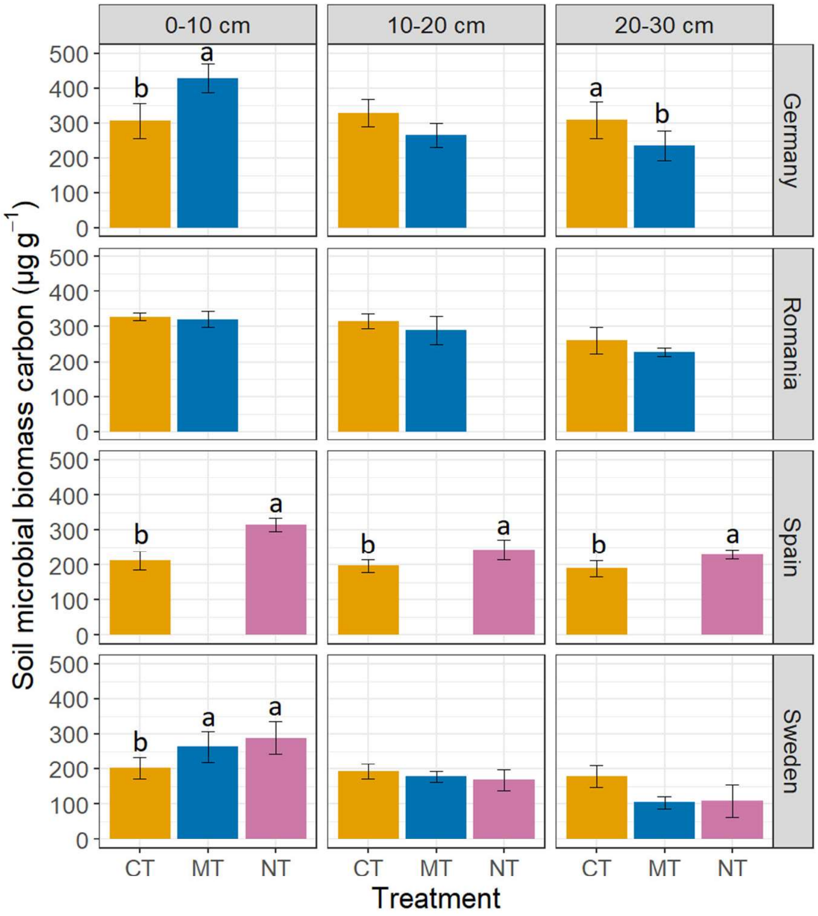

Figure 1. Effect of tillage treatments (CT = Conventional tillage, MT = Minimum tillage, NT = No-tillage) in Germany (*n* = 4), Romania (*n* = 3), Spain (*n* = 3) and Sweden (*n* = 3) on soil microbial biomass carbon at the soil depths (0–10 cm, 10–20 cm, 20–30 cm) given as means ± standard

deviation. Means followed by different letters (a, b) are significantly ($p < 0.05$) different from each other at each soil depth. Tillage treatments were carried out in a site-specific way (Table 1).

Table 3. Mean stocks (standard deviation) of soil organic carbon (SOC) and microbial biomass carbon (MBC) at the four field sites under different tillage treatments; CT = conventional tillage, MT = minimum tillage, NT = no-tillage. Information of tillage techniques are given in Table 1.

Site	**Equivalent Soil Mass (t ha^{-1} 0–30 cm)**	**SOC (t ha^{-1})**			**MBC (t ha^{-1})**		
		CT	**MT**	**NT**	**CT**	**MT**	**NT**
Säby ($n = 3$)	3790	108.2 (1.5)	86.7 (7.4)	76.5 (13.7)	0.72 (0.09)	0.66 (0.04)	0.68 (0.12)
Garte Süd ($n = 4$)	5060	75.3 (9.7)	76.2 (5.6)		1.62 (0.09)	1.53 (0.13)	
Turda ($n = 3$)	2760	59.0 (1.4)	58.8 (2.0)		0.83 (0.05)	0.77 (0.04)	
La Hampa ($n = 3$)	3790	32.5 (1.1) b		36.0 (1.1) a	0.78 (0.07) b		1.04 (0.02) a

Different letters (a, b) indicate a depth and site-specific significant difference between the tillage treatments ($p < 0.05$).

2.2. Garte Süd, Germany

Garte Süd is a long-lasting tillage experiment where the comparison between CT and MT already started 47 years ago [46]. Contents of SOC varied around 15.0 mg g^{-1} and the SOC/total N ratio was about 10 at Garte Süd. At 0–10 cm SOC contents were significantly ($F = 36.07$; $p < 0.01$) greater under MT compared with CT (Table 2). At the same soil depth, also total N contents were significantly greater ($F = 169.00$; $p < 0.001$) at MT plots than at CT plots (Table 2). MBC contents varied from 176 to 488 µg g^{-1} soil. In contrast to MT, the application of a mouldboard plough (CT) resulted in a homogenous distribution of SOC, total N and MBC concentrations in the sampled soil profile, presumably due to the strong mixing effect of this tool. At 0–10 cm soil depth, MBC contents were significantly ($F = 14.50$; $p < 0.05$) higher at MT compared with CT (Figure 1). Similar results have been repeatedly observed in Germany [32,37,41].

The soil at this site, a Haplic Luvisol, was characterised by high BD (mean of 1.69 g cm^{-3}). At 20–30 cm soil depth BD was significantly ($F = 31.23$; $p < 0.05$) higher at MT compared with CT. The generally high BD is most likely caused by heavy machinery, especially for sugar beet harvesting in wet autumns [47]. Load-induced compaction is most likely the reason for the extremely high BD at 20–30 cm under MT. Generally, field crops have less difficulty with homogeneously high BD levels than with escalating increases [48]. This is in line with the study of Murugan et al. [32], who did not observe any yield difference between CT and MT at four sites in Germany for winter wheat and sugar beet.

This high BD led to the maximum equivalent soil mass, which is partly reflected by the SOC and MBC stocks. SOC and MBC stocks showed no differences between tillage practices (Table 3). In contrast, Heinze et al. [19] and Murugan et al. [32] observed approximately 10% higher SOC stocks and 20% higher MBC stocks on Luvisols in central Germany under MT in comparison with CT. However, these differences might be partly explained by a different sampling and calculation procedure [32]. The mean MBC/SOC ratio was 2.1% at Garte Süd, which is typical for central European Luvisols [19,32,37]. The mean metabolic quotient $q\mathrm{CO_2}$ value was on average 98 without significant differences between tillage practices. In conclusion, the field site Garte Süd is shaped by relatively high BD levels. The contents of SOC, total N and MBC differed in terms of tillage practices in the upper soil depth (0–10 cm), whereas stocks were not affected.

2.3. Turda, Romania

Turda was the only site, where MT and CT were carried out on a similar working depth. The soil, a Phaeozem, was characterised by a high clay content (>50%) and a low BD level with a mean of 0.92 g cm^{-3}. MBC contents (0–30 cm depth) varied around 290 µg g^{-1} soil (Figure1). SOC contents were on average 21.4 mg g^{-1} soil at Turda with a mean

SOC/total N ratio of 10 (Table 2). Among tillage treatments, total N contents ranged from 19 to 22 mg g^{-1} soil in the upper 30 cm. Phaeozems, typical for Romanian cropland, are known for their high natural fertility [49]. The clay content at Turda was considerably above that of other European Phaeozems [50,51], which led to generally low BD values and might improve its resilience against tillage-induced compaction [52,53].

The changes of MBC contents with depth were moderate for both tillage techniques (Figure 1). The MBC decline at 20–30 cm under CT indicates that the mouldboard plough did not always reach a working depth of 30 cm. In accordance with current results, no differences in SOC contents with depth between mouldboard ploughing and deep non-inversion chisel tillage have been measured on a Mollisol in Ohio, USA [54]. In contrast, much higher SOC contents have been observed in the top layers of a deep non-inversion tillage treatment than in a mouldboard ploughing treatment on a typical Ukrainian Chernozem [51]. This discrepancy cannot be explained by the current study. Other experiments did not find differences in SOC stocks between 12 and 25 cm deep non-inversion tillage [55]. For this reason, a reduced tillage depth should be tested at Turda, especially considering the low BD. The mean SOC and MBC stocks were relatively low at Turda, which points towards low C inputs at this site. This would lead to a starving and aged microbial community, as indicated by the relatively low qCO_2 values [27,28]. This suggestion was confirmed by a mean metabolic quotient qCO_2 of 59 at Turda. Also, the relatively low MBC/SOC ratios with a mean of 1.4% indicate a relatively low C availability to soil microorganisms [28,30]. In addition, strong bonding of relict SOC to clay minerals may further reduce C availability [56]. None of the soil physical (BD), chemical (SOC and total N) and microbial properties (MBC, MBC/SOC and qCO_2) were strongly affected by tillage. This could be explained by the fact that both techniques were working at approximately the same depth (Table 1), which indicates that parameters depend more on working depth than on tillage techniques. The field site in Romania was characterised by high SOC concentrations, high clay contents and a low BD. The results from this site indicate that even different tillage techniques can lead to similar microbial conditions, which could by related to the soil type plus the choice of the same working depth for both machineries.

2.4. La Hampa, Spain

At the field site La Hampa, SOC contents were on average 8.7 mg g^{-1} soil with BD of around 1.31 g cm^{-3} (Table 2). SOC contents were significantly higher ($F = 27.77$; $p < 0.05$) in the upper 10 cm soil depth at NT plots compared with CT (Table 2). Total N contents (mean of 10 mg g^{-1} soil) were not affected by tillage reduction. MBC contents of NT plots exceeded those of CT plots (Figure 1) in the upper soil layer ($F = 30.56$; $p < 0.05$), at 10–20 cm ($F = 33.04$, $p < 0.05$) as well as in the lowest soil depth of 20–30 cm ($F = 51.07$; $p < 0.05$). The soil was characterised by high sand content and an alkaline soil pH and high MBC/SOC ratios (mean of 2.7%). Increased MBC/SOC ratios with increasing aridity of the climate have been repeatedly observed [57,58], due to shortening of the period for strong microbial activity. Consequently, qCO_2 values were low at La Hampa with a range of 31.5 to 64.6 mg CO_2 C g^{-1} MBC d^{-1}, indicating a low demand of the microbial community for maintenance energy. This view is in line with the meta-analysis of Zuber and Villamil [59] who showed that sandy soils have lower qCO_2 values under NT than under CT, whereas tillage effects were less in soils with finer particles.

The SOC stocks (mean of 32.5 t ha^{-1}) were relatively low at this site. The latter was significantly greater under NT compared with CT for both, SOC ($F = 339.97$; $p < 0.01$) and MBC ($F = 50.10$; $p < 0.05$) (Table 3). These higher stocks were combined with a less pronounced depth gradient, which suggests a larger C input rate into the 10–30 cm layers at La Hampa, as proposed by Virto et al. [60]. Another reason for these positive NT effects on SOC and MBC stocks at this semi-arid region might be a slower turnover. Without mechanical disturbance under NT, the mineralisation of aggregate occluded SOC is most likely reduced [61,62]. This lowers the qCO_2 values of a starving microbial population

[32,63] followed by increased MBC contents and later by a higher contribution of microbial necromass-derived SOC.

Low $q\mathrm{CO_2}$ values also indicated that the low SOC stocks at La Hampa are not caused by microbial mineralisation but by low C inputs combined with a low microbial turnover. However, the possibility cannot be excluded that the SOC contents were already different at the start of the experiment, as sandy Fluvisols often exhibit a considerable sedimentation-induced spatial variability [44,57]. This is often not considered, as the initial soil properties are usually analysed by a so-called representative bulk sample, pooled from several cores and not from analysing each plot separately. Results from La Hampa in Spain, where the soil is characterised by a high sand content and an alkaline pH value, showed a strong positive effect of no-till management. This was reflected by higher MBC concentrations and stocks as well as greater SOC stocks. Low $q\mathrm{CO_2}$ values and a high MBC/SOC level indicate good conditions for microbial activity.

2.5. Effects among Sites

In general, the application of CT with a mouldboard plough resulted in a more homogeneous distribution of SOC, total N, and MBC contents, due to the strong mixing effect of this tool, whereas MT and especially NT created site-specific depth gradients. This decrease of approximately 30% from the top to the bottom soil layer was similar at Garte Süd and Säby. Hence, confirming our first hypothesis, most effects of reduced tillage on soil parameters were visible at the upper 10 cm soil depth, which was particularly strong at Garte Süd. Stocks of SOC and MBC were 34% higher and 55% lower, respectively, at Säby compared with Garte Süd. The difference in study duration might also explain differences between the site-specific effects. At the German site, reduced tillage was already applied for more than 40 years, whereas at the Swedish site it was just > 10 years. Based on 17 tillage experiments in the study from Smith et al. [64], SOC levels by sequestration needs 50 to 100 years to reach a new equilibrium. These differences are also reflected by the MBC/SOC ratio. Acidification is probably the main reason for the low MBC/SOC ratio at Säby [29], increasing the microbial demand for maintenance energy [45], which is also reflected by the high $q\mathrm{CO_2}$ values. In Romania deep non-inversion MT down to 25 cm had no specific positive or negative effects on soil parameters in comparison with CT, suggesting that shallow MT down to 10 cm should be used preferentially.

Contradicting our hypotheses two and three, SOC and MBC stocks were not affected in different ways by tillage. However, the effectiveness of conservation tillage on C stocks was lower than expected. Most evidently, La Hampa was the only site where the SOC and MBC stocks of the NT treatments significantly exceeded those of the CT treatment by 11% and 33%, respectively. SOC stocks at Säby were approximately three times higher, compared with Spain, but MBC stocks were slightly lower due to the acidic soil pH. The main reason for the positive NT effect at La Hampa is most likely the slower turnover of the microbial biomass due to drier climatic conditions, which was also reflected by low $q\mathrm{CO_2}$ values, indicating that the low SOC stocks at La Hampa are not the result of strong microbial mineralization.

In line with our fourth hypothesis, the effectiveness of tillage reductions on soil parameters varied strongly between sites. Generally, less effects were found in Sweden, which is mostly related to soil pH and climate. As expected, the differences between the reduced tillage (MT/NT) systems were quite small at Säby. Results indicate that MT also has the potential to improve soil parameters to a similar extent as NT. In contrast, the strong effect of NT at La Hampa implies that no-till techniques are able to enhance microbial soil properties in semi-arid areas in a large extent. Further, marginal differences between tillage treatments in Romania (non-inversion vs. inversion tillage) indicate that soils with high clay contents and a good fertility might generally be affected less by tillage.

3. Material and Methods

3.1. Field Sites Descriptions

Across Europe (Sweden, Germany, Romania, and Spain), four different long-term experimental field sites that focus on tillage were selected for sampling. As CT treatment, inversion mouldboard ploughing down to 30 cm was present in each country, whereas the reduced tillage treatments (MT and/or NT) varied in terms of machinery and working depths (Table 1). All sites are located on flat areas without inclination, so that they could not be affected by water erosion and colluvial processes.

In Sweden, the long-term experimental site Säby is located near Uppsala (59°49′ N 17°42′ E) and was established 11 years before sampling in 2006, using a randomized block design with a plot size of 9 × 20 m and three replicates. The mean annual temperature (MAT) at this site is 6.7 °C with 547 mm mean annual precipitation (MAP) (mean of the years 1988–2017). The soil is an Eutric Cambisol [65] with a soil texture of 25% sand, 52% silt and 23% clay [66] and a pH-H_2O of 5.6. The crop rotation consisted of winter wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), and peas (*Pisum sativum* L.). Prior to sampling, winter wheat was sown in 2017 and 2016, and peas in 2015. Crop yields were 5.0 t ha^{-1}, 5.6 t ha^{-1}, and 4.2 t ha^{-1} in 2016, and 9.8 t ha^{-1}, 9.9 t ha^{-1}, and 9.0 t ha^{-1} in 2015 for CT, MT, and NT, respectively. The soil received mineral fertiliser depending on the cultivated crops, i.e., in total 139 kg N ha^{-1}, 82 kg N ha^{-1}, and 141 kg N ha^{-1} in the years 2017, 2016, and 2015, respectively.

In Germany, the long-term experimental site Garte Süd is located near Göttingen in southern Lower Saxony (51°29′ N 9°56′ E) and was established 47 years before sampling in 1970. The soil is a Haplic Luvisol [67] with 12% sand, 73% silt, and 15% clay [46] and a pH-H_2O of 7.2. Average temperature is 9.5 °C MAT with a precipitation of 621 mm MAP (average from 1989–2018). Plots with a size of 20 × 40 m are arranged in a randomized block design with four replicates. Crop rotations varied inconsistently and were mainly based on cereals. In the two years before sampling, winter wheat (*Triticum aestivum* L.) in 2016 and a mixture of peas (*Pisum sativum* L.) and oat (*Avena sativa* L.) in 2015 were grown on the site. Crop yields were 7.7and 7.4 t ha^{-1} in 2016 as well as 3.5 and 3.0 t ha^{-1} in 2015 for CT and MT, respectively. As for fertilisation, Garte Süd received no inorganic fertiliser in 2015, 188 kg N ha^{-1} in spring 2016, and 207 kg N ha^{-1} in spring 2017.

In Romania, the long-term experimental site Turda is located near Cluj-Napoca (46°35′ N, 23°48′ E) as a combined tillage and crop rotation experiment with a plot size of 30 × 12 m and three replicates. It was established 11 years before sampling in 2007. The MAT is 9.0 °C at this site with 540 mm MAP [68]. The soil is a Phaeozem with 16% sand, 28% silt and 56% clay as soil texture [69] and a pH-H_2O of 7.0. Crop rotations of both tillage systems were soy (*Glycine max* L.), winter wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.). Crop yields were 7.3 and 7.4 t ha^{-1} in 2016 and 3.2 and 3.3 t ha^{-1} in 2015 for CT and MT, respectively. The crops were fertilised with 40 kg N ha^{-1} and 40 kg P ha^{-1} as complex fertiliser in autumn, while 30 kg N ha^{-1} was added as NH_4NO_3 in spring.

In Spain, the field site is part of the experimental farm La Hampa in the southwest near Sevilla (37°17′ N 6°3′ W). The trial was set up 10 years before sampling in 2008 as a randomized block design with a plot size of 14 × 22 m and three replicates per treatment. The average temperature is 19.0 °C MAT with 497 mm MAP [70]. The soil is a Calcic Fluvisol with a pH-H_2O of 8.3. Soil texture is 58% sand, 18% silt and 24% clay [16]. The crop rotation contained cereals, sunflowers (*Helianthus annuus* L.) and legumes. The crops prior to sampling were winter durum wheat (*Triticum durum* L.) in 2017, broad bean (*Vicia faba* L.) in 2016 and again winter durum wheat in 2015. In 2016, crop yields were very low at 1.2 t ha^{-1} for CT and 0.1 t ha^{-1} for NT, due to extreme weather conditions, but in 2015 they were 4.6 t ha^{-1} for CT and 2.5 t ha^{-1} for NT. Wheat received a complex fertiliser at a rate of approximately 60 kg N ha^{-1}, 26 kg P ha^{-1}, and 50 kg K ha^{-1}. Sunflowers and legumes were not fertilised.

3.2. Sampling and Soil Chemical Analysis

Samples were collected from 29 to 30 May 2017 at Garte Süd, from 12 to 22 June 2017 at Säby, from 15 to 25 May 2018 at Turda and on 04 April 2018 at La Hampa from three soil depths (0–10, 10–20, 20–30 cm). In all countries and years, samples were taken under flowering of winter wheat with a soil corer (5 cm diameter and 30 cm length). Four soil samples were taken from each plot and combined for analysis. SOC and total N were analysed from dried, sieved (<2 mm) and ball milled soil samples using a Vario Max CN elemental analyser (Elementar, Hanau, Germany). HCl was added to the soils from Turda and La Hampa to remove inorganic C, for the other field sites total C corresponds to SOC. Soil pH was detected in deionized water with a soil to solution ratio of 1:2.5. BD was calculated by dividing the soil core volume by the soil weight determined after drying the soil at 105 °C.

3.3. Soil Biological Analysis

To determine MBC fumigation extraction [71] was used. Then, 10 g of field-moist soil were extracted pairwise, i.e., fumigated (24 h, ethanol-free CHCl3) and non-fumigated, with 40 mL of 0.05 K_2SO_4 [72]. Organic C in the extracts was measured using a multi N/C 2100S (Analytik Jena, Jena, Germany). MBC was calculated as EC/k_{EC}, where E_C = (organic C from fumigated soil sample)–(organic C from non-fumigated soil sample) and k_{EC} = 0.45 [73]. Basal respiration was determined by the MicroResp method [74]. In brief, the soil was adjusted to a water content of 15% and stored at 22 °C for 3 days before measurements; soil equivalent to 400 mg dry soil was placed in 1.1 mL deep-well microtiter plates and incubated for 6 h in a closed system. The system includes a detection microtiter plate with a colorimetric CO_2 trap containing 1% noble agar, 150 mM KCl, 2.5 mM $NaHCO_3$ and 12.5 μg g^{-1} cresol red. The colour change in the CO_2 trap was measured at the beginning (T0) and after 6 h of incubation (T6) at 570 nm with a microplate reader (BioTek, Winooski, USA). The difference in the absorption between T6 and T0 was converted into CO_2-C (μg g^{-1} soil h^{-1}). The metabolic quotient qCO_2 was calculated as mg CO_2-C g MBC^{-1} d^{-1}.

3.4. Calculations and Statistical Analyses

Data analyses were carried out using the statistical software R (version 3.6.1, R. Core Team, 2019). Stocks of SOC and MBC were calculated for equivalent soil masses to consider differences in BD [75]. All soil properties presented at 0–10, 10–20, and 20–30 cm as well as MBC and SOC stocks at 0–30 cm were analysed by linear mixed effect models using the package nlme (version 3.1-152, [76]). 'Tillage' was used as fixed factor, 'block' was considered as random factor. Analysis of variance was performed on the final models for each soil parameter. Residuals of the final model were checked for homoscedasticity. To examine significant differences between groups, a post-hoc test (Tukey test) was carried out, using the package lsmeans (version 2.30-0, [77]). All field sites were evaluated separately from each other. Results of the Romanian field site are presented in a descriptive way, because the experimental field site Turda was originally established as a combined crop and tillage experiment. Due to sampling only under one crop, randomization could not be secured as samples were only taken from winter wheat. Values in the text are given as mean ± standard deviation.

4. Conclusions

Comparing different practises of tillage reductions, our study showed that it is quite important to distinguish between different expressions of conservation tillage. Further, in order to give recommendations for tillage applications, the region, including climate and soil characteristics, has to be considered in each case. Therefore, direct comparison of different sites is limited, and environmental conditions must be considered when evaluating the effectiveness of conservation tillage systems. Especially in regions where agronomical disadvantages of NT could occur, other reduced tillage systems might be preferred without putting soil quality (as indicated by microbial properties) at risk. Therefore, the choice of machinery should also be based on other factors such as fuel consumption or harvest yields. However, our study showed that ploughless tillage systems are recommended as MT and NT resulted in an MBC, C, and N accumulation near the surface independent from site-specific conditions and appeared to have the potential to enhance microbial indicators as well as C stocks in most cases.

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Chapter 5

Study 2

The role of Collembola for litter decomposition under minimum and conventional tillage

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RESEARCH ARTICLE

The role of Collembola for litter decomposition under minimum and conventional tillage

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Abstract

Background: The role of soil mesofauna in decomposition processes still is debated and this applies in particular to arable systems.

Aim: This study investigates the role of Collembola in decomposition processes of crop residues in two different tillage systems.

Methods: We conducted a litterbag experiment in a long-term field site in Germany managed by conventional tillage (CT; mouldboard ploughing) and minimum tillage (MT). Litterbags filled with maize leaf litter of two mesh sizes (2 mm and 48 μm) were used. Litterbags were buried at 23 cm (CT) and 5–8 cm (MT), and retrieved after 2, 5 and 7 months. Litter mass, concentrations of carbon and nitrogen, litter C/N ratio as well as the abundance and community structure of Collembola and the incorporation of maize-derived carbon into Collembola were investigated.

Results: Mesofauna enhanced the loss of litter carbon, while litter mass loss was reduced. Litter C/N ratio in MT was generally lower than that in CT and decreased faster in litterbags with coarse mesh size. Abundance of Collembola in litterbags in CT exceeded that in MT, but species composition remained unaffected by tillage. Overall, Collembola effectively colonised the litter irrespective of tillage system, but benefited in particular from translocation deeper into the soil by conventional tillage.

Conclusions: Mesofauna accelerates litter carbon loss and increases litter nitrogen accumulation irrespective of tillage system. This may reduce nitrogen losses due to leaching in winter and facilitate nitrogen capture from decomposing litter material by crops in the following season, thereby contributing to the sustainable management of arable systems.

KEYWORDS
crop residues, litter breakdown, mesofauna, mouldboard plough, reduced tillage

1 | INTRODUCTION

Litter decomposition is based on the activity of soil microorganisms and soil fauna. Although soil fauna generally accelerates litter decomposition, there are still major knowledge gaps on the role of soil mesofauna in decomposition processes (Kampichler & Bruckner, 2009). Soil mesofauna mainly consists of Acari and Collembola, which typically account for 95% of the mesofauna individuals and are thought to play an important role in litter decomposition (Kampichler & Bruckner, 2009; Seastedt, 1984). Detritivore soil animals affect litter decomposition by fragmentation, digestion and regulation of bacterial and fungal populations (Giller, 1996; Hättenschwiler et al., 2005).

Litterbags of different mesh size have been employed intensively to investigate the impact of the soil mesofauna on decomposition processes (Kampichler & Bruckner, 2009). Although access by mesofauna typically accelerates litter decomposition processes (Yang et al., 2018), it may also reduce decomposition rates via overgrazing of fungal populations (Vreeken-Buijs & Brussaard, 1996). Generally, effects of soil mesofauna on litter decomposition vary with the quality of the litter material and the type of ecosystems (Bokhorst & Wardle, 2013; Gergócs & Hufnagel, 2016).

Decomposition of plant residues is essential for the cycling of elements and the provisioning of nutrients to plants (Casado-Murillo & Abril, 2013), and therefore for the sustainable management of agricultural systems (Hättenschwiler et al., 2005; Lavelle et al., 1993; Prescott, 2005). Litter breakdown depends on physical and chemical characteristics of the environment, litter nutrient concentrations and structure and the decomposer community (Couteaux et al., 1995; Knacker et al., 2003). The role of decomposers for litter decomposition depends on their community structure and activity as well as on interactions among different groups of decomposers, in particular between decomposer animals and microorganisms (Prescott, 2010; Zhang et al., 2008). Early stages of litter decomposition are dominated by saprotrophic microorganisms and characterised by a decrease in the litter C/N ratio resulting in higher quality of litter resources for animal consumers (Hättenschwiler et al., 2005; Martínez-García et al., 2021; Seastedt, 1984) and contributing to the release of nitrogen from litter materials at later stages of litter decay (Martínez-García et al., 2021).

Contact of crop residues to the surrounding soil enhances their accessibility to microorganisms and soil fauna (Faust et al., 2019; Potthoff et al., 2008). However, soft-bodied soil detritivore animals incapable of burrowing such as Collembola may be unable to access the incorporated litter material representing an important food resource (Dittmer & Schrader, 2000; Stinner et al. 1988). In addition, the mechanical disturbance by tillage and the displacement of surface living animals to deeper soil layers is a main threat to soil animal biodiversity adding to chemical and drought stress (van Capelle et al., 2012). To reduce the mechanical disturbance due to ploughing, reduced tillage practices are increasingly adopted (Vignozzi et al., 2019). In addition to reducing mechanical disturbance, reduced tillage facilitates the accessibility of plant residues to detritivore soil animals, and reduces soil surface runoff and erosion (McLaughlin & Mineau, 1995; Rodgers et al., 2018). Switching to minimum tillage practices therefore is expected to beneficially affect soil fauna and thereby crop residue decomposition. However, evidence from previous studies is controversial as not all taxa may benefit from reduced tillage. For instance, the review of van Capelle et al. (2012) stressed that the abundance of Collembola in no-tillage systems might be lower than in conventional tillage.

Collembola as major detritivore mesofauna in soil significantly affect litter decomposition, nutrient cycling and plant growth (Filser, 2002; Neher & Barbercheck, 2019; Seastedt, 1984). Reaching high density also in arable systems, they play a key role for the recycling of plant residues (House & Stinner, 1987; Ke et al., 2005). However, little is known on how tillage practices modify the role of Collembola and mesofauna in general in the decomposition of plant residues (House & Stinner, 1987; Kladivko, 2001; Reddy et al., 1994).

Stable isotope analysis in combination with stable isotope mixing models have proven to be valuable tools for investigating trophic relationships (Boecklen et al., 2011; Scheu & Falca, 2000). Since the $^{13}C/^{12}C$ ratio of consumers changes little compared to their diet, it reflects the basal food resources consumed. By contrast, the $^{15}N/^{14}N$ ratio increases in a consistent way per trophic level by 2–4 δ units, and therefore reflects the trophic position of consumers (Layman et al., 2012; Potapov et al., 2019). By using plant resources of different ^{13}C signatures, the proportion of these food resources in the diet of consumers can be determined (Scheunemann et al., 2015). Since the ^{13}C signatures of C4 and C3 plants differ markedly, C4 plant litter material placed in an agricultural field planted with C3 plants can be used to quantify the incorporation of litter-derived C into detritivores (Albers et al., 2006; Layman et al., 2012; Scheunemann et al., 2015).

The aim of this study was to investigate the importance of Collembola for litter decomposition by using stable isotope analysis to quantify the proportion of C derived from maize litter in the diet of Collembola. We chose a litterbag experiment in order to assess the influence of tillage reduction applied in a long-term field experiment running for more than 40 years on litter decomposition and the use of crop residues as food resource for soil mesofauna using Collembola as model group. We used two different mesh sizes to exclude part of the soil food web; small mesh size (48 μm) only allowed access of the litter by microorganisms, whereas coarse mesh size (2 mm) also allowed access by mesofauna.

We hypothesised that (1) access of litter by soil mesofauna increases litter decomposition, (2) reduced tillage accelerates litter decomposition, (3) Collembola are more abundant and more diverse in litterbags in minimum tillage fields, (4) Collembola species composition differs between tillage treatments and changes during litter decomposition and (5) carbon stable isotope signatures differ between tillage treatments reflecting differential use of crop residues in conventional and minimum tillage fields.

2 | MATERIALS AND METHODS

2.1 | Field site

The experiment was conducted in October 2017 at an experimental field site in Lower Saxony, Germany, close to the city of Göttingen (51°29 N 9°56 E). The soil type of the field site (Garte Süd) is Haplic Luvisol (Ehlers et al., 2000) with a pH of 7.2. The soil texture is 12% sand, 73% silt and 15% clay. Mean annual temperature is 9.5°C with a mean annual precipitation of 621 mm (Climate Data Center, 2018). In 1970, two treatments were established: (1) conventional tillage (CT) by mouldboard ploughing to a depth of 25 cm followed by seed bed preparation and shallow cultivation using a rotary harrow, and (2) minimum tillage (MT) to 5–8 cm soil depth using a rotary harrow. The crops grown varied, but were mainly based on cereals. During the experiment, however, winter oilseed rape (*Brassica napus* L.) was grown. The field received 207 kg mineral N ha^{-1} in spring 2017. Data of soil temperature were downloaded from the Climate Data Center (2018), using the hourly measurements in 10 and 20 cm, corresponding to 5–8 cm soil depth for MT and 23 cm soil depth for CT (the depths the litterbags were placed, see below). Data were obtained from the station 'Wetterwarte Göttingen' (51°30 N, 9°57 E), which is approximately 2 km away from to the field site (see Figure S1). Soil pH was determined with a soil to solution ratio of 1:2.5 in deionised water. Values were averaged among soil depths and treatments.

2.2 | Analysis of litter material

Nylon litterbags measuring 10 × 15 cm of two mesh sizes (48 μm and 2 mm) were buried. The larger mesh size allows mesofauna such as Collembola to access the litterbags, whereas the small mesh size only allows access by microorganisms and microfauna (protozoa and nematodes) (Powers et al., 2009; Swift et al., 1979). Litterbags were filled with 5 g of dried maize (*Zea mays* L.) leave litter material with a C concentration of 42% and 1.7% N, having a C/N ratio of 42.23 ± 5.38 (mean ± standard deviation). Prior to placement into the litterbags, the maize litter was fragmented into pieces of about 5 cm. Litterbags were buried approximately at the working depth of the machinery to simulate litter dispersal by the respective tillage treatment, that is at 10 cm in MT and at 20 cm in CT. Litterbags were placed in the field on 18 October 2017, and retrieved on 12 December 2017, 15 March 2018 and 15 May 2018, to follow temporal changes in litter decomposition. After harvesting, soil adhering to the litterbags was removed, the bags were opened and the litter was taken out. Litter from half of the litterbags was dried at 60°C for 24 h, weighed, ground in a ball mill and total C and total N were determined using an elemental analyser (Vario Max; Elementar, Hanau, Germany). The amount of C and N remaining was evaluated following the calculations of Jacobs et al. (2011). To correct for the contamination of litter by C and N derived from soil particles, the aluminium content was used assuming that it did not change during the experiment following the methodology of Potthoff and Loftfield (1998). Aluminium concentrations were measured by inductively coupled plasma–optical emission spectrometer (ICP–OES Optima 8000; Perkin Elmer, Bremen, Germany).

2.3 | Extraction of mesofauna

From the second half of the litterbags retrieved on 12 December 2017 and 15 May 2018, Collembola were extracted using high gradient heat extraction (Macfadyen, 1961) and collected in 1:1 diethylene glycol–water solution. For storage until determination, animals were transferred into 70% ethanol. Collembola species were identified using the keys of Hopkin (2007) and Fjellberg (1998, 2007). In addition, each Collembola species was assigned to one of the following ecological groups: epedaphic, hemiedaphic and euedaphic. The classification was based on traits including length of furca, coloration, number of ocelli, length of antenna and the presence of scales and hairs. The combination of these traits represents the adaptation of the species to live above or below ground. Each trait received a score between 0 and 4, with low scores for traits well adapted to above-ground live and high scores for traits well adapted to below-ground live (see Table S1). For each species, the trait scores were added up resulting in species scores between 0 and 18. The species receiving scores between 0 and 6 were classified as epedaphic, those with scores between 7 and 12 as hemiedaphic and those with scores between 13 and 18 as euedaphic (see Table S2).

2.4 | Stable isotope and soil analyses

Stable isotope analysis of Collembola, plant material and soil was conducted at the Centre for Stable Isotope Research and Analysis Göttingen (KOSI) using a combination of an elemental analyser (NA 1110; CA-Instruments, Milano, Italy) and an isotope mass spectrometer (Delta Plus; Finnigan MAT, Bremen, Germany; Reineking et al., 1993). Stable isotope ratios were analysed for each Collembola species separately using a set-up modified for measuring small sample sizes (Langel & Dyckmans, 2014). To reach the required amount of tissue, Collembola individuals from each litterbag were pooled. Individual Collembola and milled plant and soil material were placed in tin capsules, dried at 60°C for 24 h and weighed.

Stable isotope abundance (δX) was expressed using the δ notation with:

$$\delta X\,(\text{‰}) = \left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}} \times 1000, \qquad (1)$$

with X represents the target isotope (^{13}C or ^{15}N) and R_{sample} and R_{standard} represent the respective target isotope ratio (^{13}C/^{12}C or ^{15}N/^{14}N). Atmospheric nitrogen was used as standard for ^{15}N and Vienna Peedee Belemnite limestone as standard for ^{13}C. For internal calibration, acetanilide (C_8H_9NO, Merck, Darmstadt) was used.

The relative contributions of *Zea mays* and *Brassica napus* to the diet of Collembola were calculated using the Bayesian mixing model FRUITS version 2.1.1 Beta (Fernandes et al., 2014), taking into account the carbon signature of *Zea mays* leaves in the litterbags and *Brassica napus* plants on the experimental field site as possible food sources. The fractionation factor for carbon including the standard deviation was set to 2.0‰ ± 0.5‰ and was based on the average difference in δ^{13}C values of Collembola (–27.0‰ ± 0.9‰; mean ± SD) and soil organic matter (–25.3 ± 3.1) at our study site ('detrital shift'; Potapov et al., 2019).

2.5 | Statistical analysis

Statistical analyses were done using R (R Core Team, 2020). Decomposition (amount of litter mass, C and N remaining and C/N ratio) as well as δ^{13}C signatures of Collembola and proportion of C of *Zea mays* in Collembola tissue were analysed by linear mixed-effects models using the lme function of the nlme package (Pinheiro et al., 2020). For the analysis of decomposition data, 'Tillage' (CT and MT) and 'Mesh size'

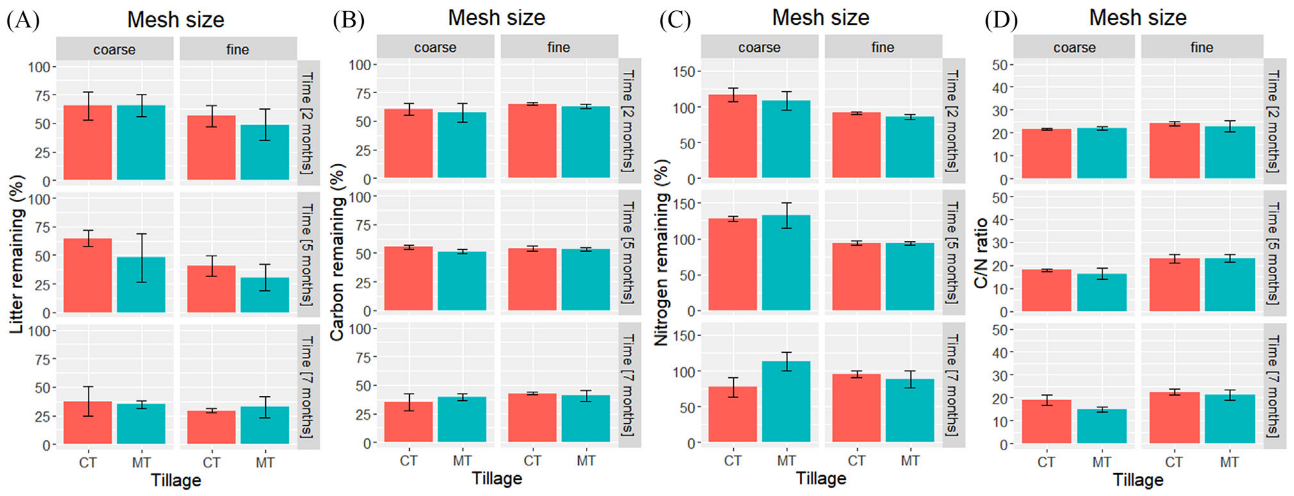

FIGURE 1 Amount of (A) litter remaining (%), (B) carbon remaining (%), (C) nitrogen remaining (%) and (D) C/N ratio of litter exposed in litterbags in conventional tillage (CT) and minimum tillage (MT) fields for 2, 5 and 7 months; means ± standard deviation; for statistical analysis, see Table 1.

(coarse and fine) were included as factors. 'Time' (first sampling, second sampling and third sampling) was included as ordered factor to allow inspecting for linear and quadratic changes in decomposition with time. Abundance and species richness of Collembola were analysed using generalised liner mixed-effects models using the *glmer.nb* and *glmer* function of the *lme4* package (Bates et al., 2015). For the analysis of Collembola abundance data, 'Tillage' and 'Time' (first sampling, third sampling) were included as factors. Non-independence of litterbags retrieved from the same plot was taken into account by using plotID as random factor in linear as well as generalised linear mixed-effects models. Statistical models calculated with the *lme*, *glmer* or *glmer.nb* function were chosen by stepwise exclusion of non-significant factors after starting with the full model including all interactions. The *DHARMa* package (Hartig, 2020) was used to check model quality for overdispersion, model conversion, outliers and zero inflation.

Non-metric multidimensional scaling using Bray–Curtis dissimilarity was used to analyse Collembola community composition (metaMDS, *vegan* package) (Oksanen et al., 2019). Differences in community composition between treatments and sampling time were identified using multivariate analysis of variance (manova, *stats* package). Linear discriminant analysis (lda, *MASS* package) (Venables & Ripley, 2002) followed by Hotelling's T^2-test (HotellingsT^2, *ICSNP* package) (Nordhausen et al., 2018) was used for two-dimensional graphical depiction of communities using the first two dimensions of the NMDS, and for pairwise comparisons of treatments and sampling times.

3 | RESULTS

3.1 | Litter decomposition

Litter mass remaining in the litterbags varied significantly with Time (linear term only) and Mesh size, but not with Tillage (Table 1, Figure 1A). On average, litter mass remaining decreased in a linear way from 65.37% ± 9.54% (mean ± SD) at the first sampling to 36.14% ± 8.29% at the last sampling in the coarse mesh size litterbags. Respective values for the fine mesh size litterbags were 52.52% ± 10.90% and 30.96% ± 5.95%. The amount of litter remaining was generally lower in litterbags with fine than in those with coarse mesh size (overall means of 41.74% ± 13.90% and 50.76% ± 17.13%) regardless of Tillage and Time.

Similar to litter mass remaining, changes in litter C remaining significantly varied with Time and Mesh size, but not with Tillage (Table 1, Figure 1B). However, the amount of C remaining was higher in litterbags with fine than in those with coarse mesh size (overall means of 52.79% ± 11.42% and 48.10% ± 12.24%, respectively). Litter C remaining decreased from 58.91% ± 6.02% (mean ± SD) at the first sampling to 37.28% ± 5.42% at the last sampling in coarse mesh size litterbags. Respective values for the fine mesh size litterbags were 63.91% ± 17.86% and 41.66% ± 3.21%.

Litter N remaining varied significantly with Time, Mesh size and Tillage with a significant three factor interaction between Time, Mesh size and Tillage (linear term) (Table 1, Figure 1C). The amount of N remaining in fine mesh size litterbags remained relatively constant regardless of tillage treatment increasing slightly from 88.60% ± 3.92% (mean ± SD) at the first sampling to 91.49% ± 8.75% at the last sampling, but increased in the coarse mesh size litterbags from 116.52% ± 8.31% and 108.43% ± 11.47% (for CT and MT treatments, respectively) at the first sampling to 128.17% ± 2.86% and 132.61% ± 15.77% at the second sampling before decreasing at the last sampling. This final decrease in N remaining was stronger in the CT (77.15% ± 11.80%) than in the MT treatment (112.69% ± 11.19%) (significant Tillage × Time × Mesh size interaction; Table 1).

The C/N ratio of litter material varied significantly with Time, Mesh size and Tillage, with the effect of Time (both linear and quadratic term) varying with Mesh size (Table 1, Figure 1D). The C/N ratio decreased in fine mesh size litterbags with time regardless of tillage treatment from 23.46 ± 1.67 (mean ± SD) at the first sampling to 21.81 ± 1.79

TABLE 1 Linear mixed-effects model table of *t*- and *p*-values on the effect of Tillage (Till; conventional tillage, minimum tillage), Mesh size (Mesh; fine: 45 μm, coarse: 2 mm) and Time (first sampling, second sampling, third sampling) on mass, amount of carbon (C), amount of nitrogen (N) and C/N ratio of maize litter exposed in litterbags in the field for 5 months. Time was fitted as linear (L) and quadratic term (Q); df = degrees of freedom; Int = Intercept; non-significant effects were excluded in a stepwise procedure to obtain reduced models with only significant effects (see Section 2); the Tillage × Mesh size × quadratic term was generally not significant

		Int	Till	Mesh	L	Q	Till × L	Mesh × L	Mesh × Q	Till × Mesh × L
Litter mass	df	41		41	41					
	t-value	15.63		–4.19	–6.74					
	p-value	<0.01		<0.01	<0.01					
Litter C remaining	df	41		41	41	41				
	t-value	58.92		2.71	–14.98	–2.34				
	p-value	<0.01		<0.01	<0.01	0.02				
Litter N remaining	df	33	33	33	33	33	33	33	33	33
	t-value	37.76	2.64	–3.40	–5.66	–5.20	–2.71	4.33	3.59	–3.23
	p-value	<0.01	0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
Litter C/N ratio	Df	38	38	38	38	38		38	38	
	t-value	44.32	–2.51	8.17	–5.66	3.04		2.66	–2.43	
	p-value	<0.01	0.02	<0.01	<0.01	<0.01		0.01	0.02	

at the last sampling. In coarse mesh size litterbags, the C/N ratio in the MT treatment also decreased with time, but the decrease from the first (22.08 ± 0.70) to the last sampling (14.84 ± 0.89) was much more pronounced. Compared to fine mesh size litterbags, the decrease in C/N ratio in coarse mesh size litterbags in the CT treatment from the first (21.63 ± 0.20) to the last sampling (19.03 ± 1.83) also was more pronounced, but the decline was less steep than in the MT treatment. Overall, the linear decrease in C/N ratios with time was stronger in coarse than in fine mesh size litterbags (significant Mesh size × Time interaction; Table 1). In addition, the C/N ratio was generally lower in coarse than in fine mesh size litterbags and lower in the MT than in the CT treatments.

TABLE 2 Generalised linear mixed-effects model table of *Z*- and *p*-values on the effect of Tillage (Till; conventional tillage, minimum tillage) and Time (first sampling, third sampling) on total abundance, abundances of different ecological groups and species richness of Collembola; df = degrees of freedom; Int = intercept; non-significant effects were excluded in a stepwise procedure to obtain reduced models with only significant effects (see Section 2); the Tillage × Time interaction was generally not significant

		Int	Till	Time
Total abundance	df	11	11	11
	Z-value	8.46	–3.48	2.77
	p-value	<0.01	<0.01	<0.01
Epedaphic	df	11	11	11
	Z-value	–2.09	2.39	2.38
	p-value	0.04	0.02	0.02
Hemiedaphic	df	12		12
	Z-value	1.27		3.78
	p-value	0.2		<0.01
Euedaphic	df	11	11	11
	Z-value	8.82	–5.27	1.73
	p-value	<0.01	<0.01	0.08
Species richness	df	12		12
	Z-value	7.7		2.84
	p-value	<0.01		<0.01

3.2 | Abundance and community composition of Collembola

Total abundance of Collembola significantly increased with time from 21.38 ± 24.53 individuals g^{-1} litter (mean ± SD) at the first sampling to 77.13 ± 82.69 individuals g^{-1} litter at the last sampling independent of tillage treatment (Table 2). Overall, total abundance of Collembola was considerably higher in CT than in MT treatments (82.75 ± 79.68 and 15.75 ± 20.01 individuals g^{-1} litter, respectively; Figure 2).

Likewise, epedaphic Collembola increased with time from 0.63 ± 0.70 individuals g^{-1} litter (mean ± SD) at the first to 2.13 ± 2.26 individuals g^{-1} litter at the last sampling, but they reacted differently to tillage practices with the abundances in CT being lower than in MT treatments (0.63 ± 1.32 and 2.13 ± 1.96 individuals g^{-1} litter, respectively; Table 2, Figure 2). Hemiedaphic Collembola, by contrast, were not affected by tillage treatments but followed the general pattern of increasing abundance with time from 1.88 ± 1.27 individuals g^{-1} litter at the first to 22.25 ± 24.31 individuals g^{-1} litter at the last sampling. Euedaphic Collembola followed the same pattern as total Collembola with their abundance increasing from 19.38 ± 23.87 individuals

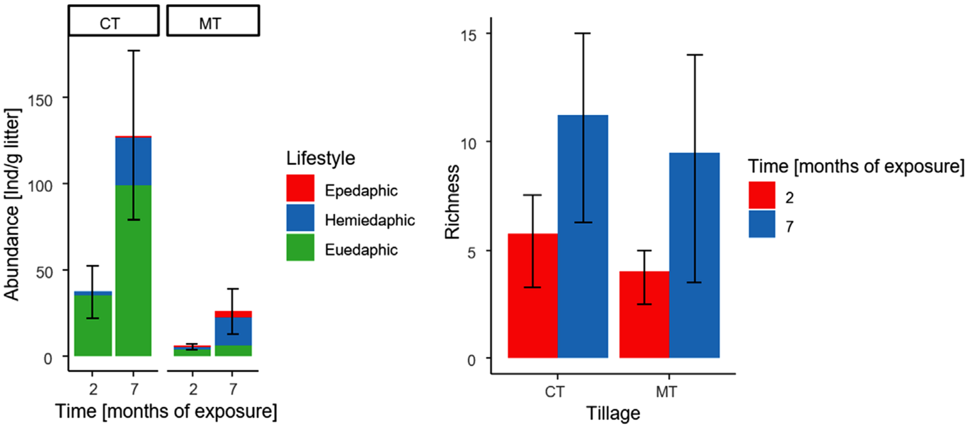

FIGURE 2 Abundance (A) and species richness of Collembola (B) in conventional tillage (CT) and minimum tillage (MT) fields after 2 and 7 months of exposure; means ± standard deviation

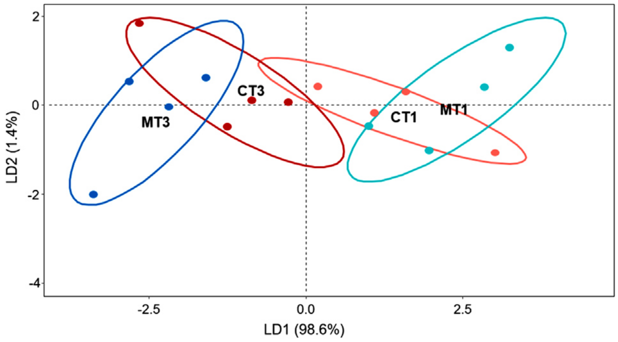

FIGURE 3 Collembola community composition in litterbags with maize (*Zea mays*) litter exposed in conventional tillage (CT) and minimum tillage (MT) fields for 2 (CT1 and MT1) and 7 months (CT3 and MT3). LDA plot based on NMDS scores of Collembola species composition grouped by tillage treatments and sampling dates. LD1 accounted for 98.6% and LD2 for 1.4% of the total variance.

g^{-1} litter at the first to 52.63 ± 67.37 individuals g^{-1} litter at the last sampling, and being more abundant in CT than MT treatments (67.13 ± 60.93 and 4.88 ± 3.55 individuals g^{-1} litter, respectively; Figure 2).

In contrast to total abundance, Collembola species richness did not differ significantly between tillage treatments, but overall, it increased from 4.88 ± 2.03 (mean ± SD) at the first to 10.38 ± 4.90 at the last sampling (Table 2; Figure 2). Manova based on NMDS scores (k = 2, stress = 0.095, R^2 = 0.99) indicated that the community composition of Collembola significantly differed between sampling dates ($F_{2,13}$ = 22.97, $p < 0.01$), but not between tillage treatments ($F_{2,13}$ = 0.11, $p = 0.89$). Pairwise comparisons of species composition at different sampling dates within the tillage treatments revealed that species composition in both tillage treatments changed with time ($F_{2,5}$ = 9.84, $p = 0.02$ for conventional tillage and $F_{2,5}$ = 51.77, $p < 0.01$ for minimum tillage; Figure 3).

3.3 | Incorporation of maize carbon by Collembola

On average, the δ^{13}C signature of Collembola decreased significantly from the first (−18.90‰ ± 3.08‰; mean ± SD; Figure 4) to the third sampling (−22.65‰ ± 3.19‰) regardless of tillage treatment (t-value = −5.08, df = 64, $p < 0.01$). In addition, the average δ^{13}C signature of Collembola was generally lower in MT (−22.75‰ ± 3.28‰) than in CT treatments (−20.38‰ ± 3.45‰) regardless of the time of exposure (t-value = −3.28, df = 64, $p < 0.01$).

Based on our mixing models, the proportion of maize carbon in Collembola tissue varied strongly and did not differ significantly between tillage treatments (t-value = −1.28, df = 18, $p = 0.22$). Overall, the average proportion of maize carbon in Collembola tissue was 41% ± 21% (mean ± SD). However, the proportion of maize carbon decreased significantly between sampling dates from 55% ± 19% at the first to 34% ± 18% at the third sampling (t-value = −3.74, df = 18, $p < 0.01$).

4 | DISCUSSION

Contradicting our first hypothesis that decomposition is faster with mesofauna access, the mass of litter remaining was higher in litterbags allowing access by mesofauna. By contrast, however, decomposition, as measured by the amount of litter C remaining, was lower in the fauna exclusion litterbags compared to litterbags allowing access by mesofauna. Initial losses of C were low during winter (December to March) and increased in spring (March to May) reflecting reduced activity of soil microorganisms and fauna at low soil temperature (see Figure S1). Irrespective of season, however, the effect of mesofauna on litter C loss was generally small, and, although being somewhat larger in the first than the second half of the experiment, the interaction between Mesh size and Time was not significant. The contradicting results of the effect of soil mesofauna on litter mass and litter C remaining presumably were due to the transport of soil and/or faecal material into the litterbags, which might not have been fully captured by the method

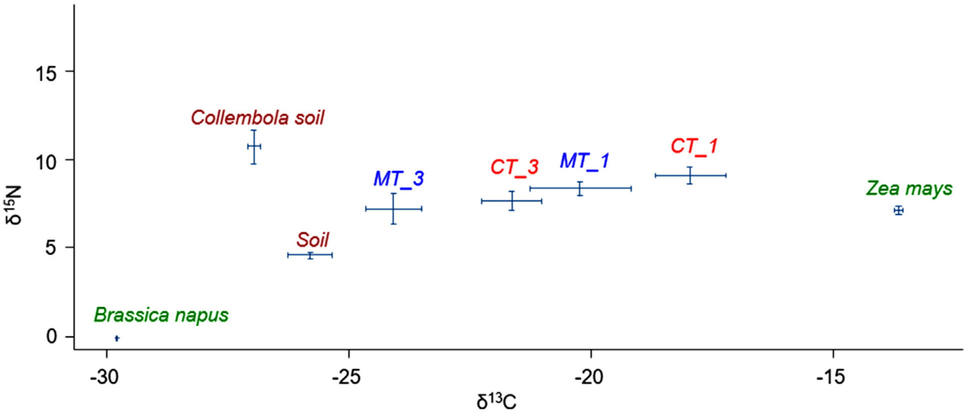

FIGURE 4 δ^{13}C and δ^{15}N values (mean ± SD) of Collembola in litterbags with maize (*Zea mays*) litter exposed in conventional tillage (CT) and minimum tillage (MT) fields cultivated with rape (*Brassica napus*) after 2 (CT_1 and MT_1) and 7 months (CT_3 and MT_3); δ^{13}C and δ^{15}N values of rape and maize, the surrounding soil (Soil) and Collembola outside of the litterbags (Collembola soil) are given as control.

used for correcting mass loss data (see Section 2). In fact, the amount of N in the coarse mesh size litterbags increased continuously during the experiment, whereas it decreased in the fine mesh size litterbags. Further, as indicated by higher litter C/N ratio in the fauna exclusion litterbags and the faster decrease in the C/N ratio with time in the coarse mesh size litterbags, mesofauna contributed to the transport of N into the litterbags, presumably by depositing faecal material rich in N and/or by facilitating N transfer by fungi into the litterbags (Lummer et al., 2012). Increased transfer of N into litterbags by soil fauna has also been observed by Jacobs et al. (2011) for wheat straw. Interestingly, the increase in the amount of N in the litterbags by mesofauna and the resulting lower C/N ratio later in the experiment, on one side, may contribute to preventing N losses due to leaching in winter, and on the other may facilitate nutrient mobilisation in spring and summer from decomposition of crop residues of the previous year. Generally, the decrease in the litter C/N ratio reflects an increase in litter quality during decomposition (García-Palacios et al., 2013; Hättenschwiler et al., 2005), suggesting that mesofauna facilitates nutrient mobilisation from decomposing litter material at later stages of decay, that is spring and summer, favouring nutrient capture by plants and thereby crop production. Although mesofauna may accelerate litter decomposition (García-Palacios et al., 2013; Seastedt, 1984) and decrease litter C/N ratio by increasing litter N concentration (Frouz et al., 2015), the direct effect of detritivore mesofauna on litter decomposition may generally be low (Hättenschwiler et al., 2005; Lavelle et al., 1993; Parker et al., 1984). Rather, detritivore soil animals may indirectly stimulate microbial litter decay, for example by increasing the supply of N (Lavelle et al., 1993; Seastedt, 1984), and this is supported by results of our study. Further, soil mesofauna may stimulate microbial activity by grazing, which may contribute to increased mobilisation of nutrients in litter, in particular at later stages of decay (Hättenschwiler et al., 2005; Lavelle et al., 1993; Parker et al., 1984; Seastedt, 1984). Interestingly, our results oppose one of the main criticisms of litterbag studies, that is increased mechanical loss or active transport of litter out of the litterbags of larger mesh size (Kampichler & Bruckner, 2009; Seastedt, 1984). Rather, our results indicate that mesofauna actually transport materials rich in N into the litterbags, which likely comprise predominantly faecal material.

Contradicting our second hypothesis, litter decomposition, as indicated by both litter mass and amount of C remaining, was not faster in MT than in CT fields. Only the litter C/N ratio was lower in MT than in CT fields. This indicates that reduced tillage, although it may not speed up litter decomposition, is associated with faster conversion of litter into litter of higher quality. Presumably, this is due to favouring the colonisation of litter by fungi from surrounding soil, thereby transporting N into the litter resulting in lower litter C/N ratio (Berg & Staaf, 1981; Lummer et al., 2012). Besides increasing litter quality and facilitating nutrient capture by plants, increased nutrient availability is likely to speed up litter decomposition and this is likely to be more pronounced in MT fields (Lavelle et al., 1993; Parker et al., 1984; Prescott et al., 2010; Zhang et al., 2008).

Contradicting our third hypothesis, the abundance of Collembola in litterbags of CT fields exceeded that in litterbags of MT fields, and tillage treatments did not affect Collembola species richness. Higher abundance of Collembola in CT fields was mainly due to euedaphic species living deeper in soil. The abundance of epedaphic Collembola, by contrast, was higher in litterbags of MT than CT fields, but the abundance of epedaphic species was generally lower than that of euedaphic species. Total Collembola abundance and the abundance of each of the three ecological Collembola groups as well as Collembola species richness increased with time indicating the immigration of additional species into the litter later in the experiment and the build-up of populations at higher temperature in spring. This supports results of earlier studies suggesting that Collembola follow the spatial distribution of their food resources (Verhoef & Nagelkerke, 1977; Vignozzi et al., 2019), and underlines the importance of plant litter as a food resource and habitat for Collembola in agricultural fields. Soil moisture often functions as limiting factor for Collembola in soil (Berg et al., 1998; Jucevica & Melecis, 2006). Translocation of litter deep into the soil by ploughing where moisture conditions are more constant therefore may favour Collembola, but this likely varies between ecological groups of Collembola. Translocating litter deeper into the soil by tillage is likely to improve in particular resource supply for euedaphic species, whereas resource supply for epedaphic and hemiedaphic species is likely to decline (Krab et al., 2010). Our results therefore likely reflect resource shortage of Collembola deeper in soil, which is alleviated by the translocation of litter by tillage serving as additional food resource allowing euedaphic Collembola to reproduce and increase in abundance.

Our fourth hypothesis was confirmed in part as species composition did not differ between tillage treatments, but changed with sampling time. Notably, the changes with time were similar in both tillage treatments indicating that the decomposing litter material favoured similar Collembola species irrespective of tillage treatments. The similar changes in Collembola communities in both tillage treatments are remarkable as the litterbags were placed at different depths in CT and MT fields. This further highlights the dominating role of the stage of decomposition of litter as driving force for Collembola community composition irrespective of tillage treatment and placement of litter in the soil profile. The similar changes, however, hamper drawing general conclusions on the impact of tillage practices on Collembola species and community composition. The uniform changes in Collembola community composition with litter decomposition presumably reflect that early colonising species predominantly feed on the litter material itself, whereas species dominating at later stages of decomposition predominantly feed on bacteria and fungi colonising the litter. As indicated by the increase in abundance at later stages of decay, resource quality and supply for Collembola increases with litter decomposition and the colonisation of the litter by bacteria and fungi.

Conform to our fifth hypothesis, the incorporation of maize C into Collembola in CT fields exceeded that in MT fields, but due to high variability the differences were not significant. However, they suggest that Collembola in CT fields fed more intensively on maize litter, whereas in MT fields they also fed on resources outside the litterbags, thereby diluting the maize signal. Generally, irrespective of tillage treatments the proportion of maize C in Collembola tissue decreased later in the experiment. This decline in maize C in Collembola tissue indicates a switch to alternative resources at later stages of litter decomposition. As the quality of maize litter increased, as indicated by increased C/N ratio, this suggests that the availability of alternative food resources of high quality increased. Potentially, this was due to increased availability of root-derived resources later in the experiment. In fact, Scheunemann et al. (2015) showed that the importance of root-derived C in arable fields may outweigh the importance of litter-derived C for the nutrition of soil food webs. As roots of *Brassica napus* were hardly available at the first sampling, but were fully developed at the last sampling, the importance of root-derived C from *Brassica napus* likely increased. Presumably, this was associated by increased availability of bacteria and fungi serving as food resource for Collembola (Parker et al., 1984; Seastedt, 1984). Nevertheless, our results show the importance of litter material incorporated into the soil for Collembola nutrition, in particular at the end of the cropping season when the roots of the winter crop are not yet fully developed.

5 | CONCLUSIONS

Results of the present study highlight the dependency of Collembola on litter material incorporated into the soil by tillage in agricultural systems. Translocation of litter into deeper soil layers provided additional resources and habitable space for Collembola, especially for euedaphic species vulnerable to desiccation. Stable isotope ratios indicated that Collembola depend on the incorporated litter material to a higher degree in deeper soil layers where root-derived C is barely available. Further, the results showed that, although affecting litter decomposition only little, soil mesofauna stimulate the transport of N into litter thereby decreasing litter C/N ratio, which is likely to speed up nutrient turnover and contribute to the fertility and productivity of agricultural systems. Although litter C loss did not vary significantly between tillage systems, the amount of litter N and the litter C/N ratio indicated that reduced tillage increases the mobilisation of litter N suggesting that it favours nutrient uptake by the crop species in the following year and mesofauna contributes to this mobilisation. Overall, the incorporation of crop residues into the soil by both CT and MT fostered Collembola abundance and the mobilisation of litter N, and thereby may contribute to the sustainable management of agricultural systems.

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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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SUPPORTING INFORMATION

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Chapter 6

Study 3

Crop residue displacement by soil inversion: Annelid responses and their impact on carbon and nitrogen dynamics in a lab-based mesocosm study

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Crop residue displacement by soil inversion: Annelid responses and their impact on carbon and nitrogen dynamics in a lab-based mesocosm study

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ABSTRACT

In the context of sustainable agriculture, a deeper knowledge of the effects of soil management on soil annelids is needed, as they play an important role in many soil processes. In a laboratory mesocosm experiment, we compared the simulated effect of ploughing by inverting the top soil (crop residues at 15 cm depth) to a non-inversion treatment (crop residues on the soil surface) using the soil type Haplic Luvisol. We investigated the response of earthworms and enchytraeids and the consequences for microbial and chemical soil parameters. Four treatments with soil fauna were established by adding: (i) endogeic earthworms (*Octolasion cyaneum*), (ii) anecic earthworms (*Lumbricus terrestris*), (iii) a combination of two enchytraeid species (*Enchytraeus crypticus* and *Enchytraeus christenseni*) and (iv) having control columns (without annelids). Feeding behaviour of annelids was investigated using isotopic analysis (δ^{13}C, δ^{15}N), and chemical and microbial soil properties were measured. Carbon and nitrogen losses in the form of gas emissions (CO_2, N_2O) and leachate were recorded during the time of incubation.

We found no interactions of soil inversion and annelid addition on chemical and microbial soil properties; these properties were closely related to crop residue placement, indicated by the effect of soil inversion between 0 and 20 cm. Below 20 cm, this effect disappeared. Here, the presence of enchytraeids enhanced soil microbial properties, regardless of soil inversion. Stimulating microbial activity and increasing soil aeration seem to be the most important factors that increase CO_2-C emissions in the presence of anecic earthworms. N_2O-N emissions were consistently higher (+188%) in the inverted columns. Our results show that regardless of the placement of crop residues, anecic earthworms and enchytraeids fed more on crop residue derived carbon than endogeic earthworms, while endogeic earthworms appeared to avoid feeding at the soil surface. Moreover, it was found that the inversion and the annelid effects did not interact in an experimental setting where soil inversion is carried out in a form without detracting or impairing the annelids directly.

1. Introduction

Major knowledge gaps regarding earthworm behaviour, their biology, and their impact on soil function and ecosystem services persist (Blouin et al., 2013). Soil annelids (earthworms, enchytraeids) react sensitively to human impacts, e.g. agricultural management practices. Many studies have reported that conventional ploughing decreases abundance and biomass of anecic earthworms in the field (e.g. van Capelle et al., 2012; Briones and Schmidt, 2017). Anecic earthworms preferably feed at the soil surface (Bouché, 1977), and soil inversion due to ploughing relocates their food source belowground. In addition, ploughing destroys the vertical burrows of anecic earthworms and takes away their day time shelter (Briones and Schmidt, 2017). In contrast, biomass and abundance of endogeic earthworms increases in ploughed fields (Chan, 2001). In order to recommend soil management practices that support annelids and their functions, it is imperative to understand how these management practices affect annelids activity (e.g. feeding behaviour as a response to ploughing) and how microbial and chemical

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soil properties are altered.

Earthworms act like ‘engineers’ in the soil system (Lavelle et al., 1997; Jouquet et al., 2006; Blouin et al., 2013). They translocate soil particles and organic matter during feeding and egesting (Taylor et al., 2018), contribute to the transfer of carbon in the soil (Jégou et al., 1998), and boost the turnover of soil organic material by stimulating microbial activity (Hoang et al., 2017). Briefly, anecic earthworms are primary decomposers, which feed directly on partially decayed organic material, whereas endogeic earthworms are secondary decomposers and feed on soil and its organic matter (Curry and Schmidt, 2007). Seeber et al. (2006) showed that earthworms belonging to primary decomposers (*L. rubellus*) strongly increased litter breakdown, whereas secondary macro-decomposers (*O. lacteum*) had no or even negative effects on litter breakdown. To investigate feeding ecology and trophic relationships of annelids, analysing stable isotope ratios (C, N) offers a powerful instrument (Scheu and Falca, 2000; Schmidt et al., 2004; Curry and Schmidt, 2007).

Greenhouse gas emissions in agriculture are known to be influenced by soil fauna and soil disturbances. For instance, studies have shown an increase in CO_2 (Lubbers et al., 2013) and a decrease in N_2O (Kuiper et al., 2013) due to the presence of earthworms. Gorbunova et al. (2020) even report on suppressive effects of earthworms on the emission of CO_2. The impact of soil invertebrate fauna on soil gas emissions seems variable as three different experiments of Kuiper et al. (2013) demonstrate; soil fauna can suppress, increase or on a temporal scale delay and accelerate N_2O emissions.

Enchytraeids are an often neglected part of the soil mesofauna. Compared with earthworms, much less is known about enchytraeids even though they are widely distributed in agricultural systems (Vavoulidou et al., 2009; Severon et al., 2012) and react sensitively to changes in management practices (Pelosi and Römbke, 2016). Enchytraeids contribute to the comminution and mixing of litter and soil. Thus, they enhance the surface area for microbial colonization along with passively transporting microorganisms through the various soil depths (Scheu et al., 2005). Enchytraeids are mainly secondary decomposers (Gajda et al., 2017) and feed on partially degraded plant fragments. Additionally, they regulate microbial communities by grazing on bacteria, fungi and microalgae (Gajda et al., 2017). This strong interaction of enchytraeids with microorganisms in soil is able to induce both increasing microbial activity by stimulation and also reducing activity due to stronger reductions of the microbial biomass by feeding (van Vliet, 2000). Concerning the effects of enchytraeids on CO_2 and N_2O emissions from soil van Vliet et al. (2004) could show strong dependencies to soil type and litter placement. Furthermore, the results of John et al. (2019) indicate that enchytraeids can promote the immobilization of carbon.

The influence of annelids on microorganisms is not yet fully understood. Many studies have reported that annelids decrease microbial biomass (Butenschoen et al., 2007; Sandor and Schrader, 2012; Wachendorf et al., 2014) due to feeding and may as a result alter microbial community composition (Eck et al., 2015). Microorganisms are considerably involved in many processes of the carbon and nitrogen cycle. For instance, they contribute to the storage and loss of nutrients. Earthworm burrows are known to be microbial hotspots (Kuzyakov and Blagodatskaya, 2015) and can improve the efficiency of organic matter decomposition by microorganisms (Hoang et al., 2017). Microbial parameters like basal respiration, microbial biomass and fungal abundance are indicators that reflect the ability of the soil system to store and cycle organic carbon over time (Creamer et al., 2016).

The aim of this study was to investigate how soil inversion including different crop residue placements in the soil profile (buried vs. unburied) affects ecological and functional impacts of certain annelids known to be dominating the annelid fauna in agricultural soils. We chose three different organisms or organism groups to represent (1) anecic earthworms, (2) endogeic earthworms and (3) endogeic enchytraeids. We further aimed to assess if this has consequences for soil microbial and chemical properties. To investigate impacts on nutrient fluxes, we quantified gas emissions (CO_2 and N_2O) and leachate losses (C and N). Corn (a C4 plant) leaves were selected as crop residues to follow the feeding behaviour of annelids, which was tracked using isotopic shifts in $\delta^{13}C$. We expected ecological groups to differ based on the proportion of crop residue derived C in the annelids tissue. We hypothesised that incorporating crop residues into the soil will be beneficial for endogeic earthworms, whereas litter on the surface will be advantageous for anecic species. We assumed that endogeic earthworms and enchytraeids (secondary decomposers, feeding on organic material that is already decomposed and colonised by microorganisms), absorb less crop residue derived C than anecic earthworms (primary decomposer, feeding on fresh organic material). Overall, we expected combined effects of both factors (annelid addition and soil inversion) on the chemical and microbial properties of the soil.

2. Material and Methods

2.1. Sampling for soil mesocosms

After harvest of winter wheat in August 2017 we sampled undisturbed soil columns for the mesocosm experiment at the study site “Garte Süd” (5°29′ N, 9°56′ E), which is located in the south of Göttingen, Lower Saxony, Germany (Jacobs et al., 2009). Referring to the complete randomized block design of “Garte Süd” we took 32 undisturbed soil columns from the minimum tilled plots (8 columns per block).

Plexiglass cylinders (15 cm diameter, 30 cm length, 0.018 m^2 surface area) were inserted into the soil and then carefully extracted using a spade. For annelid-defaunation, the columns were frozen for one week at −20 °C. Defaunation of the soil columns was successful. This was verified at the Institute of Applied Soil Biology, Hamburg, Germany by extracting enchytraeids from 12 randomized sub-samples of four columns from the three depths (0–10 cm, 10–20 cm, 20–30 cm) using a wet-funnel technique without heating (ISO 23611-3, 2007).

The soil type in our columns was a Haplic Luvisol (Ehlers et al., 2000; Reiter et al., 2002) derived from loess with a pH of 7.2. It consists of 15.1% clay, 72.7% silt and 12.2% sand (Ehlers et al., 2000). Average annual precipitation and temperature at the sample location was 649 mm and 9.5 °C, respectively (Climate Data Center (CDC), 2018). Prior to column sampling, the soil was fertilized at a rate of 20.7 kg ha^{-1} mineral N in spring 2017 and winter wheat (2017 and 2016) and a mixture of peas and oat (2015) was grown.

2.2. Experimental design

The two factors soil inversion and annelid treatment were investigated under laboratory conditions in a randomized block ($n = 4$) experiment. The factor ‘soil inversion’ consisted of two levels: (i) Soil inversion with crop residues buried at 15 cm depth (SI) and (ii) non-inversion with crop residues at the soil surface (NI). For SI, the upper soil layer (0–15 cm) was inverted manually without destroying its natural structure by turning the layer over and replacing it back in the soil column. For NI, the undisturbed soil columns were left in their natural state and crop residues were placed on the soil surface. However, one has to keep in mind that mechanical or manual tillage in an arable field always leads to stochastic destruction and inversion.

In both, the SI and the NI treatment, 5 g of corn (*Zea mays* L.) leaves with a C/N ratio (mean ± standard deviation) of 42 ± 5 (43 ± 0.2% C, 1 ± 0.1% N) were used as crop residues. Leaves were collected in September 2017 from a field-site in the South of Germany, dried for 24 h at 60 °C and cut into pieces of 2–4 cm. The amount of corn leaves added to each soil column corresponded to ~2.8 Mg ha^{-1} on a field level. Compared to other earthworm mesocosm studies, such as Frazão et al. (2019) (2.2 Mg ha^{-1} winter wheat stubble + straw, radish residues) and Giannopoulos et al. (2010) (~2.6 Mg ha^{-1} radish residues) the amount of crop residues was in a similar range.

The factor ‘annelid treatment’ consisted of four levels: Addition of (i) anecic earthworms (EW_{anecic}), (ii) addition of endogeic earthworms ($EW_{endo.}$), (iii) addition of a combination of two enchytraeid species (ENCH), and (iv) a control (CON) without any addition of worms to the SI and NI mesocosms. Adult individuals of earthworms from two different species (*Lumbricus terrestris*, *Octolasion cyaneum*) were collected five days prior to the start of the experiment. *L. terrestris* is one of the most abundant earthworm species in temperate Europe and belongs to the ecological group of anecic earthworms; it is widely spread in grassland, pastures and arable land (Sims and Gerard, 1999; Krück, 2018). As an endogeic earthworm *O. cyaneum* is widely spread over most soil habitats dwelling in forest soils, grassland and arable soils and has no preference for certain soil types (Sims and Gerard, 1999, Krück, 2018).

Earthworms were collected by hand-sorting from the same field-site, where the soil columns were sampled and were kept fresh at 4 °C. The indicators established by Fründ et al. (2010) were used to check if the earthworms were in good condition. Earthworms were cleaned with cold water and placed on a wet tissue for 24 h for defecation. For the EW_{anecic} treatment, two individuals of L. *terrestris* were added per column and for $EW_{endo.}$ four individuals of *O. cyaneum*, which corresponded to 113 and 226 individuals m^{-2}, respectively. The chosen number of individuals m^{-2} was in the same range as abundances in the field. Ulrich et al. (2010) reported 221 worms m^{-2} in reduced tilled (chisel plough) plots at a long-term experimental field-site in Germany. For the ENCH treatment, a combination of 150 individuals of *Enchytraeus crypticus* and 200 individuals of *Enchytraeus christenseni* was used, which corresponded to ~20,000 ind. m^{-2} and was similar to values reported under conventional tillage (22,567 ind. m^{-2}) and reduced tillage (12,318 ind. m^{-2}) at a field-site in Germany (Severon et al., 2012). The biology and ecology of both species is similar. Both enchytraeid species originated from the same lab culture of the Institute of Biodiversity, Thünen-Institute, Braunschweig, Germany.

2.3. *Incubation*

The soil columns were placed on ceramic plates (pore diameter of 1 μm), to which a constant suction of 50 bar was applied. The leachates were collected weekly in glass bottles and frozen in polyethylene bottles until measurements began. All mesocosms were hermetically sealed and were randomly placed in a dark climate chamber at 10 °C. They were continuously supplied with fresh air and automatically sprinkled (3 times day^{-1} 10 mL; refers to 1.67 mm precipitation per day, which corresponds to annual precipitation at the field-site, where soil columns were sampled) with a 0.01 M $CaCl_2$ solution – to simulate the ionic strength of the soil solution. This irrigation procedure resulted in a soil moisture of 46% of the maximal water holding capacity of our soil. However, one has to keep in mind that the results of these mesocosm experiments are not directly transferable to field conditions with variations in moisture and temperature.

The lids were connected to a gas chromatograph by plug valves. Gas fluxes (CO_2 and N_2O) were measured every 4.5 h utilizing a gas chromatograph (Shimadzu Gas Chromatograph GC-14A, Duisburg, Germany), with an electron-capture detector (ECD) for determination of CO_2 and a flame ionization detector (FID) for N_2O (Loftfield et al., 1997). After a pre-incubation period of 20 days, the experiment run for 114 days.

2.4. *Extraction of annelids*

At the end of the incubation experiment, mesocosms were sampled destructively, keeping three soil depths (0–10 cm, 10–20 cm, 20–30 cm) separately. Earthworms were hand-sorted and weighed (fresh weight). Enchytraeids were extracted from soil samples by using a wet-funnel technique without heating by placing soil subsamples under water for 48 h (ISO 23611-3, 2007). In modification to ISO 23611-3 (2007), the water-solution containing the enchytraeids was sieved (20 μm) for collecting the enchytraeids. Thereafter, they were counted using a grid and a dissecting microscope with magnification 10 to 40 times.

2.5. *Analyses of soil samples and leachate*

Soil samples were taken at the three different depths from the mesocosms at the end of the experiment: 0–10 cm, 10–20 cm, 20–30 cm. Soil dry weight was measured by oven-drying (105 °C, 24 h). Ergosterol was measured as marker of saprotrophic fungi applying the method of Djajakirana et al. (1996). The determination of ergosterol was done by high-performance liquid chromatography (HPLC), followed by UV detection at 282 nm (Dionex UVD 170 L). Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were determined by Chloroform-Fumigation-Extraction (CFE) (Brookes et al., 1985; Vance et al., 1987; Wu et al., 1990; Joergensen, 1996). Half of the samples (10 g of homogenized sediment) were fumigated (24 h, 25 °C) with chloroform ($CHCl_3$). Afterwards, samples (non-fumigated and fumigated) were extracted with 40 mL 0.05 M K_2SO_4 on a horizontal shaker (200 rpm) and filtered. Estimations for the microbial activity (mg CO_2-C g C_{mic}^{-1} d^{-1}) were calculated based on the metabolic quotient from Anderson and Domsch (1990); not using basal respiration rates, but total CO_2-C fluxes from the soil columns. The soil pH was measured in deionized water with a soil to solution ratio of 1:2.5. Percolates were analysed for DOC (dissolved organic carbon), TDN (total dissolved nitrogen) and DIN (dissolved inorganic nitrogen: NH_4^+, NO_3^-). DOC and TDN extracts were analysed by dry combustion with a multi N/C 2100 S (Analytik Jena, Jena, Germany). Dissolved inorganic nitrogen (DIN) was measured by ion chromatography (930 Compact IC Flex, Metrohm, Filderstadt, Germany). Dissolved organic nitrogen (DON) was calculated as TDN minus DIN.

2.6. *Isotopic analysis*

Isotopic ratios ($\delta^{13}C$, $\delta^{15}N$) as well as total masses for soil organic carbon (C_{org}) and total nitrogen (N_{total}) were measured for soil samples, for corn leaves and for annelid body tissues after defecation as described above (see 2.2). For soil, total C corresponded to C_{org} as no carbonates were detectable. Measurements were done by isotope-ratio mass spectrometry Delta V Advantage (Thermo Fisher Scientific, Bremen, Germany). Soil samples were dried, ball milled and weighed in 5 × 9 mm sized tin capsules. Four samples of non-incubated corn leaves (dried at 60 °C, 24 h) were ball milled and analysed in the same way. Annelid tissues were dried (60 °C) before analysis; prepared as single subsamples of each worm, using the first body segments of every individual earthworm and a number of whole bodies of the enchytraeids (without separating the two species) until reaching a sample weight suitable for analysis (mean: 151 μg). Control samples (four replicates) for earthworms consisted of non-incubated earthworms from the same field-site; both earthworm species were tested separately and for enchytraeids the control were animals from the lab-culture (non-incubated). The analyses took place at the Centre for Stable Isotope Research and Analysis, University of Göttingen, Germany. Dual stable isotopes (δ^{13} C and δ^{15} N) were measured on the same sample. The results are expressed as the delta notation in parts per thousand: $\delta\ ^{13}C/^{15}N$ [‰]. V-PDB was used as the standard for ^{13}C and atmospheric dinitrogen for ^{15}N, respectively. The proportion of crop residue derived C in annelid tissue C (ATC_{crd} %) was calculated with the following equation based on Balesdent and Mariotti (1996):

$$ATC_{crd}\% = \left(\delta^{13}C_{AT} - \delta^{13}C_{CAT}\right) / \left(\delta^{13}C_{CR} - \delta^{13}C_{CAT}\right)$$

where $\delta\ ^{13}C_{AT}$ is the $\delta\ ^{13}C$ value from each individual annelid tissue at the final sampling day, $\delta\ ^{13}C_{CAT}$ is the initial $\delta\ ^{13}C$ value of the control annelid tissues at day 0, $\delta\ ^{13}C_{CR}$ is the $\delta\ ^{13}C$ value of the crop residues (corn leaves) at day 0.

2.7. Statistics

Data were analysed using the statistical software R (Version 3.6.1, R Core Team, 2019) with packages *car* (V. 3.0-3, Fox and Weisberg, 2019) and *multcomp* (V. 1.4-13, Hothorn et al., 2008). Analysis of variance (ANOVA) was carried out to determine if there were significant differences between treatments. ANOVAs were carried out separately for each depth with $n = 32$. The effects of block (4 factor levels), soil inversion (2 factor levels), annelid treatment (4 factor levels) and the interaction of soil inversion and annelid treatment were tested. Stepwise model reductions were carried out. We first eliminated a non-significant interaction, then non-significant main effects (Crawley, 2012), independent of the factor 'block'. Residuals of the final model for each variable were checked for homoscedasticity graphically and using Levene's test and for normal distribution by the Shapiro–Wilk test and graphically by inspecting QQ-plots. In the case of a significant treatment effect in the ANOVA, Tukey's HSD tests were applied to conduct pairwise comparisons between treatment levels.

For soil analysis, the three different soil depths were evaluated separately. In order to meet parametric assumptions, the following variables were transformed using logarithmic transformation (ergosterol (0–10 and 10–20 cm), ergosterol:C_{mic} (10–20 cm), DOC, TDN, NO_3^--N) or Box-Cox transformation (C_{org} (10–20 cm)). Data were considered significant for $p \leq 0.05$. For C_{mic}:C_{org} ratio, Pearson product-moment correlation (C_{org}) and Spearman rank correlation analyses (N_{total}) were carried out.

3. Results

3.1. Survival and growth of annelids

After the incubation period, the total number of enchytraeids was increased approximately tenfold; from 350 to 3481 ± 976 (mean ± standard deviation) individuals (total number of juvenile and adult individuals per column (0.018 m^2)) in the NI treatment and up to 3432 ± 649 (mean ± standard deviation) individuals in the SI treatment. In the SI treatment, the enchytraeid distribution was decreasing with soil depth: 44% of the total community at 0–10 cm, 38% at 10–20 cm and 18% at 20–30 cm soil depth. For the NI treatment, distribution was quite balanced throughout the soil column, containing 35%, 30% and 35% of the total community from the top to the bottom for the three soil depths, respectively.

Earthworm mortality was low during the experiment; on average, only 6% of the anecic earthworms (corresponding to one individual earthworm) died while all endogeic earthworms survived the incubation. Biomass (mean ± standard deviation) of anecic earthworms for SI was 5.17 ± 0.18 g fresh weight (FW) before incubation and 5.66 ± 0.96 g FW after the experiment and for NI it was 4.97 ± 0.19 g FW and 5.21 ± 0.67 g FW, respectively. In contrast, biomass of endogeic earthworms increased in SI columns during the experiment from 3.47 ± 0.07 g FW to 4.82 ± 0.20 g FW. For NI, it was from 3.76 ± 0.16 g FW to 4.63 ± 0.62 g FW. The activity of the anecic earthworms in the respective columns could be observed with the naked eye. Already after one month, the soil surface area had changed visibly due to the presence of anecic earthworms in the non-inverted columns; at the end of the experiment, nearly all crop residues had disappeared from the soil surface (Fig. 1).

3.2. Isotopic ratios of annelids

Averaged over all treatments, isotopic values of soil samples ranged between −27 to −25 δ^{13}C (‰) and 4 to 6 δ^{15}N (‰). The corn leaves had an isotopic signature of −13.65 δ^{13}C (‰) and 7.12 δ^{15}N (‰). A comparison of isotopic ratios of body tissues of earthworms from the field-site where the soil originated (control) and annelids in the experiment showed a shift in δ^{13}C (Fig. 2). However, anecic earthworms differed significantly ($F(2, 6) = 49.38$; $p < 0.001$) in δ^{13}C values; Tukey post-hoc analysis indicated that only control earthworms ($p < 0.001$) differed from anecic earthworms in the lab (SI and NI) without differences between soil inversion treatments (Fig. 2). On average, anecic earthworms had assimilated crop residue derived C corresponding to 29% of their body tissue C in the NI and 30% in the SI treatment.

Endogeic earthworms differed in δ^{13}C values ($F(2, 6) = 144.47$; $p < 0.001$). Tukey post-hoc analysis revealed not only a differences between control earthworms and earthworms from the lab experiment for SI ($p < 0.001$) and NI ($p < 0.001$), but also differences between earthworms from SI and NI columns: endogeic earthworms had a significantly ($p = 0.02$) greater proportion of crop residue derived C in their tissue C in the SI columns (18%) compared with NI (14%) (Fig. 2).

Similar to the earthworms, isotopic ratio (δ^{13}C) of enchytraeid tissues differed significantly ($F(2, 6) = 21.91$, $p < 0.01$). The results of the Tukey post hoc analysis showed that enchytraeids from SI ($p < 0.01$) and NI columns ($p < 0.01$) had higher δ^{13}C values compared with control enchytraeids from laboratory culture. Assimilated crop residue derived C of enchytraeids corresponding to approximately 25% (SI) and 30% (NI) of their body tissue C (Fig. 2).

3.3. Chemical and microbial soil parameters

We found no significant interactions of soil inversion and annelid addition at any of the soil depths. At the soil depths 0–10 cm and 10–20 cm, only soil inversion affected microbial and chemical soil parameters (Tables 1, 3), regardless of annelid addition. At 20–30 cm, only differences in annelid treatments occurred, but no soil inversion effect (Tables 2, 3).

Concentrations of C_{mic} and N_{mic}, ergosterol and the ergosterol:C_{mic} ratio were, like C_{org} and N_{total} concentrations higher in NI columns (vs.

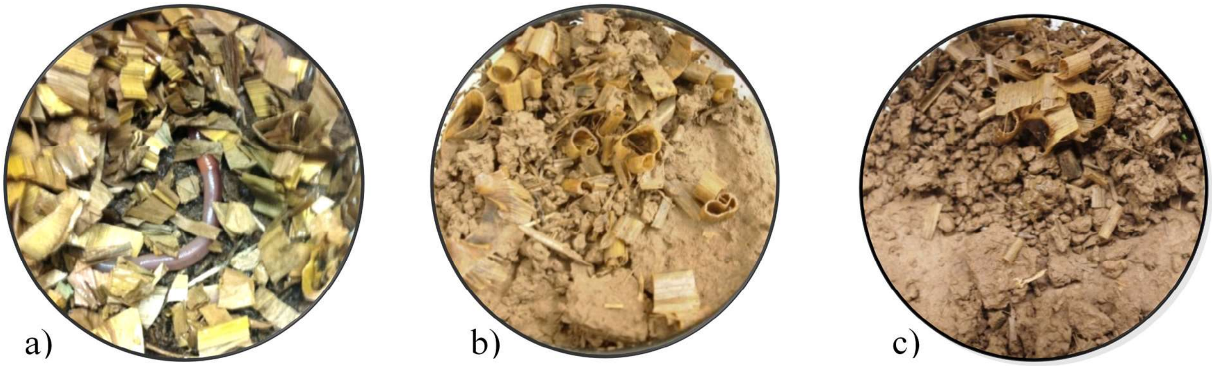

Fig. 1. Change of the surface area (diameter: 15 cm) over time (a) start of the experiment, (b) after 23 days, (c) after 114 days, from a soil mesocosm of the non-inversion treatment with chopped corn residues on the soil surface (NI) and addition of anecic earthworms (*Lumbricus terrestris*).

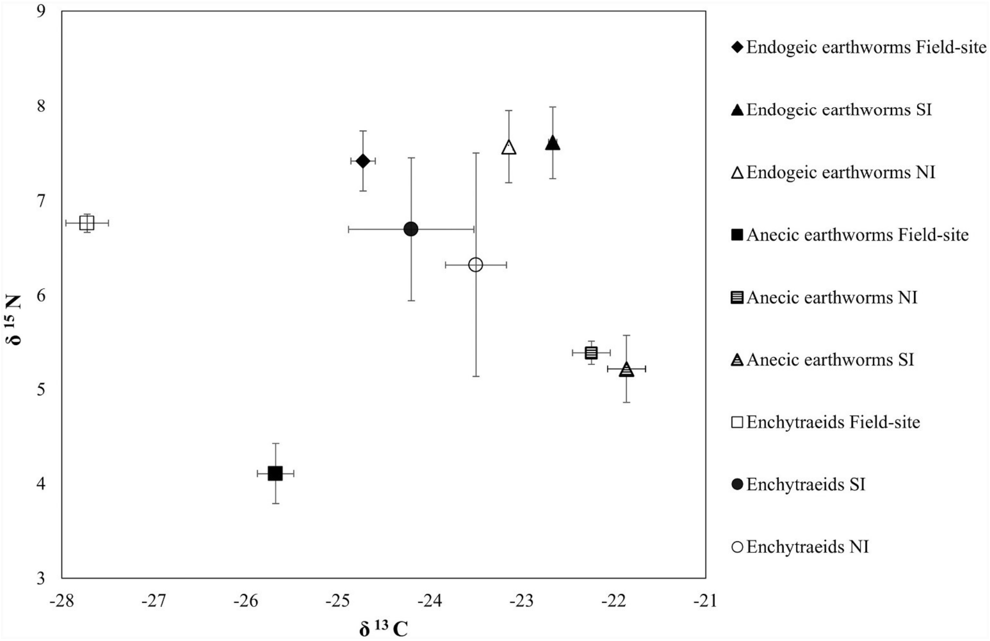

Fig. 2. Means ± standard errors of dual stable isotopes ($n = 4$) of annelid tissues before (start) and after incubation in soil columns treated either with soil inversion with residues buried (SI) or non-inversion with residues on the soil surface (NI). EW_{anecic} = Anecic earthworms (*Lumbricus terrestris*), $EW_{endo.}$ = Endogeic earthworms (*Octolasion cyaneum*), ENCH = Enchytraeids (*Enchytraeus crypticus* and *Enchytraeus christenseni*). Shift of isotopic values reflect an uptake of corn leaves as residues during the time of incubation (114 d). Values of corn leaves (non-incubated) were 7.12 $\delta^{15}N$, −13.65 $\delta^{13}C$, for soil samples before incubation 5.2 $\delta^{15}N$, −26.7 $\delta^{13}C$ and soil samples after incubation 5.3 $\delta^{15}N$, −26.4 $\delta^{13}C$. Means of $\delta^{13}C$ followed by different letters are significantly ($p < 0.05$) different; lower case letters indicate differences for endogeic earthworms, upper case letters for enchytraeids and framed letters for anecic earthworms.

Table 1

Effect of the factor soil inversion on concentrations of soil organic carbon (C_{org}), total nitrogen (N_{total}), microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), the ratio of C_{mic} to C_{org}, ergosterol and the ratio of ergosterol to C_{mic} at the soil depths 0–10 cm and 10–20 cm. Means ($n = 32$) for the levels non-inversion (NI) and soil inversion (SI) and standard errors for differences of means are shown. Means followed by different letters are significantly ($p < 0.05$) different from each other at each soil depth.

Soil property	Soil depth	NI	SI	Standard errors for differences of means
C_{org} [%]	0–10 cm	1.57 a	1.37 b	0.02
	10–20 cm[†]	0.19 b	0.48 a	0.04
N_{total} [%]	0–10 cm	0.17 a	0.15 b	0.00
	10–20 cm	0.13 b	0.14 a	0.00
C_{mic} [µg g^{-1}]	0–10 cm	358 a	278 b	26
	10–20 cm	174 b	255 a	20
N_{mic} [µg g^{-1}]	0–10 cm	49.3 a	40.9 b	4.5
	10–20 cm	25.7 b	40.7 a	3.5
C_{mic}:C_{org} [%]	0–10 cm	2.27	2.04	0.17
	10–20 cm	1.49 b	1.88 a	0.14
ergosterol [µg g^{-1}]	0–10 cm[‡]	0.38 a	−0.33 b	0.16
	10–20 cm[‡]	−0.67 b	0.20 a	0.11
ergosterol:C_{mic} [%]	0–10 cm	0.44 a	0.30 b	0.05
	10–20 cm[‡]	−5.78 b	−5.27 a	0.10

In these depths, neither the interaction between the factors soil inversion and annelid treatment nor the annelid treatment were significant. There were no significant effects of the factor soil inversion at 20–30 cm.

[†] Data were Box-Cox transformed.

[‡] Data were log-transformed.

Table 2

Effect of the factor annelid treatment on concentrations of microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) and the ratio of C_{mic} to soil organic carbon (C_{org}) at 20–30 cm. Means (n = 32) for the levels anecic earthworms (EW_{anecic}), endogeic earthworms ($EW_{endo.}$), enchytraeids (ENCH) and control columns (CON) and standard errors for differences of means are shown. Means followed by different letters are significantly ($p < 0.05$) different from each other.

Soil property	EW_{anecic}	$EW_{endo.}$	ENCH	CON	Standard errors for differences of means
C_{mic} [µg g^{-1}]	80.94 ab	91.15 ab	101.36 a	68.00 b	10.82
N_{mic} [µg g^{-1}]	11.71 ab	11.21 ab	14.83 a	8.55 b	1.99
C_{mic}:C_{org} [%]	0.89 ab	1.04 ab	1.11 a	0.75 b	0.13

In this depth, neither the interaction between the factors soil inversion and annelid treatment nor the factor soil inversion were significant. There were no significant effects of the factor annelid treatment at 0–10 and 10–20 cm.

Table 3
ANOVA for the effect of soil inversion on concentrations of soil organic carbon (C_{org}), total nitrogen (N_{total}), microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), the ratio of C_{mic} to C_{org}, ergosterol and the ratio of ergosterol to C_{mic} at the soil depths 0–10 cm and 10–20 cm plus ANOVA for the effect of annelids on microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) and the ratio of C_{mic} to C_{org} at the soil depth 20–30 cm. Non-significant (n.s.) results are not shown.

Soil depth	Source	DF	C_{org} [%]		N_{total} [%]		C_{mic} [µg g^{-1}]		N_{mic} [µg g^{-1}]		ergosterol [µg g^{-1}]		ergosterol:C_{mic} [%]		C_{mic}:C_{org} [%]	
			F ratio	P	F ratio	P	F ratio	P	F ratio	P	F ratio	P	F ratio	P	F ratio	P
0–10 cm	Soil inversion	1	95.11	<0.001	68.33	<0.001	9.61	<0.01	3.37	0.08	41.09	<0.001	8.91	<0.01	1.94	0.43
	Block	3	3.84	0.02	8.10	<0.001	0.55	0.65	1.94	0.15	6.54	<0.01	7.01	<0.01	0.94	0.18
	Residual	27														
10–20 cm	Soil inversion	1	35.16	<0.001	30.22	<0.001	17.47	<0.001	18.77	<0.001	58.18	<0.001	26.00	<0.001	8.10	<0.01
	Block	3	8.32	<0.001	13.04	<0.001	8.32	<0.001	10.03	<0.001	1.61	0.21	3.07	<0.05	6.65	<0.01
	Residual	27														
20–30 cm	Annelids	3	n.s.		n.s.		3.48	<0.05	3.35	<0.05	n.s.		n.s.		3.33	<0.05
	Block	3					4.72	<0.01	2.05	0.13					6.79	<0.01
	Residual	25														

SI) at 0–10 cm and lower at 10–20 cm. C_{mic}:C_{org} was significantly higher in SI columns compared with NI at 10–20 cm. The C_{mic}:C_{org} ratio was closely correlated with C_{org} ($r = 0.74$) and N_{total} ($r = 0.76$) among soil depths. At 20–30 cm, C_{mic} and N_{mic} contents as well as the C_{mic}:C_{org} ratio was lowest in the control columns, compared with the ENCH treatment.

3.4. Carbon and nitrogen losses

On average, among all treatments, the cumulative emissions of CO_2-C was 128.5 g m^{-2} after 114 days of incubation; without differences in soil inversion treatments. The amount of cumulative CO_2-C emitted in the soil columns was 22% higher in the EW_{anecic} columns compared with control columns (F(3, 25) = 3.51; $p = 0.03$). In contrast, cumulative N_2O-N emissions increased due to SI (vs. NI) by 188%, independent from annelids (F(1, 27) = 13.81; $p < 0.001$). The ratio of CO_2 flux to C_{mic} was significantly (F(3, 25) = 4.28; $p = 0.014$) increased by 37% in the presence of anecic earthworms, compared with enchytraeids (Table 4). After adding the substrate at the start of the experiment, the daily CO_2-C emissions led to a steadily increasing curve of the cumulative CO_2-C emissions, which flattens out slightly over time. In the case of the N_2O-N emissions, differences over time can be seen between the two inversion levels: with soil inversion, the increases of cumulative values are on average constantly higher than for the values of non-inversion columns, which is also reflected in the cumulative sums (Table 4).

The amount of soil solution that had leached after 114 days of incubation was 3263 g in NI and 3178 g in SI columns. For DOC, TDN and DIN no effect of soil inversion or annelid treatment was found. Leaching of DON was small; averaged over all treatments, 71% of the N had leached as DIN in form of NO_3^-. Cumulative TDN and NO_3^--N losses averaged 10.39 g m^{-2} and 7.33 g m^{-2}. Ammonium-N concentrations were below detection rates. Cumulative DOC values were in a range from 0.82 to 2.49 g m^{-2}.

4. Discussion

4.1. Conditions for annelids

Compared with other laboratory experiments, earthworm mortality in this mesocosm experiment was very low (0–6%). This underlines that earthworms had suitable conditions during the experiment, although one single earthworm died. Giannopoulos et al. (2010) reported a mortality of 15% and 14% for L. *rubellus* and *A. caliginosa,* respectively, in a 90-days mesocosm experiment. In contrast to our hypothesis, we could not find a body weight loss in any soil inversion treatment for anecic earthworms. This result contrasts the mesocosm study of Frazão et al. (2019). In their study, running for 61 days, incorporation (burying) of crop residues led to a 30% reduction in body weight for L. *terrestris*; the body weight loss of the endogeic earthworm species (*A. caliginosa*) did not differ between the treatments (incorporated crop residues vs. surface applied). Thus, in addition to crop residue placement, other factors might play a role for changes in earthworm biomass during laboratory experiments, such as the number of individuals per column, the study duration and possibly also the choice of crop residues.

Experimental conditions seemed also suitable for enchytraeids. The strong (tenfold) increase of enchytraeid individuals during the experiment can be explained by sufficient food supply and the lack of predators. Under favourable conditions, enchytraeids are known for their rapid reproduction rates, as reported by van Vliet et al. (2004) for *E. minutus* and by Sandor and Schrader (2012) for the two species, that were also used in this experiment. In our case, the enchytraeids were evenly distributed over the entire length of the soil columns in the NI treatment, whereas they were concentrated in the upper 20 cm in the SI treatment. Under field-conditions, Severon et al. (2012) reported an even distribution of enchytraeids in the entire soil profile examined (0–20 cm) in conventionally tilled plots (inverted soil), whereas for reduced tilled plots (non-inversion), enchytraeids were mainly found

Table 4

Effect of the factors soil inversion (SI: soil inversion with residues buried, NI: non-inversion with residues on the surface) and annelid treatment (EW_{anecic}: anecic earthworms, $EW_{endo.}$: endogeic earthworms, ENCH: enchytraeids, CON: control columns) on cumulative N_2O-N and CO_2-C emissions after 114 days and on the ratio of CO_2 and microbial biomass carbon (C_{mic}). Values followed by different letters are significantly different ($p < 0.05$) different (n = 32 for each response variable in the ANOVA and subsequent Tukey's HSD test).

	N_2O-N [mg m^{-2}]	CO_2-C [g m^{-2}]	CO_2-C:C_{mic} [mg CO_2-C g^{-1} C_{mic} d^{-1}]
Factor soil inversion: NI	45 b	132	16.0
SI	129 a	125	15.2
Standard errors for differences of means	23	7	1.3
Factor annelid treatment: EW_{anecic}	77	141 a	18.0 a
$EW_{endo.}$	114	135 ab	17.1 ab
ENCH	109	122 ab	13.2 b
CON	49	115 b	14.1 ab
Standard errors for differences of means	38	9	1.6

n.s.: not significant.

There were no significant interactions between the soil inversion and annelid treatment factors.

treatment factors.

between 0 and 10 cm. In our experiment, the uneven distribution of enchytraeids in the SI columns could be due to the manual soil inversion, which caused an interruption at a soil depth of 15 cm, so that the deepest layer (20–30 cm) could no longer be reached by the enchytraeids and their dispersion was limited.

4.2. Annelid feeding behaviour

The isotopic signature ($\delta^{13}C$, $\delta^{15}N$) showed a clear separation between ecological earthworm groups (anecic vs. endogeic). This difference between the anecic species (*L. terrestris*) that is predominantly a litter feeder and therefore a primary decomposer and the endogeic species (*O. cyaneum*) that as secondary decomposer predominantly feeds on soil affirms the findings of Scheu and Falca (2000) in beech forests and of Schmidt et al. (1997) in arable soils. Similar to the results of Schmidt et al. (2004), litter-feeding earthworms were notably lighter in both, $\delta^{13}C$ and $\delta^{15}N$, compared with soil feeders. In accordance with Schmidt et al. (2016), $\delta^{15}N$ values of enchytraeids were similar to those of endogeic earthworms, but not to anecic earthworms.

The shift in isotopic $\delta^{13}C$ values for all investigated groups of annelids reflect that earthworms and enchytraeids had a preference for fresh crop residue material over older native soil organic matter (Briones et al., 1999), but with differences between the ecological groups. Anecic earthworms showed a greater uptake of crop residue derived C, compared with endogeic earthworms. Our results indicate that even though the secondary decomposer *O. cyaneum* fed on crop residues, it preferred to feed on well mineralised organic matter (cf. Eck et al., 2015), whereas anecic earthworms and enchytraeids favoured the fresh organic material or grazed on microorganisms dwelling on the litter. These findings are also in line with other studies (Briones et al., 1999; Bossuyt et al., 2006). In contrast to our hypothesis, *L. terrestris* (known as a species feeding at the soil surface) did not show a higher uptake of crop residue derived C in the NI columns.

Our results showed that deep burrowing earthworms access and feed on buried crop residues to an equal extend as if crop residues are placed at the soil surface. This result indicates that under field conditions it is more likely the destruction of natural bio-pores and the killing and injuring of worms by machinery that has a negative effect on anecic earthworms than the belowground placement of food sources due to ploughing. Nevertheless, in this mesocosm study our conclusions with regard to crop residue placement are only valid for adult individuals. In order to predict the community development under such conditions, other factors such as reproductive activity and hatchling survival should also be taken into account (cf. Briones and Schmidt, 2017).

4.3. Microbial biomass and soil organic matter

An important result is the absence of any interaction among the soil inversion treatment and annelids on the response variables, indicating that soil inversion, as carried out here, and annelid treatment have simple additive effects on the response variables. Most literature reflects the combined detractive effects of tillage on earthworm abundance and their activity (Briones and Schmidt, 2017). Our experiment shows that the two effects (annelids and pure soil inversion) are independent, when the inversion is not detracting or impairing the annelids directly as a side effect of the used machinery. For this reason, the findings of this model study cannot be directly translated to field conditions, where soil inversion is hardly realisable without soil structure destruction.

Concentrations of C_{org} and N_{total} were only affected by soil inversion and enhanced where crop residues were buried, which indicate that both parameters were closely related to the organic material. Similar results were observed for ergosterol contents and the ratio of ergosterol to C_{mic}. Incorporation of crop residues in the soil is known to promote fungi (Allison and Killham, 1988) as it provides a habitat for decomposing microorganisms (Potthoff et al., 2005). A decrease of ergosterol has been reported in the presence of the endogeic earthworm species *O. tyrtaeum* by Butenschoen et al. (2007) and *A. caliginosa* by Wachendorf et al. (2014). Such an effect of annelids on fungal biomass was not detected in our experiment. As shown for total microbial biomass reducing and supporting effects of annelids are balanced. Hence, the annelid feeding on fungi might be compensated or less pronounced due to a variety of other food sources in our setting using quite large soil columns (~5.8–7 kg of dry soil) containing undisturbed soil.

In the present experiment, higher C_{mic} and N_{mic} concentrations and a greater C_{mic}:C_{org} ratio were found in columns with enchytraeids, compared with control columns, at 20–30 cm for both, NI and SI. Sandor and Schrader (2012) reported for a mesocosm experiment that the presence of enchytraeids reduced microbial biomass. Van Vliet et al. (2004) reported quite small impacts of enchytraeids on microbial biomass in a lab incubation experiment. As known for earthworms two effects directed towards microbial communities might be balanced also for enchytraeids. On the one hand, there is a reduction of microbial biomass by direct feeding (Potthoff et al., 2001) and, on the other hand, earthworm bioturbation might enhance availability of resources and support microbial growth.

The ratio C_{mic}:C_{org} was in the upper 10 cm on average 2.1%, similar to values reported by Heinze et al. (2010) and Murugan et al. (2014) for Luvisols. From the top to the bottom, C availability for microorganisms decreased by approximately 55%, indicating a decreasing C availability for microorganisms (Anderson and Domsch, 1989; Anderson and Domsch, 2010). As the C_{mic}:C_{org} ratio was closely correlated to C_{org} and N_{tot} concentrations, it reflects the reduced amount of organic material at 20–30 cm soil depth.

4.4. Gas emissions and leaching

For all treatments, C losses were mostly due to gas emissions in the form of CO_2-C. Generally, DOC levels were in the same range like in the mesocosm study of Sänger et al. (2011). We found no effect of annelids on NO_3^--N unlike reported in other studies (Araujo et al., 2004; Sandor and Schrader, 2012). Furthermore, we found no effect of annelids on N_2O emissions. In contrast, Nieminen et al. (2015) showed an increase in N_2O (27%) and CO_2 (13%) in the presence of L. *terrestris* in a mesocosm experiment running for 15 weeks. Lubbers et al. (2013) reported that earthworms increase CO_2 and N_2O emissions in soils by an average of 33% and 42%, respectively. Our results showed a strong and pronounced soil inversion effect (+188%) for N_2O-N emissions. This might have masked the annelid effect, as this effect could be expected as much smaller. Comparable results were reported by Giannopoulos et al. (2010), who investigated the role of earthworms (*A. caliginosa* and L. *rubellus*) in a treatment with incorporated crop residues vs. a treatment with crop residues placed on the soil surface. Here, the incorporation of crop residues led to much higher N_2O emissions than when crop residues were placed on the soil surface.

We observed a 22% increase in cumulative CO_2-C emissions in the presence of L. *terrestris*, compared with control columns. Lubbers et al. (2015) noticed an increase of 25% in no-till mesocosms (*A. caliginosa*, *L. rubellus*) after 750 days. In one of our columns one L. *terrestris* died throughout incubation, which might induce a temporary flush of CO_2-C emissions. We did not observe such an effect. The increase in CO_2-C might be explained by stimulation of the microbial turnover. Concerning the microbial respiration in this study, in the presence of anecic earthworms 37% more CO_2-C was respired per unit C_{mic} over time, compared to when only enchytraeids were present. This was less pronounced for endogeic earthworms, since anecic earthworms as primary decomposers are closer related to plant residues where microbial mineralization runs faster and in higher rates than for soil organic matter. Therefore, the anecic impact takes place at a hot spot of decomposition with high rates. In addition, Binet et al. (1998) name higher soil aeration due to bioturbation due to L. *terrestris* in their experiment as an explanation for an increase in respiration rates. A reduced availability of O_2 reduces CO_2, but enhances N_2O fluxes (Vor et al., 2003).

Earthworms boost soil aeration due to their burrowing activity that creates soil pores. We assume soil aeration to be increased in the presence of both earthworm species (*L. terrestris* and *O. cyaneum*), though bioturbation rates of anecic species are known to be generally lower than for endogeic species (Taylor et al., 2018). As both earthworm species differ in their feeding and burrowing behaviour (Bouché, 1977), their burrow constructions are not the same. While 40–50% of the burrows of endogeic earthworms are refilled with earthworm casts, this is only the case for 20% of the burrows by anecic earthworms that preferably cast on the soil surface (Capowiez et al., 2014). Therefore, soil aeration might be greater in soil columns with anecic compared with endogeic earthworms. For enchytraeids, we found no significant effect on gas fluxes. Van Vliet et al. (2004) also report no effect on CO_2 emissions. However, N_2O emissions were increased by enchytraeids in a loamy sand soil and decreased in a sandy clay loam. In contrast, John et al. (2019) observed a reduction of the average CO_2 emissions by 35% by *E. buchholzi* in a paddy soil. In summary, a strong interaction of enchytraeid effects with soil type is indicated. Based on the results from their 64-day mesocosm experiment, Wu et al. (2015) highlight, that only earthworms enhanced CO_2 emissions, whereas the smaller mesofauna had less impact, which indicate that effects may also be size-dependent.

5. Conclusion

The experiment showed that *O. cyaneum* benefit when crop residues are buried in the soil profile (due to soil inversion). Contrasting our hypothesis, *L. terrestris* took no advantage from the non-inversion treatment. However, the amount of crop residue derived C in the earthworm tissue and its isotopic signature clearly differed between L. *terrestris* on the one hand and *O. cyaneum* and enchytraeids on the other hand, which may indicate, that there is a difference between primary and secondary decomposers. Surprisingly, there was no interaction between both factors (soil inversion x annelid treatment) for soil microbial and chemical parameters. In the upper 20 cm, the concentrations were only dependent on soil inversion, indicating a strong relationship to the organic material. We observed an effect of annelids regarding nutrient losses only for carbon, but not for nitrogen. Here, stimulation of microbial activity by annelids seems to be an important factor increasing CO_2-C emissions. In contrast, N_2O-N emissions were dependent on soil inversion. Results indicate that the effect of annelids on soil processes clearly depend on life form of earthworms and might be also partially size-dependent, being lower for mesofauna than for macrofauna.

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.

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PART IV

Chapter 7

General Discussion

The following section highlights key findings and gives overarching conclusions from all three studies to contribute to the conservation of soil biodiversity and the integration of soil biota in agricultural management. Tillage and soil ecology are the two major partners for sustainable agriculture (Roger-Estrade et al., 2010). In the setting of the SoilMan project both aspects were investigated in an European framework. This thesis focuses on (i) the effect of reduced tillage on soil physicochemical and microbial parameters under field-conditions, (ii) soil processes and functions related to litter decomposition driven by soil mesofauna under field-conditions and (iii) the effects of crop residue placement in the soil profile under laboratory conditions on annelids and microorganisms. Based on the assumption that soil biodiversity benefits agriculture when agricultural management is adapted to foster soil biodiversity, the experiments were based on different approaches:

Study 1 (Chapter 4) mainly shows how agriculture can care for biodiversity, namely by a reduction in tillage intensity, whereas **study 2** (Chapter 5) contains both aspects - on the one hand, the abundance and community composition of Collembola indicate what agriculture can do for biodiversity, and on the other hand, litter decomposition by soil fauna shows how biodiversity works for agriculture. **Study 3** (Chapter 6) basically showed how biodiversity can work for agriculture, investigating the wide range of functions, processes and services soil biota can provide.

The experiments of the **studies 1 & 2** were chosen in order to investigate the effects of reduced tillage under field conditions, which had the advantage that the results can be transferred quite well to practice. As a disadvantage, results will be affected by and may even partly depend on the prevailing environmental conditions. In contrast, the laboratory experiment of **study 3** took place under controlled conditions, which allows a detailed investigation of the various effects of soil biota in combination with soil inversion and crop residue placement. However, results can only be transferred on field conditions to a limited extent. Both approaches have proven to be important for scientific knowledge and complement each other.

Modifications of the soil habitat by tillage

Soil as a complex habitat for soil organisms is characterised by temporal and spatial heterogeneities (Roger-Estrade et al., 2010). Anthropogenic impacts strongly shape agricultural ecosystems (Woodward, 2011; Sandor and Homburg, 2017). For instance, repeated ploughing can result in a plough pan which modifies the distribution of soil fauna because they generally avoid compacted zones in the soil (Kretzschmar, 1991; Cluzeau et al., 1992). A strong compaction effect of tillage on soil was shown in **study 1** (Chapter 4). Here, the soil under mouldboard ploughing at the German field site showed high bulk densities at 20-30 cm soil depth. Similar results were found for MT (10-12 cm working depth) at the field site in Sweden, where bulk densities increased strongly from 0-10 cm to 10-20 cm soil depth. Besides the spatial restrictions for soil organisms, such cultivator layers can also reduce C inputs by crop roots which in the long-term has adverse effects for microorganisms (Schjønning and Thomsen, 2013; Tian et al., 2016). Such lasting effects could not be identified in **study 3** (Chapter 6), because ploughing was simulated by turning the upper part of the soil columns upside down (15 cm) and therefore lacked the compacting effect of machinery. Nevertheless, the disruption of the natural soil structure by inverting the soil columns may have restricted the distribution of enchytraeids during the experiment. Enchytraeids were less abundant in the deepest soil layer in inverted soil columns (Chapter 6). Earthworms are generally less affected by such barriers in the soil and play a crucial role in the regeneration process of soil being already compacted (Larink and Schrader, 2000).

Soil inversion and the burial of residues in deeper soil layers removes a food resource for soil organisms from the soil surface. Although anecic earthworms are known to preferentially feed at the soil surface, soil inversion with displacement of residues showed no negative effects on *L. terrestris* in **study 3** (Chapter 6). Endogeic earthworms reacted even positively to the incorporation of crop residues in this mesocosm experiment, indicated by a greater biomass and a higher uptake of litter-derived C in the inverted soil columns. These results suggest that earthworms show a great adaption ability to changes in crop residue placement due to tillage (Chapter 6). Nevertheless, deep ploughing should be avoided to promote and preserve anecic species at agricultural field sites. In practice, a way should be sought to meet the demands of both ecological groups of earthworms, an incorporation of crop residues close to the surface without a deep disturbance in the soil structure (< 15 cm). In **study 2** (Chapter 5) euedaphic Collembola were highly present in conventionally tilled plots. Here, incorporated crop residues provided a new habitat and additional food sources at deeper soil layers where other substrates are less available. This study supports that Collembola follow the spatial distribution of their food sources (Verhoef and Nagelkerke, 1977; Vignozzi et al., 2019).

No matter if meso- or macrofauna, in the experiments of this thesis only one of the studied life form groups was supported by CT, namely euedaphic collembolans (**study 2**) and endogeic earthworms (**study 3**). The associated incorporation of crop residues into deeper soil layers, while the other ecological group, harmed epidaphic collembolans (**study 2**) and had no effect on anecic earthworms (**study 3**). But the other life form groups, which are not supported by ploughing, may have a greater influence on soil processes like nutrient release and the related ecosystem service of plant growth. This became clear in **study 2** by the fact that the litter quality was improved by MT (here more epidaphic species were present) and in **study 3** by the influence of the anecic earthworm species (*L. terrestris*) on C fluxes. Even though the importance and benefits of biodiversity are readily understood, assessing the value of one species is still difficult (Gascon et al., 2015). The results of **study 2 & 3** suggest species-specific effects of management strategies should always be thoroughly assessed. The modification by intensive tillage may provide new habitats but also damages the soil structure close to the surface. Especially anecic earthworms are at risk in agricultural fields, because they react sensitive to ploughing (Briones and Schmidt, 2017). They are particularly important in C cycles in the soil (Borken et al., 2000; Don et al., 2008), which is discussed in the following section.

The importance of annelids in the soil carbon cycle

The effects of earthworms on the biomass of microorganisms was investigated in **study 3** (Chapter 6). Generally, earthworms stimulate the growth and activity of microbial biomass but also reduce the microbial biomass by grazing (Medina-Sauza et al., 2019). Although earthworms did not alter the biomass of microorganisms, anecic earthworms increased the microbial activity by 37% more CO_2-C respired per unit MBC (Chapter 6). Previous studies confirm that there is a large variability in the results on the effect of annelids on microbial parameter. Gajda et al. (2017) showed that enchytraeids are able to alter microbial communities via grazing. In contrast, other studies report relatively low effects of enchytraeids on microbial biomass (van Vliet et al., 2004). In the mesocosm experiment (**study 3**) the presence of enchytraeids increased MBC in the lowest soil depth at 20-30 cm, compared with control columns, independent of soil inversion. Due to the fact that the two species of enchytraeids (*Enchytraeus crypticus* and *Enchytraeus christenseni*) in the **study 3** experiment have similar habits and feeding guilds, a differential effect of a single species on microorganisms was not expected. Enchytraeids may have contributed to a better nutrient availability due to their activity in the soil column. Although their distribution in SI columns was relatively limited, they occur at all three soil depths (in SI and NI columns) and may have enhanced microbial conditions at the lowest soil depth (20-30 cm) where maize leaves were not available. Presumably, this increased nutrient availability, for example, through feaces at the lower soil depth, which favoured microorganisms.

It is a controversial topic whether earthworms increase gas emissions or lead to C sinks by stabilizing C in the soil (Lubbers et al., 2013; Zhang et al., 2013). Carbon dioxide (CO_2) is a good indicator of energy flows (mineralization of nutrients) due to decomposition processes (Muñoz et al., 2010). Although earthworms play a crucial role for soil carbon sequestration, the review of Lubbers et al. (2013) shows that they increase GHG emissions. In the microcosm experiment of **study 3** (Chapter 6) anecic earthworms increased CO_2-C emissions by 22% compared with control columns. As soil derived CO_2 fluxes mainly reflect the activity of microorganisms (Binet et al., 1998; Kuzyakov and Domanski, 2000) the higher CO_2-C fluxes in **study 3** are likely due to increased microbial activity due to the presence of *L. terrestris*. The outcome from the microcosm experiment of Speratti and Whalen (2008) suggests that the presence of endogeic (*A. caliginosa*) and anecic earthworms (*L. terrestris*) stimulate microbial respiration rates due to the interactions between these species. The combination of both ecological groups of earthworms was not investigated in **study 3** and would therefore require further investigation to quantify the effect of interactions between earthworm species on microbial activity.

The contribution of anecic earthworms to the soil C cycle was clearly demonstrated in **study 3**, but the contribution of endogeic earthworms to this cycle remains an open question. Previous studies showed that endogeic earthworms mostly influence the soil C cycle due to grazing on litter-decomposing fungi and thereby alter C translocation in the soil (Butenschoen et al., 2007). The results of Wachendorf et al. (2014) demonstrated that the endogeic species *A. caliginosa* reduced the microbial biomass and ergosterol concentrations and that the selective feeding on litter by endogeic earthworms probably depends on SOC contents. However, their presence is associated with an increase in Bacteroidetes, which in turn increases mineralization of organic residues (Medina-Sauza et al., 2019). Overall, endogeic earthworms are known to enhance the mineralization of soil C (Bernard et al., 2012; Wachendorf et al., 2014). Especially fresh casts may contribute to the availability of nutrients (Tiunov and Scheu, 2000). Generally, the excrements of geophages enhance organic-inorganic interactions as faeces are a mixture of SOM and mineral soil (Wolters, 2000).

In **study 3** it remains uncertain whether the uptake of litter-derived C occurred through direct feeding of the maize litter by ingestion of microorganisms which fed as primary decomposer directly on the litter. However, Butenschoen et al. (2007) showed that leaves (rye leaf material) can be an additional food source for endogeic earthworms indeed although the direct effect of endogeic earthworms on litter decay is generally negligible (Huang et al., 2020). Overall, studies on the effects of endogeic earthworms on ecosystem services seem to be less represented, compared with anecic earthworms. The most likely reason for this is the fact that their impact on soil properties are mostly indirect and cannot be measured as easily as for anecic earthworms. However, this ecological group of earthworms also contributes significantly to the promotion of soils, such as through bioturbation. Due to their higher egestion rates (Taylor and Taylor, 2014) and their feeding preference (soil organic mixtures), they often contribute to soil bioturbation in agricultural settings in a larger extent than anecic earthworms, especially in ploughed fields (Torppa and Taylor, 2022).

One reason why no effect of endogeic earthworms occurred in **study 3**, neither on microbial biomass and ergosterol concentrations nor on CO_2-C emissions rates, may be the fact that the soil was generally rich in nutrients. A stronger effect might appear in nutrient poor arable soil, where an increased nutrient availability due to the presence of earthworms might be of more importance (Butenschön, 2007). However, the global meta-analysis of Huang et al. (2020) demonstrated that the presence of all three functional groups (anecic, epigeic, endogeic) at the same time enhance litter mass loss strongest, compared with a single group. Thus, a way of tillage should be chosen supporting a wide range of soil biota in agricultural soils as every single ecological group contributes to ecosystem services, which are needed to ensure a sustainable agriculture.

Effects of tillage on greenhouse gas emissions

Tillage causes a disturbance of the soil system, increasing microbial metabolic activity and leading to short-term CO_2 emissions (Jia et al., 2021). The review of Smith and Chalk (2021) highlights that NT systems reduce C losses compared with ploughing. Commonly, a "flush" of CO_2 occurs immediately after tillage (Reicosky, 1997). Also in the mesocosm experiment in **study 3**, these flushes were observed when soil columns were inverted manually before the actual start of the experiment (Chapter 6).

As most CO_2 is evolved by heterotrophic microorganisms (Kuzyakov, 2005), favorable conditions for microorganisms and ergo a high efficiency are crucial in order to mitigate GHG emissions. There are different locations of CO_2 production in the soil, from which one important is the soil rhizosphere (Kuzyakov, 2006). The process of microbial SOM decomposition is the main process affecting the atmospheric CO_2 (Kuzyakov, 2005). Most C is respirated by microbes as CO_2, whereas only a small amount of C is retained and stays as stable organic matter (Buragohain et al., 2019). Previous studies showed that an additional C source such as biochar can reduce the degradation of native SOC and decrease the metabolic quotient

(Bamminger et al., 2014). An increase in one unit of microbial respiration per unit MBC (qCO_2) can lead to an even larger increase in CO_2 an N_2O fluxes (Haohao et al., 2017). Therefore, the aim of sustainable agriculture should be to develop systems where C inputs exceed losses, which in turn increase SOC levels and contribute to C sequestration. However, more important than a high conversion rate, which is generally desirable (e.g. the degradation of crop residues), is the efficiency of the microorganisms, which may be influenced also by abiotic factors. Significant differences in the metabolic activity of microorganisms between tillage treatments could not be found at any of the sites in **study 1** (Chapter 4). In contrast, previous studies showed that long-term CO_2 emissions in NT fields are usually lower than in MT or CT fields (Omonode et al., 2007; Ussiri and Lal, 2009). In **study 1**, qCO_2 levels were relatively low at the Romanian (high fertility, clay-rich). Clay is known to be a key factor in the process of C sequestration (Churchman et al., 2020). Reactive surfaces which are able to bind organic matter, contribute to its persistence (Schweizer et al., 2021). The review of Smith and Chalk (2021) emphasizes that shifts in $\delta^{13}C$ are greatest in the sand fraction, whereas smallest changes are observed in the clay fraction which means turnover of SOC is relatively low in fine fractions, whereas a faster turnover (replacement of original carbon) is associated with coarse and fine sand. Further, at the Swedish site the acidification might have increased qCO_2 values, indicating stress for microbes by the environment (Anderson and Domsch 1990, 2010). To sum up, in the long-term, other abiotic factors may have a higher impact on microbial respiration rates than differences in tillage. In the perspective of soil management all measures that allow or even support high C sequestration in relation to a low energy demand for biological activity should be preferred, in order to increase C levels. This is especially the case when soil conditions are given, which are favourable for soil organisms.

In addition, tillage may also strongly affect N_2O gas emissions from soils. Microbial processes (nitrification and denitrification) are the source of nitrous oxide emissions in soils (Freney, 1997). The process of microbial denitrification occurs when the demand of oxygen exceeds its supply (Muñoz et al., 2010). This situation can occur when ploughing disrupts the connectivity of pores and channels to deeper soil depths (Dowdell et al., 1979), leading to a poor aeration (Stepniewski et al., 1994). Simulated tillage (SI) in **study 3** (Chapter 6) greatly increased N_2O-N emissions. The results of a comparison of 25 different sites by Rochette (2008) showed that no-tilled fields with poor aeration generally had higher N_2O emissions than tilled fields. This points to aeration being one of the main factors controlling N_2O fluxes. In contrast, in the microcosm experiment in **study 3** N_2O-N emissions under SI (simulated ploughing) were higher compared with soil NI (simulated no-tillage). However, this may be due to the fact that the original soil columns used for both treatments had the same tillage history (minimum tilled since 1970). Inversion of these columns in the SI treatment changed the connectivity of biopores and hampered soil aeration. As a result N_2Oemissions increased to higher values than in the undisturbed, NI columns. This study shows how important undisturbed biopores are for gas emissions and highlights the importance of soil aeration as an ecosystem services driven by soil biota. The results suggest that agricultural management needs to support and maintain the natural soil structure in order to enhance oxygen flows and thus reduce N_2O emissions. Further research is be needed to give recommendations on a field-level.

How tillage alters soil microbial properties

The effect of tillage on the fungal biomass was investigated using ergosterol concentrations as a proxy in **study 3** (Chapter 6). Here, similar to microbial biomass (C and N) values, ergosterol concentrations were closely linked to the placement of crop residues in the soil profile and seemed less affected by a disruption of the soil column due to tillage simulation. This was also confirmed by the results of **study 1** (Chapter 4) where tillage altered the distribution of SOC and MBC and in the three soil depths (0-30 cm). In particular, soil MBC was increased in the upper 10 cm at plots with reduced tillage (MT, NT), although this increase was partially balanced by an enrichment in deeper layers. Such a stratification is characteristic for conservation tillage systems (Ahl et al., 1998; van Capelle et al., 2012) and high concentrations of MBC close to the soil surface are essential for the soil-plant-interaction. Plants foster microorganisms around their roots, e.g. by the release of photosynthates that are an important C source for microbes (van Veen et al., 1989). Good conditions for microorganisms can promote their activity and accelerate the access to soil-borne nutrients for the crops (Jacoby et al., 2017). Therefore, the promotion of microbial activity is also important for nutrient uptake and crop development. Further, microorganisms are known to have a key role in aggregate stability and soil formation (Artz et al., 2010; Siddiky et al., 2012), which in turn enhance the soils resilience to erosion, e.g. due to extreme weather conditions (Barthès and Roose, 2002; Haddaway et al., 2017).

Results from **studies 1 & 3** (Chapter 4 & 6) emphasize how important organic material e.g. crop residues as food resources for microorganisms are. An increased availability of SOM for microbes, indicated by a greater ratio of MBC to SOC could be observed in inverted soil columns between 10-20 cm soil depth where residues were incorporated at a depth of 15 cm (Chapter 6). For the mesofauna **study 2** (Chapter 5) showed that a change in collembolan community composition over time was associated with an increasing litter quality, indicated by a lower C/N ratio. The decomposition of plant-derived material by soil biota is known to improve the nutrient availability for crops (Ma et al., 2012; Malik et al., 2013). Collembola access to litter increased litter C losses. Generally, the direct effect of detritivorous mesofauna on decomposition processes is considered to be rather low, whereas the indirect effects via the stimulation of microbial activity are of greater importance (Parker et al., 1984; Lavelle et al., 1993; Hättenschwiler et al., 2005).

The significant effect of NT on soil parameters was most evident in Spain on a sandy *Calcic Fluvisol* (**study 1**, Chapter 4). A mulch layer can help to increase soil water availability, decreases water evaporation (Vita et al., 2007) and promotes microbial activity (Aziz et al. 2013; Badagliacca et al. 2015; Balota and Auler 2011), especially in dry regions (López-Fando et al., 2007). This might be the most likely reason for enhanced microbial conditions at the Spanish site of

study 1. Further, sandy soils are more affected by disturbances while fine soil particles are a key component for soil recovery and resilience (Gregory et al., 2007; Bonetti et al., 2017). The clay-rich *Phaeozem* at the Romanian site may have buffered disturbances due to tillage. Indeed, differences in microbial soil parameters between tillage applications could not be detected under the given conditions at this site. Zuber and Villamil (2016), based on a meta-analysis, emphasize that finer particles, and clay in particular, play an important role in the cation exchange capacity as well as the water holding capacity of soils. Therefore, soil conditions in Romania probably promoted microorganisms and reduced the effect of tillage.

Abiotic factors influencing decomposition processes

The application of MT and NT in Sweden at an *Eutric Cambisol* was of special interest, since the comparison between different reduced tillage systems on the same field site is rare (**study 1**, Chapter 4). Especially in cold-temperate regions, NT can cause problems like reduced yields (Arvidsson et al., 2014). Climate is one important factor steering litter decomposition rates (Couˆteaux et al. 1995). Decomposition processes are usually slower under NT, compared with CT (Burgess et al., 2002; Lupwayi et al., 2004). The main reason for these differences might be the fact that crop residues are more susceptible to fluctuations in temperature on the soil surface compared with incorporated residues.

The decay of organic matter in soils is difficult to predict as it does not follow a constant rate, slow down over time and depend on litter quality, temperature and moisture (Sierra et al. 2017; Waksman and Gerretsen 1931). Soil temperature has a strong impact on the activity of soil biota (Gongalsky et al., 2008). In **study 2**, differences in soil temperature between CT measured at 20 cm soil depth and at 10 cm soil depth for MT were marginal and seemed negligible, indicating that only difference for NT can be expected. Although there was no differences in soil temperature between the two tillage systems, litter decomposition rates of maize leaves in the litterbags in **study 2** differed in time. After an initial litter C loss of 36-41% from October to December, C losses during winter (December to March) were quite small (10-16%). Besides the effect of litter quality (e.g. C/N ratio), these differences in C losses could be associated to a low activity of the soil fauna during days with low soil temperatures. Gongalsky et al. (2008) showed that there is no feeding activity of enchytraeids, collembolans and mites at freezing temperature (-4 °C).

The choice of the 'right' tillage system

Although the negative effects of ploughing are well-known, CT is one of the most common management in European agroecosystems. The main reasons why, particularly organic farmers, in Germany are still reluctant to switch to conservation tillage practices are weed pressure and possible yield-reductions (Zikeli and Gruber, 2017). However, ploughing can decrease soil quality as indicated in the present thesis by a reduction in SOC and MBC stocks and concentrations (**study 1**), a lower rate of litter decomposition (**study 2**) and an increase in N_2O-N emissions (**study 3**) due to soil inversion. Nevertheless, the positive effects of reduced tillage practices on soil parameters depend strongly on abiotic factors (e.g. climate, soil texture), as indicated by the results of **study 1** (Chapter 4). Similar results are presented in the review of Jia et al. (2019), investigating the efficiency of conservation tillage in different regions of China. It can be deduced that the effectiveness of conservation tillage practices for microbial and physicochemical soil parameters can only be predicted to a limited extent.

The present thesis shows that ploughing often supports only the functional faunal groups, which are mostly independent from an undisturbed soil surface (**study 2 & 3**). To attain the wide range of services provided by soil biota and thus to reach and maintain soil stability and health in the long-term, the performance of the different organisms groups must receive more attention. Decisions for tillage applications need to be taken on the basis of supporting a wide range of soil faunal groups and species and enhancing SOC as well as MBC (most importantly close to the soil surface). To make farmers adopt reduced tillage systems that promote soil life, the advantages must be noticeable for them. Therefore, it is still necessary to emphasize the value of soil biodiversity and the services provided by soil organisms as a benefit to farmers, e.g. the potential of soil biodiversity to ensure yield stability in the long-term (Biodiversa, 2021). With regard to ecosystem services, diversity in functional traits is the key (Kardol et al., 2016). If services provided by soil biota decline, changes in SOC, a reduction in maximum yields and a lower fertiliser-use efficiency can follow (Brady et al., 2015).

In summary, the advantages of conservation tillage counteract the arguments for CT. Nevertheless, farmers will only change their systems if the benefits of alternative tillage applications outweigh their arguments for ploughing. What farmers can expect from reduced tillage is an enhanced biological activity and an improved nutrient availability for plant growth. Further, by preserving the natural structure of the soil, water infiltration and soil aeration can be advanced. Still, concerns about weed pressure are a major issue. Here, alternatives in weed control need to be found, like surface cover with crop residues, cover crops and an adapted crop rotation (Pesticide Action Network Europe, 2017). To reduce the risk of lower yields, conservation tillage should be adapted to regional conditions, as NT can lead to yield losses, especially in cold-temperate regions (Arvidsson et al., 2014). The present thesis also shows that NT is not necessary needed to promote soil biota but that also MT has a great potential to improve soil conditions. Depending on the region, MT can be just as advantageous for soil life or even better compared to NT. Based on the results of the present work, a combination of conservation tillage and crop residue retention are recommended as valuable tools for the promotion of the entire soil biota and their functions and services.

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Chapter 8

General Conclusion

In the present thesis, soil biota showed different responses to tillage reductions. The positive effect of reduced tillage was indicated in **study 1** by enhanced microbial biomass and activity as well as optimized physicochemical soil properties in terms of sustainability and fertility. Nevertheless, **study 2 & 3** highlight that even ploughing can promote specific functional groups of soil organisms mainly those living in the deeper soil and that are therefore not dependent on food source at the soil surface. Hence, the question of sustainable tillage systems seems still to be important and complex. The impact of agricultural management on soil biota and its diversity is highly specific. Moreover, the present thesis showed that soil fauna is able to promote the decomposition of organic substances, enhances nutrient availability and increases microbial activity. The presence of soil biota can thereby help to ensure nutrient availability and support the resilience of soils by improving soil structure and stabilizing carbon pools. Most importantly, these services are not provided by a single specific faunal group, but depend on the interaction of many soil organism groups, that means the entirety of the biodiversity in the soil ecosystem. Therefore, tillage strategies are needed which support soil biodiversity as a whole. One important factor to enhance soil life is the retention of crop residues in the field as it provides an important food and habitat source for earthworms and the soil mesofauna. With regard to microorganisms, ploughing should be avoided in order to achieve an increase in microbial activity close to the surface and thus improve the nutrient supply for the crop. One of the consequences of soil disturbance due topsoil inversion is the contribution of agriculture to global GHG emissions. Therefore, a tillage system with less intensity is urgently needed. Whether NT or MT should be applied depends on the region and in particular on the local climatic conditions and soil properties. In arid areas, covering the soil surface with crop residues in NT systems can be beneficial to maintain the soil moisture that soil organisms depend on. Additionally, NT can support a wider range of species as it also provides opportunities for surface-feeding organisms. In cold-temperate areas, MT might also be an appropriate tool as suggested by the results of the present thesis. To sum up, supporting soil biota by certain management decisions in cropping systems enables farmers to take advantages of the services provided by soil organisms, which allows further reductions of artificial inputs like fertiliser in the long-term. The benefits for both, biodiversity and farmers can only be achieved if there are profitable alternatives for famers to current technologies, which need to be developed together with economists and policy makers.

PART V

Chapter 9

Thesis Declaration

I hereby confirm that I have written this doctoral thesis independently, that I have not used other sources or facilities other than the ones mentioned, that I have not used unauthorized assistance and that I have not submitted this thesis previously in any form for another degree at any university or institution.

Göttingen, 20th of April 2022

Ilka Engell

Chapter 10

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Chapter 11

List of publications

Peer-reviewed articles

ENGELL, I., LINSLER, D., SANDOR, M., JOERGENSEN, R. G., MEINEN, C., POTTHOFF, M. (2022): The effects of conservation tillage on chemical and microbial soil parameters at four sites across Europe. *Plants* 11 (13), 1747.

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Other publications

Jacobs, A., Schrader, S., Babin, D., Beylich, A., Brunotte, J., Dauber, J., Emmerling, C., **Engell, I.**, Flessa, H., Hallmann, J., Hommel, B., Klages, S., Lehmhus, J., Meyer, M., Meyer-Wolfarth, F., Potthoff, M., Runge, T., Scherber, C., Schikora, A., Schorpp, Q., Schulz-Kesting, K., Smalla, K., Tebbe, C. C., van Capelle, C., Varrelmann, M. (2022): Lebendige Böden - fruchtbare Böden. Herausgeber: Bundesanstalt für Landwirtschaft und Ernährung, Bundesinformationszentrum Landwirtschaft. Erstauflage, 48 Seiten.

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Conference contributions

Engell, I., Graefe, U., Linsler, D., Beylich, A., Guernion, M., Guzmán, G., Hoeffner, K., Landa, B. B., Sandor, M., Taylor, A., Torppa, K., Peres, G., Potthoff, M.: Earthworm communities in European arable sites as affected by different tillage reduction measures according to local practices – Results from the SoilMan-Project. ISEE 12, Rennes, France, July 2022 (poster).

Potthoff, M., Faber, J., van Doorn, A.; Pérès, G. Taylor, A.; Plaas, E.; Beylich, A.; Nicolai, A., Meyer-Wolfarth, F., Schrader, S., **Engell, I.**, Linsler, D.: The economy of earthworms; values and incentives beyond ecology. ISEE 12, Rennes, France, July 2022 (oral presentation).

SCHMOOCK, I., LINSLER, D., SANDOR, M., JOERGENSEN, R.G., POTTHOFF, M.: Long-term effects of tillage intensity on the distribution of microbial biomass and activity in four arable field sites across Europe. EGU General Assembly, Vienna, Austria, May 2020 (oral presentation via video).

SCHMOOCK, I., LINSLER, D., SANDOR, M., JOERGENSEN, R.G., POTTHOFF, M.: Long-term effects of tillage intensity on the distribution of microbial biomass and activity at different soil depths across European field sites. 8th Rhine-Waal Soil and Plant Ecology Seminar, Kleve, Germany, February 2020 (oral presentation).

SCHMOOCK, I., LINSLER, D., SCHRADER, S. , TAYLOR, A., POTTHOFF, M.: Effects of tillage intensity in arable land on ecosystem services driven by soil biota. Conference "Soil Biota driven Ecosystem Services in European Agriculture", Braunschweig, Germany, October 2019 (poster).

SCHMOOCK, I., LINSLER, D., SCHRADER, S. , TAYLOR, A., POTTHOFF, M.: Effect of varying food placements due to tillage on two earthworm species (*Lumbricus terrestris* vs. *Octolasion cyaneum*), representing different ecological groups. Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Münster, Germany, September 2019 (poster).

LINSLER, D., BENDER, S., **SCHMOOCK, I.**, POTTHOFF, M.: Einfluss von Anneliden und der Lage von organischem Material auf die Verteilung von wasserstabilen Bodenaggregaten. Annual Meeting of the Soil Science Society of Switzerland and the German Soil Science Society, Bern, Switzerland, August 2019 (poster).

GERIGK, J., LINSLER, D., **SCHMOOCK, I.**, POTTHOFF, M., JOERGENSEN, R.G.: Mikrobielle Bodeneigenschaften von landwirtschaftlichen Böden in Europa. Annual Meeting of the Soil Science Society of Switzerland and the German Soil Science Society, Bern, Switzerland, August 2019 (poster).

SCHMOOCK, I., LINSLER, D., SCHRADER, S. , TAYLOR, A., POTTHOFF, M.: Impacts of agricultural management practices: Is the contribution of soil biota to litter breakdown affected by different positioning of organic material due to tillage? Annual Meeting of the Soil Science Society of Switzerland and the German Soil Science Society, Bern, Switzerland, August 2019 (oral presentation).

LINSLER, D., BENDER, S., **SCHMOOCK, I.**, POTTHOFF, M.: Influence of annelids and the location of organic material on water-stable aggregate distribution. EGU General Assembly, Vienna, Austria, April 2019 (poster).

SCHMOOCK, I., LINSLER, D., SCHRADER, S., TAYLOR, A., POTTHOFF, M.: Soil functional impacts of annelids affected by different vertical placement of crop litter due to tillage. EGU General Assembly, Vienna, Austria, April 2019 (poster).

Hanisch, J., **Schmoock, I.**, Linsler, D., Potthoff, M., Scheu, S.: Soil biodiversity in agricultural fields across Europe as affected by farm-based soil management practices and regional constraints. Building Bridges Conference in Göttingen, Germany, March 2020 (oral presentation).

Schmoock, I., Linsler, D., Schrader, S., Taylor, A., Potthoff, M.: Ökosystemleistungen sichtbar machen - Bodenorganismen auf der Spur. 2. Symposium der Kommission VIII der DGB, Wahrnehmung und Bewertung von Böden in der Gesellschaft, Leipzig, Deutschland, October 2018 (oral presentation).

Schmoock, I., Linsler, D., Schrader, S., Taylor, A., Potthoff, M.: The effect of ploughing on earthworms and enchytraeids – Soil biotic properties and carbon dynamics. 48th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland, Vienna, Austria, September 2018 (poster).

Schmoock, I., Linsler, D., Schrader, S., Taylor, A., Potthoff, M.: Can annelid induced soil functions be managed by the choice of different tillage practices? Effect of enchytraeids and earthworms on soil biotic properties and carbon dynamics. International earthworm congress, Shanghai, China, June 2018 (oral presentation).

Schmoock, I., Linsler, D., Potthoff, M.: Indicating resilience and sustainability in arable soil systems across Europe. Response of soil microbial biomass to tillage reductions in three European countries. EGU General Assembly, Vienna, Austria, April 2018 (poster).

Schmoock, I., Linsler, D., Potthoff, M.: Indicating resilience and sustainability in arable soil systems across Europe; Response of the soil microbial biomass to tillage reductions in four European countries. GfÖ, Ghent, Belgium, December 2017 (poster).

Schmoock, I. & Gehrt, E.: Verbreitung und Charakterisierung der Wölbackerböden in Niedersachsen. DBG Conference 2017, Göttingen, Germany, September 2017 (poster).

Potthoff, M., Pérès, G., Taylor, A., Schrader, S., Landa, B., Nicolai, A., Sandor, M., Öpik, M., Guzmán, G., Bergmann, H., Cluzeau, D., Banse, M., Bengtsson, J., Guernion, M., Zaller, J., Roslin, T., Scheu, S., Gómez Calero, J-A., **Schmoock, I.**, Linsler, D., and all collaborators: Ecosystem services driven by the diversity of soil biota – understanding and management in agriculture. EGU General Assembly, Vienna, Austria, April 2017 (oral presentation).