

# **Ecological groups of Collembola in agroecosystems as affected by farm-based management practices**

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# Summary

Present day agriculture is identified as a main threat to biodiversity. At the same time, the productivity of agricultural systems is relying on ecosystem services provided by biota. In order to conserve critical ecosystem services for future generations preserving the functions of the soil is one main goal of sustainable agriculture. Nevertheless, the impact of management practices on soil biota providing critical ecosystem services remains ambiguous. The high level of complexity in combination with limited taxonomic knowledge and the opaqueness of the soil hampers general conclusions. To develop sustainable management practices a better understanding of how farm-based management practices shape soil biota communities is needed. Thereby, trait based approaches can be used to increase mechanistic understanding while at the same time circumvent time consuming species identification. In the present thesis we utilized trait based approaches to assess effects of farm-based management practices on Collembola communities.

In Chapter 2 we investigated how Collembola communities are affected by reduced tillage in comparison to conventional tillage practices. We evaluated Collembola communities in five long-term tillage field trials across European countries (Sweden, Germany, France, Romania and Spain). The study included differing bioclimatic regions to assess general effects of agricultural practices on soil Collembola across Europe. We found different ecological groups of Collembola defined by morphological traits to be differentially affected. Epedaphic Collembola were detrimentally affected while especially euedaphic Collembola were fostered by conventional tillage. Further, we found Collembola communities to be differentially affected depending on the dominating ecological groups at each field site. Reactions of Collembola communities resembled effects of tillage practices on organic carbon and total nitrogen revealing similar changes in depth distributions. Our results suggest nutrient supply in terms of organic carbon and total nitrogen in combination with favourable soil moisture conditions to be of critical importance for soil Collembola. The displacement of litter resources by tillage into deeper soil where they are available under preferable moisture conditions turned deeper soil layers into habitable space utilized especially by euedaphic species.

In Chapter 3 we investigated the effect of Collembola and tillage on litter decomposition. We conducted a litterbag experiment in a long-term tillage field trial in Germany with reduced and conventional tillage. We buried litterbags filled with maize litter at the respective depth of tillage. Further, we investigated the incorporation of litter derived carbon into Collembola to quantify their dependence on litter resources under different tillage regimes. Collembola promoted decomposition by enhancing carbon loss and the transformation of litter into high quality resources as indicated by C/N ratios. Conventional tillage favoured colonization of litterbags by Collembola, especially that of

euedaphic species. Furthermore, in conventional tillage fields Collembola depended more on litter material than in reduced tillage fields. In conclusion, Collembola accelerate litter transformation by increasing carbon loss and nitrogen accumulation. Facilitative effects on nitrogen capture from decomposing litter material by crops may contribute to the sustainability of arable systems.

In Chapter 4 we investigated the effect of intercropping and genetic variation of crop species on Collembola communities. We sampled Collembola communities in two field trials in Germany containing monocultures of four genotypes of faba bean and intercropped stands of each genotype with winter wheat. The field sites differed in soil carbon and nitrogen content. Intercropping only promoted Collembola in the low carbon field site, but the effects were restricted to hemiedaphic Collembola while eu- and epedaphic Collembola remained unaffected. Further, at the low carbon site Collembola benefited from the bean genotype characterized by high tillering and short shoots which yielded the highest root biomass. The results suggest that root and shoot overyielding in intercropped stands led to increased availability of carbon resources for Collembola. Our results indicate that intercropping and the choice of plant genotypes promote Collembola communities if resources are scarce. Presumably the beneficial effects are mediated by the provision of litter and root resources and improved moisture conditions as well as habitat diversification.

Overall, the results of Chapters 2 and 4 indicate the availability and quality of resources in combination with preferable moisture conditions to be of critical importance for Collembola. These abiotic factors determine habitat suitability and they surpass the importance of mechanical disturbance and soil compaction. Results of Chapter 3 highlighted the dependency of Collembola on litter resources in agricultural systems. In addition, it proved that Collembola accelerate litter decomposition thereby contributing to crucial ecosystem services. Altogether, the results of this thesis indicate that the response of Collembola to farm-based management practices depend on underlying changes in abiotic conditions. Generally, we showed the suitability of trait based approaches as tool to improve mechanistic understanding of the response of Collembola communities to farm-based management practices, which is urgently needed for the sustainable management of arable systems.

# 1 General Introduction

## 1.1 Sustainable agriculture

Agriculture is considered a main contributor to global biodiversity loss (McLaughlin and Mineau 1995). Agroecosystems are highly manipulated production systems characterized by tillage, fertilization and the use of pesticides, which are used to optimize yields (McLaughlin and Mineau 1995), but also form important habitat conditions for biota (Moonen and Barberi 2008). Arable systems typically are characterized by low diversity, while increased diversity is assumed to promote ecosystem resilience and therefore sustainability (Moonen and Barberi 2008). Fostering biodiversity in agroecosystems therefore may improve the ecosystem services provided by these systems including primary production and the provisioning of food, fiber and other natural products (Moonen and Barberi 2008; Kibblewhite et al. 2008).

Biodiversity in the soil is particularly high due to spatial heterogeneity in combination with large inhabitable area and the wide range of biota colonizing soils (Giller 1996; Gardi et al. 2009). Importantly, biodiversity in soil is critical for ecosystem processes including decomposition, nutrient cycling, maintenance of soil fertility and pest control, which are essential for human wellbeing (Giller 1996; Gardi et al. 2009; Kibblewhite et al. 2008). Therefore, healthy agricultural soils and their sustainable management is essential for the production of high quality food and fibre while at the same time delivering further ecosystem services essential for human wellbeing such as carbon transformation, soil structure maintenance and biological population regulation (Kibblewhite et al. 2008). Although it is critical to understand which processes in managed ecosystems affect the diversity and functioning of biota, knowledge on these processes is limited and this applies in particular to the belowground system (Giller 1996). This is mainly due to the opaqueness of soil and the limited taxonomic knowledge on soil biota (Giller 1996; Gardi et al. 2009). To preserve the long-term functioning of and services provided by soils is the main objective of sustainable agriculture (McLaughlin and Mineau 1995). This is of particular importance as soils are considered the most precious non-renewable resource (Giller 1996). Sustainable management of soil faces the challenge to ensure the long-term provisioning of ecosystem services, and at the same time optimize yield and maximize biodiversity (Kibblewhite et al. 2008).

## 1.2 Soil fauna

Although key steps in major elemental cycles are ultimately conducted by soil microorganisms, the soil fauna has a profound regulatory role in soil processes (Bender et al. 2016). Soil animals incorporate and comminute litter material, control microbial communities, protect plants against pests and maintain the structural porosity and build aggregations in soils through burrowing, casting and nesting activities (Lavelle et al. 2006; Bender et al. 2016). These activities lead to increased water infiltration and supply, decomposition and nutrient cycling, soil formation and primary production (Lavelle et al. 2006). Therefore, detritivore soil fauna are crucial for ecosystem services like decomposition contributing to fertility and productivity of agroecosystems (Seastedt 1984; Kampichler and Bruckner 2009). In agricultural systems they heavily depend on the input of plant residues or organic fertilization as food source (Kautz et al. 2006). Effects of soil fauna on the decomposition of plant residues are mediated through the stimulation of the soil microflora, but also the fragmentation and digestion of the litter increase decomposition and therefore the fertility of the soil (Seastedt 1984; Whalen and Hamel 2004). Further, faecal pellets produced by soil mesofauna provide an increased surface area for decomposition and build an integral component of soil structure (Behan-Pelletier 2003; Potapov et al. 2020). Collembola in agricultural soils are among the most abundant soil animals and together with Acari account for approximately 95 % of total soil arthropods (Seastedt 1984; Winter et al. 1990; Rodgers et al. 2018; Potapov et al. 2020). Collembola occupy all trophic levels, but most of them are microphages or detritivores feeding on litter material and associated fungi (Chahartaghi et al. 2005; Potapov et al. 2018, Pollierer and Scheu 2021). In addition, they promote fungi by dispersing spores on their body surface or through their digestive system, but also protect crops by reduction of pest species (Behan-Pelletier 2003; Meyer-Wolfahrt et al. 2017; Potapov et al. 2020). Due to their high abundance they are of agronomic importance. The distribution of Collembola in soil correlates with the availability of food resources and beneficial moisture and temperature conditions (Verhoef and Nagelkerke 1977; Berg and Bengtsson 2007; Vignozzi et al. 2019). Additional limiting factors can be bulk density and soil compaction (Winter et al. 1990; Jucevica and Melecis 2006; Dubie et al. 2011). Collembola can be assigned to three ecological groups which are closely related to their adaptation to vertical distribution in soil (Vandewalle et al. 2010; Salmon et al. 2014). Epedaphic Collembola are adapted to life at the soil surface, bear long antennae and furca in combination with well-developed visual apparatus and adaptations against desiccation in form of dark coloration and presence of hairs or scales covering the body. Hemiedaphic species mainly inhabit the litter layer and therefore show a combination of adaptations suitable for life above- as well as belowground. They often have a furca and long antennae, but reduced numbers of ocelli and often lack coloration and protective structures like hairs and scales. Fully adapted to belowground life are euedaphic species. In these species adaptations to aboveground life are reduced, reflected by short antennae, or absent including furca,



ocelli, coloration and protective hairs and scales (Parisi et al. 2005; Vandewalle et al. 2010). Traits reflecting the level of adaptation of animals to the soil environment can be used to assess characteristics of the habitat (Parisi et al. 2005). Collembola are considered useful indicators for soil quality (Greenslade et al. 2007; Rodgers et al. 2018). Such indicators have to be sensitive to changes in soil management, correlate with soil functions, help in elucidating ecosystem processes, and have to be easy and cheap to assess (Doran and Zeiss 2000). Since species identification is often time consuming and expensive, the use of traits of species accessible by non-specialists might be a promising approach (Parisi et al. 2005). In order to circumvent time consuming species identification, approaches focusing on traits and ecological groups can be used as indicators across communities and ecosystems with differing species compositions allowing simplified assessment (Parisi et al. 2005; Pey et al. 2014; Moretti et al. 2017). Further, traits are important for understanding the mechanisms which drive community assemblages as well as their response to stress and management practices (Parisi et al. 2005; Vandewalle et al. 2010; Widenfalk et al. 2015). In soil trait based approaches have been used to indicate soil quality using a number of animal groups including earthworms, nematodes, mites, enchytraeids and collembolans (Vandewalle et al. 2010). Morphological characters reflecting adaptation to life in soil include the reduction of appendices, adaptations for flying or jumping, pigmentation, protection against desiccation and ocelli as explained above (Parisi et al. 2005). Similar traits are used to identify ecological preferences of Collembola across large spatial scales and management practices (Vandewalle et al. 2010; Salmon et al. 2014). The high complexity of the response of Collembola to disturbance can be elucidated using trait based approaches analysing the responses of different ecological groups, which may help to understand the inconsistency of results on, for example, tillage effects on Collembola (van Capelle et al. 2012). The complexity of the response of Collembola also likely applies to other soil biota. Depending on traits such as body size, burrowing ability, and food and habitat preferences, the response of soil biota to tillage varies. Therefore, generalizations on the effect of management practices on soil biota are difficult to draw (Stinner and House 1990, van Capelle et al. 2012).

### 1.3 Tillage

In conventional agriculture ploughing is used to loosen and turn the soil aiming at reducing weeds, counteracting nutrient leaching, facilitating seeding and cleaning the soil surface (van Capelle et al. 2012, Rodgers et al. 2018). Plant residues of previous crops are integrated into the soil in order to enhance decomposition (Winter et al. 1990). However, the mechanical stress exerted by tillage is considered as a main threat to soil biodiversity alongside with chemical stress (van Capelle et al. 2012). The destruction of soil structure is an undesired side effect caused by tillage leading to soil surface

sealing, erosion, soil compaction and decreased soil organic matter (Winter et al. 1990; McLaughlin and Mineau 1995; van Capelle et al. 2012). In addition, in the long-term tillage results in poor soil aggregation, reduced porosity and poor water retention (McLaughlin and Mineau 1995; Vignozzi et al. 2019). Generally, it is assumed that soil fauna is vulnerable to mechanical disturbance by tillage (Petersen 2002). Compaction, decreased soil moisture and disruption of root systems is negatively affecting soil fauna reducing diversity and biological activity in tilled soils (Dubie et al. 2011; Vignozzi et al. 2019). Displacement of individuals can bury or kill them and the displacement of litter material at unreachable soil depth are additional adverse impacts on soil fauna (Stinner et al. 1988). Therefore, the European Union is recommending conservation rather than conventional tillage (Vignozzi et al. 2019). The spectrum of reduced tillage practices ranges from non-inversion tillage to direct seeding which leaves crop residues on or close to the soil surface and reduces physical disturbance (McLaughlin and Mineau 1995). Thereby, soil compaction, erosion and runoff are reduced, while water infiltration is increased (Rodgers et al. 2018). Effects of reduction of tillage intensity on soil biota differ depending on habitat requirements, burrowing ability, food preferences and body size (van Capelle et al. 2012). Large organisms tend to be more sensitive to high tillage intensities while smaller soil biota like mites and Collembola are less sensitive to mechanical disturbance and show inconsistent responses to changes in tillage intensity (Kladivko 2001; van Capelle et al. 2012). Collembola in particular vary in the displayed responses. Beneficial effects of reduced or no-till practices compared to conventional tillage have been described in a number of studies (Stinner et al. 1988; Brennan et al. 2006; Vignozzi et al. 2019). However, these effects seem to be constrained to the upper soil layers as most studies focus on the top 10 cm of the soil and differences may disappear when deeper soil layers are considered as well (Winter et al. 1990). Non-inversion as well as conservation tillage have been found to reduce Collembola abundances especially in deeper soil layers compared to conventional tillage (Petersen 2002; Reeleder et al. 2006). Overall, abundance and diversity of Collembola in reduced tillage systems is lower compared to conventional tillage systems, while the reduction varies with soil type and the ecological group of Collembola (van Capelle et al. 2012).

#### 1.4 Mixed cropping systems

Although it is widely accepted that biodiversity increases ecosystem functioning, monoculture cropping systems typically dominate agricultural systems as pesticides and synthetic fertilizers allow high yields (Horwith 1985; Machado 2009). These high input systems, however, adversely affect the quality of soil, water and air, and threaten biodiversity (Matson et al. 1997, Stoate et al. 2001; Kleijn et al. 2006). As an alternative mixed cropping systems based on growing different crops simultaneously at the same field receive increased attention (Vandermeer 1992; Lithourgidis et al. 2011). By

complementarity and facilitation mixed cropping systems bare the potential to increase resource use and crop yield (Hauggaard-Nielsen et al. 2008; Jensen et al. 2010; Duchene et al. 2017). Complementarity occurs when plants utilize different resources or the same resource at different times or at different locations, leading to more efficient exploitation of available resources and reduced competition (Hooper and Vitousek 1997; Duchene et al. 2017). Facilitation, on the other hand, is a positive effect exerted from one organism to another (Michalet and Pugnaire 2016). Both mechanisms result in increased productivity of mixed cropping systems compared to monocultures known as transgressive overyielding (Nyfeler et al. 2009). In order to optimize the beneficial effects of complementarity and facilitation and minimize niche overlap crop species for mixed cropping have to be chosen accordingly (Brooker et al. 2015; Litrico and Violle 2015). Furthermore, not only species have to be chosen accordingly. The same mechanisms apply to varieties or novel genotypes, which differ in morphological and physiological traits (Collins et al. 2003; Annicchiarico and Proietti 2010). Morphological traits like root and shoot architecture affect the use of resources, water and light, while physiological differences lead to differences in the amount and quality of litter resources for soil biota (Barot et al. 2017). Crop breeding usually aims at optimizing yield in monoculture and does not consider combinations of traits favourable in mixed cropping systems, enhancing complementarity and facilitation (Litrico and Violle 2015; Barot et al. 2017). In fact, performance of plant genotypes may differ from pure stands when cultivated in mixed stands which will ultimately affect mixture performance (Collins et al. 2003; Litrico and Violle 2015). However, genetic improvement by plant breeding may enhance plant species compatibility in mixture, stabilizing productivity of mixed cropping systems (Annicchiarico and Proietti 2010; Barot et al. 2017).

It has been shown that aboveground diversity promotes detritivore soil fauna including Collembola in terms of abundance and diversity (Eisenhauer et al. 2010; Eisenhauer et al. 2011; Sabais et al. 2011). Thereby, effects may be caused by individual links between plant and animal species or by a range of soil animals being promoted by single plant species (Hooper et al. 2000). Two main effects are supposed to result in higher abundance and diversity of soil fauna. First, a wider range of diverse resources provided by a diverse plant community including high quality litter and a more even provision of plant resources in time due to differing phenologies (Spehn et al. 2000; Sabais et al. 2011). Second, diversification of the habitat caused by different root and shoot structures is resulting in more habitable space (Eisenhauer et al. 2011). Both in combination will lead to more ecological niches promoting soil fauna diversity (Eisenhauer et al. 2011; Lemanceau et al. 2015). However, results of studies investigating effects of plant diversity on Collembola are inconsistent and reported little effects (Salamon et al. 2004) or positive relationships of plant diversity and Collembola abundance as well as species richness (Sabais et al. 2011). Further, different plant genotypes may differentially affect soil fauna as they may differ in morphology and physiology as mentioned above. In addition, Collembola

may benefit from the presence of certain plant functional groups (Salamon et al. 2004; Milcu et al. 2006; Eisenhauer et al. 2011). Especially legumes are providing resources rich in nitrogen, which is of critical importance as nitrogen often limits soil animal abundance and positively affects Collembola as well as earthworms and microorganisms (Milcu et al. 2008; Eisenhauer et al. 2011, 2012). However, grasses also have been shown to beneficially affect Collembola abundance in plant mixtures (Milcu et al. 2006; Eisenhauer et al. 2011). As results of previous studies are inconsistent neither confirming direct diversity effects nor uniform promotion of Collembola by certain functional groups further experiments need to focus on underlying mechanisms in order to clarify under which conditions Collembola can be promoted by increased plant diversity.

## 1.5 Objectives

Agriculture in Europe is characterized by high input monoculture systems using high amounts of fertilizers and pesticides to increase yields, and conventional tillage as additional weed control and to promote soil fertility. This thesis focusses on how the abundance and diversity of Collembola as major group of detritivore soil arthropods is affected by farm based soil management practices and crop diversity. Thereby, Collembola communities were assessed using two differing approaches. On the one hand a species level approach is applied and on the other hand a trait based approach is used. The two approaches are expected to allow comparing effects on the overall community, on the ecological groups and the abundance of traits allowing to better understand the mechanisms affecting the community by exerting effects on a particular group. We will focus on tillage practices on the one hand as it had been identified as main threat to soil biodiversity due to mechanical disturbance. Therefore, we assessed Collembola communities across a large geographical gradient representing diverse climatic regions in five long term field experiments in Europe located in Sweden, Germany, France, Romania and Spain. Furthermore, we conducted a litterbag experiment to investigate the importance of Collembola for litter decomposition and the importance of plant litter as food resource under different tillage regimes in a long term field trial in Germany. On the other hand, we investigated the effect of enhanced aboveground biodiversity achieved by mixed cropping on soil Collembola. Hence, we compared the Collembola community of two field sites in Germany planted with faba bean monocultures and mixed stands of faba bean and winter wheat. We expected our results to prove the usefulness of trait based approaches in general which can be used to adapt management practices for the promotion of desired soil biota delivering crucial ecosystem services.

We examined the following main hypotheses:

- (1) Reduction of tillage intensity will promote Collembola communities across Europe while differentially affecting ecological groups.
- (2) Collembola will be more abundant in reduced tillage fields and will therefore exert a more positive effect on decomposition in reduced tillage fields.
- (3) Collembola will be promoted by mixed cropping while being differentially affected by bean genotypes.

**References:**

- Annicchiarico, P., & Proietti, S. (2010). White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science*, *65*(3), 318-324.
- Barot, S., Allard, V., Cantarel, A., Enjalbert, J., Gauffreteau, A., Goldringer, I., ... & Porcher, E. (2017). Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review. *Agronomy for sustainable development*, *37*(2), 1-20.
- Behan-Pelletier, V. M. (2003). Acari and Collembola biodiversity in Canadian agricultural soils. *Canadian Journal of Soil Science*, *83*(Special Issue), 279-288.
- Bender, S. F., Wagg, C., & van der Heijden, M. G. (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution*, *31*(6), 440-452.
- Berg, M. P., & Bengtsson, J. (2007). Temporal and spatial variability in soil food web structure. *Oikos*, *116*(11), 1789-1804.
- Brennan, A., Fortune, T., & Bolger, T. (2006). Collembola abundances and assemblage structures in conventionally tilled and conservation tillage arable systems. *Pedobiologia*, *50*(2), 135-145.
- Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., ... & White, P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1), 107-117.
- Chahartaghi, M., Langel, R., Scheu, S., & Ruess, L. (2005). Feeding guilds in Collembola based on nitrogen stable isotope ratios. *Soil Biology and Biochemistry*, *37*(9), 1718-1725.
- Collins, R. P., Fothergill, M., Macduff, J. H., & Puzio, S. (2003). Morphological compatibility of white clover and perennial ryegrass cultivars grown under two nitrate levels in flowing solution culture. *Annals of Botany*, *92*(2), 247-258.
- Doran, J. W., & Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied soil ecology*, *15*(1), 3-11.
- Dubie, T. R., Greenwood, C. M., Godsey, C., & Payton, M. E. (2011). Effects of tillage on soil microarthropods in winter wheat. *Southwestern Entomologist*, *36*(1), 11-20.
- Duchene, O., Vian, J. F., & Celette, F. (2017). Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. *Agriculture, Ecosystems & Environment*, *240*, 148-161.
- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., ... & Scheu, S. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology*, *91*(2), 485-496.
- Eisenhauer, N., Milcu, A., Sabais, A. C., Bessler, H., Brenner, J., Engels, C., ... & Scheu, S. (2011). Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. *PLoS ONE*, *6*(1), e16055.
- Eisenhauer, N., Reich, P. B., & Scheu, S. (2012). Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology*, *13*(7), 571-578.
- Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., ... & Menta, C. (2009). Soil biodiversity monitoring in Europe: ongoing activities and challenges. *European Journal of Soil Science*, *60*(5), 807-819.

- Giller, P. S. (1996). The diversity of soil communities, the 'poor man's tropical rainforest'. *Biodiversity & Conservation*, 5(2), 135-168.
- Greenslade, P. (2007). The potential of Collembola to act as indicators of landscape stress in Australia. *Australian Journal of Experimental Agriculture*, 47(4), 424-434.
- Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J., & Jensen, E. S. (2008). Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renewable Agriculture and Food Systems*, 23(1), 3-12.
- Hooper, D. U., & Vitousek, P. M. (1997). The effects of plant composition and diversity on ecosystem processes. *Science*, 277(5330), 1302-1305.
- Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Dangerfield, J. M., Wall, D. H., ... & Wolters, V. (2000). Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks: We assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship—depending on the strength and type of interactions among species. *Bioscience*, 50(12), 1049-1061.
- Horwith, B. (1985). A role for intercropping in modern agriculture. *BioScience*, 35(5), 286-291.
- Jensen, E. S., Peoples, M. B., & Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. *Field crops research*, 115(3), 203-216.
- Jucevica, E., & Melecis, V. (2006). Global warming affect Collembola community: A long-term study. *Pedobiologia*, 50(2), 177-184.
- Kampichler, C., Bruckner, A., 2009. The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews* 84 (3), 375–389. 10.1111/j.1469-185X.2009.00078.x.
- Kautz, T., López-Fando, C., & Ellmer, F. (2006). Abundance and biodiversity of soil microarthropods as influenced by different types of organic manure in a long-term field experiment in Central Spain. *Applied Soil Ecology*, 33(3), 278-285.
- Kibblewhite, M. G., Ritz, K., & Swift, M. J. (2008). Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1492), 685-701. Lemanceau et al. 2014
- Kladivko, E. J. (2001). Tillage systems and soil ecology. *Soil and Tillage Research*, 61(1-2), 61-76.
- Kleijn, D., Baquero, R. A., Clough, Y., Díaz, M., De Esteban, J., Fernández, F., ... & Yela, J. L. (2006). Mixed biodiversity benefits of agri-environment schemes in five European countries. *Ecology letters*, 9(3), 243-254.
- Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., ... & Rossi, J. P. (2006). Soil invertebrates and ecosystem services. *European journal of soil biology*, 42, S3-S15.
- Lemanceau, P., Maron, P. A., Mazurier, S., Mougél, C., Pivato, B., Plassart, P., ... & Wipf, D. (2015). Understanding and managing soil biodiversity: a major challenge in agroecology. *Agronomy for Sustainable Development*, 35(1), 67-81.
- Lithourgidis, A. S., Vlachostergios, D. N., Dordas, C. A., & Damalas, C. A. (2011). Dry matter yield, nitrogen content, and competition in pea–cereal intercropping systems. *European Journal of agronomy*, 34(4), 287-294.

- Litrico, I., & Violle, C. (2015). Diversity in plant breeding: a new conceptual framework. *Trends in plant science*, 20(10), 604-613.
- Machado, S. (2009). Does intercropping have a role in modern agriculture? *Journal of soil and water conservation*, 64(2), 55A-57A.
- Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science*, 277(5325), 504-509.
- McLaughlin, A., & Mineau, P. (1995). The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment*, 55(3), 201-212.
- Meyer-Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., & Brunotte, J. (2017). Collembolans and soil nematodes as biological regulators of the plant pathogen *Fusarium culmorum*. *Journal of Plant Diseases and Protection*, 124(5), 493-498.
- Michalet, R., & Pugnaire, F. I. (2016). Facilitation in communities. *Functional Ecology*, 30(1), 3-9.
- Milcu, A., Partsch, S., Langel, R., & Scheu, S. (2006). The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional group diversity of plants. *Oikos*, 112(3), 513-524.
- Milcu, A., Partsch, S., Scherber, C., Weisser, W. W., & Scheu, S. (2008). Earthworms and legumes control litter decomposition in a plant diversity gradient. *Ecology*, 89(7), 1872-1882.
- Moonen, A. C., & Bàrberi, P. (2008). Functional biodiversity: an agroecosystem approach. *Agriculture, Ecosystems & Environment*, 127(1-2), 7-21.
- Moretti, M., Dias, A. T., De Bello, F., Altermatt, F., Chown, S. L., Azcarate, F. M., ... & Berg, M. P. (2017). Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, 31(3), 558-567.
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, 46(3), 683-691.
- Parisi, V., Menta, C., Gardi, C., Jacomini, C., & Mozzanica, E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, Ecosystems & Environment*, 105(1-2), 323-333.
- Petersen, H. (2002). Effects of non-inverting deep tillage vs. conventional ploughing on collembolan populations in an organic wheat field. *European Journal of Soil Biology*, 38(2), 177-180.
- Pey, B., Nahmani, J., Auclerc, A., Capowiez, Y., Cluzeau, D., Cortet, J., ... & Hedde, M. (2014). Current use of and future needs for soil invertebrate functional traits in community ecology. *Basic and Applied Ecology*, 15(3), 194-206.
- Potapov, A. M., Korotkevich, A. Y., & Tiunov, A. V. (2018). Non-vascular plants as a food source for litter-dwelling Collembola: Field evidence. *Pedobiologia*, 66, 11-17.
- Potapov, A., Bellini, B., Chown, S., Deharveng, L., Janssens, F., Kováč, L., ... & Berg, M. (2020). Towards a global synthesis of Collembola knowledge: challenges and potential solutions. *Soil Organisms*, 92(3), 161-188.
- Pollierer, M. M., & Scheu, S. (2021). Stable isotopes of amino acids indicate that soil decomposer microarthropods predominantly feed on saprotrophic fungi. *Ecosphere*, 12(3), e03425.



- Reeleder, R. D., Miller, J. J., Coelho, B. B., & Roy, R. C. (2006). Impacts of tillage, cover crop, and nitrogen on populations of earthworms, microarthropods, and soil fungi in a cultivated fragile soil. *Applied Soil Ecology*, 33(3), 243-257.
- Rodgers, D., McPhee, J., Aird, P., & Corkrey, R. (2018). Soil arthropod responses to controlled traffic in vegetable production. *Soil and Tillage Research*, 180, 154-163.
- Sabais, A. C., Scheu, S., & Eisenhauer, N. (2011). Plant species richness drives the density and diversity of Collembola in temperate grassland. *Acta Oecologica*, 37(3), 195-202.
- Salamon, J. A., Schaefer, M., Alpehi, J., Schmid, B., & Scheu, S. (2004). Effects of plant diversity on Collembola in an experimental grassland ecosystem. *Oikos*, 106(1), 51-60.
- Salmon, S., Ponge, J. F., Gachet, S., Deharveng, L., Lefebvre, N., & Delabrosse, F. (2014). Linking species, traits and habitat characteristics of Collembola at European scale. *Soil Biology and Biochemistry*, 75, 73-85.
- Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, 29(1), 25-46.
- Spehn, E. M., Joshi, J., Schmid, B., Alpehi, J., & Körner, C. (2000). Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant and soil*, 224(2), 217-230.
- Stinner, B. R., McCartney, D. A., & Van Doren Jr, D. M. (1988). Soil and foliage arthropod communities in conventional, reduced and no-tillage corn (maize, *Zea mays* L.) systems: a comparison after 20 years of continuous cropping. *Soil and Tillage Research*, 11(2), 147-158.
- Stinner, B. R., & House, G. J. (1990). Arthropods and other invertebrates in conservation-tillage agriculture. *Annual Review of Entomology*, 35(1), 299-318.
- Stoate, C., Boatman, N. D., Borralho, R. J., Carvalho, C. R., De Snoo, G. R., & Eden, P. (2001). Ecological impacts of arable intensification in Europe. *Journal of Environmental Management*, 63(4), 337-365.
- van Capelle, C., Schrader, S., & Brunotte, J. (2012). Tillage-induced changes in the functional diversity of soil biota—A review with a focus on German data. *European Journal of Soil Biology*, 50, 165-181.
- Vandermeer, J. H. (1992). *The ecology of intercropping*. Cambridge University Press.
- Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Doledec, S., Dubs, F., ... & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, 19(10), 2921-2947.
- Verhoef, H. A., & Nagelkerke, C. J. (1977). Formation and ecological significance of aggregations in Collembola. *Oecologia*, 31(2), 215-226.
- Vignozzi, N., Agnelli, A. E., Brandi, G., Gagnarli, E., Goggioli, D., Lagomarsino, A., ... & Gucci, R. (2019). Soil ecosystem functions in a high-density olive orchard managed by different soil conservation practices. *Applied Soil Ecology*, 134, 64-76.
- Whalen, J. K., & Hamel, C. (2004). Effects of key soil organisms on nutrient dynamics in temperate agroecosystems. *Journal of Crop Improvement*, 11(1-2), 175-207.
- Widenfalk, L. A., Bengtsson, J., Berggren, Å., Zwigelaar, K., Spijkman, E., Huyer-Brugman, F., & Berg, M. P. (2015). Spatially structured environmental filtering of collembolan traits in late successional salt marsh vegetation. *Oecologia*, 179(2), 537-549.

Winter, J. P., Voroney, R. P., & Ainsworth, D. A. (1990). Soil microarthropods in long-term no-tillage and conventional tillage corn production. *Canadian Journal of Soil Science*, 70(4), 641.

# 2 Displacement of resources in agricultural fields by tillage practices differentially affects ecological groups of Collembola across Europe

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## Abstract

Agricultural fields are highly managed ecosystems, degraded by mechanical and chemical disturbance. The most significant mechanical disturbance is caused by tillage activities. To reduce the mechanical stress for soil biodiversity reduced tillage practices are adapted in Europe. However, the beneficial effects for some soil biota are still under debate. Collembola are important decomposers in agricultural systems which display varying reactions to reduced tillage practices. To investigate effects of reduced tillage practices in agricultural systems, the Collembola community in experimental field sites in five countries across Europe was analysed. In order to increase comparability between the geographical regions a trait based approach was included. Neither total Collembola abundance nor species richness show uniform reactions to tillage practices across the field sites. Comparisons between depth distributions of Collembola and resources (represented by  $C_{org}$  and  $N_t$ ) show that the displacement of resources is affecting the Collembola community more significantly than the mechanical disturbance itself. Furthermore our results show increased abundances of euedaphic Collembola and a reduction of epedaphic Collembola in conventional tillage fields. While species community compositions displayed only regional differences between the investigated field sites, trait composition revealed higher abundances of euedaphic traits in conventional tillage fields and a higher prevalence of epedaphic traits in reduced tillage fields supporting our earlier findings. In conclusion tillage practices have to be adapted to support the local community of soil biota as the ecosystem can only profit from adapted tillage practices if the species present can benefit from them.

## 2.1 Introduction

Agriculture is considered as main factor for the worldwide loss of biodiversity because of the expansion, intensification and mechanization of management practices in combination with climate change and soil degradation, which results in soils that are compacted, prone to erosion, polluted and hosting invasive species (McLaughlin and Mineau 1995; Gardi et al. 2009; Pullemann et al. 2012). Loss of soil biodiversity leads to reduced functions and alters ecosystem processes resulting in decreased resilience and resistance (Gardi et al. 2009; Bardgett and van der Putten 2014). Mechanical and chemical stress have been proposed as the main threat to biodiversity in agricultural fields (van Capelle et al. 2012). The main mechanical stress in agricultural fields, which is also the most widespread, is tillage (van Capelle et al. 2012). Conventional ploughing is used to turn the soil and loosen it, reduce weeds, counteract nutrient leaching, cleaning the soil surface and facilitate seeding (van Capelle et al. 2012). By tillage activities aboveground crop residues are displaced from the soil surface into deeper soil layers which enhances decomposition (Winter et al. 1990), but the destruction of soil structure is an undesirable side effect of tillage, which leads to subsoil compaction, soil surface seals, erosion and a decrease in soil organic matter (Winter et al. 1990; McLaughlin and Mineau 1995; van Capelle et al.

2012). Furthermore, long-term tillage activities result in poor soil aggregation, reduced porosity, poor water retention and depletion of soil organic carbon (Mc Laughlin and Mineau 1995; Vignozzi et al. 2019). Thereby, it also negatively affects the soil fauna by compaction, decreasing soil moisture and disruption of existing root systems (Dubie et al. 2011), which leads to a decrease in biological activity and diversity in tilled soils (Vignozzi et al. 2019). In general, soil fauna is assumed to be vulnerable to mechanical disturbance by tillage (Petersen 2002).

Soil tillage affects soil fauna directly by misplacement of individuals, burying and killing them, and indirectly by displacing the litter material deeper in the soil column where it might be unreachable for some (Stinner et al. 1988). Reduced tillage practices ranging from non-inversion tillage to direct seeding have in common that they reduce the physical disturbance of the soil and leave plant residues on top of or close to the soil surface (Mc Laughlin and Mineau 1995). Reduced tillage practices reduce soil compaction, erosion and runoff, and increase water infiltration (Rodgers et al. 2018). However, soil biota react in different ways to different tillage practices (van Capelle et al. 2012). Especially Collembola display a variety of responses. Some authors reported reduced Collembola abundance due to soil tillage or beneficial effects of no-till and reduced tillage practices (Stinner et al. 1988; Brennan et al. 2006; Vignozzi et al. 2019). Winter et al. (1990) reported increased abundance and diversity of microarthropods in no-till fields. However, this only holds true if only the upper layers of soil are considered. When considering deeper soil layers the differences may disappear (Winter et al. 1990). Further, Collembola abundance also may be detrimentally affected by non-inversion tillage with the decrease in abundance being most pronounced in deeper soil layers compared to conventional tillage (Petersen 2002). Similarly, Reeleder et al. (2006) reported inconsistent results on effects of tillage on microarthropods with the abundance of Collembola varying in a similar way due to conservation tillage and conventional tillage (Reeleder et al. 2006). Reviewing the topic, van Capelle et al. (2012) concluded that overall the abundances and diversity of Collembola in reduced tillage systems is lower than in conventional tillage systems, but the reduction varies with soil type and ecological group of Collembola.

Ecological groups of Collembola are closely related to their adaptation to vertical distribution in soil (Vandewalle et al. 2010). Trait based approaches, which led to the establishment of ecological groups, allow simplified assessment of communities without having to determine all individuals to species level (Pey et al. 2014). They were proven useful for understanding the mechanisms driving the assemblage of communities, and their response to stress and management practices (Parisi et al. 2005; Vandewalle et al. 2010; Widenfalk et al. 2015). Switching from taxonomically focussed approaches to trait based approaches avoids time consuming species identification and allows identification of traits as indicators across communities and ecosystems (Parisi et al. 2005; Moretti et al. 2017). Traits can be used to identify ecological preferences of Collembola across large spatial scales and management

practices (Salmon et al. 2014). However, trait based approaches also elucidated the high complexity of the response of Collembola to disturbances including interaction effects between different ecological groups, which may explain the inconsistency of results on the effect of tillage on Collembola (van Capelle et al. 2012). The complexity of the response of Collembola likely also applies to other soil biota. Their response to tillage depends on a variety of traits including body size, burrowing ability, food and habitat preferences, and other adaptations. Therefore, generalizations on the effect of tillage practices on soil biota are difficult to draw (Stinner and House 1990, van Capelle et al. 2012).

The differential response of soil biota to tillage practices suggest that adopting certain tillage practices may allow to promote focal soil animal taxa (van Capelle et al. 2012). As the importance of soil biota for sustainable agriculture is known, their protection has become a key component for maintaining and enhancing soil fertility for food and fiber production in sustainable agricultural systems (van Capelle et al. 2012; Bardgett and van der Putten 2014). Since Collembola take part in important ecosystem services provided by agricultural soils, such as decomposition and nutrient cycling, and together with mites account for about 95% of total microarthropod numbers, they need particular attention (Seastedt 1984; van Capelle et al. 2012). Nevertheless, conservation tillage with minimized soil disturbance, which may be detrimental to Collembola populations, is recommended by the European Union (Vignozzi et al. 2019). The present study therefore focusses on the influence of reduced tillage and no-till practices in comparison to conventional mouldboard ploughing practices on Collembola abundance and diversity in agricultural fields across Europe. In particular we focus on variations in the depth distribution of Collembola in different tillage systems. We hypothesize that (1) total Collembola abundance and species richness in conventionally tilled fields is lower compared to reduced tillage and no-till systems across European countries, (2) the depth distribution of Collembola differs between tillage systems with maximum abundance in deeper soil in conventional tillage systems, (3) ecological groups of Collembola are differentially affected by conventional tillage practices, with hemi- and euedaphic Collembola being affected more negatively by conventional tillage than epedaphic Collembola, and (4) Collembola community composition as well as trait community composition differs between conventional and reduced tillage systems within a country.

## 2.2 Material and Methods

### 2.2.1 Field sites

In each of five countries across Europe (Sweden, Germany, France, Romania, Spain) arable systems planted with winter wheat were investigated. In each country replicated fields managed by conventional tillage, which included ploughing and reduced tillage without ploughing were investigated. The fields were fertilized by mineral fertilizers at conventional rates (see Supplementary Table 1). Sampling was carried out at the flowering stage of wheat in 2017 and 2018. The field site in Sweden (Säby) is located in central Sweden near Uppsala and was established in 2006. The soil is characterized as Eutric Cambisol. The treatments included (1) conventional tillage using mouldboard ploughing to a depth of 23 cm, (2) minimum tillage performed using a cultivator with a working depth of 10-12 cm, and (3) direct drilling in no tillage plots. For further information see Arvidsson (2010). The field site in Germany (Garte Süd) is located near Göttingen in Lower Saxony on a loess derived Haplic Luvisol (WRB). The treatments included (1) conventional tillage characterized by mouldboard ploughing to a depth of 25 cm and a seed bed preparation and shallow cultivation (6-8 cm) with a rotary harrow, and (2) minimum tillage to a soil depth of 5-8 cm using a rotary harrow. Before the experiment was established in 1970 the field sites had been used as arable land and were mouldboard ploughed. For further information see Ehlers et al. (2000). The field site in France (Efele) is located in western France near Rennes and was established in 2012. The soil is characterized as Luvisol. It is managed by the French National Institute of Agronomics Research (INRA) and forms part of the SOERE-PROs network (Long Term Observatory Network-Organic Residual Products). The treatments included (1) conventional tillage by ploughing using a mouldboard plough to a depth of 25 cm followed by harrowing to 12 cm and seeding, and (2) minimum tillage using a rotary harrow to a depth of 7-10 cm. For further information see Potard et al. (2017). The field site in Romania (Turda) is located near Cluj-Napoca and was established in 2007. The soil is characterized as Phaeozem. The treatments included (1) conventional tillage by ploughing to a soil depth to 25-30 cm followed by seedbed preparation using a rotary harrow, and (2) minimum tillage using a chisel cultivator after maize and winter wheat. The field site in Spain (La Hampa) is located in southwest Spain near Seville and was established in 2008. The soil is characterized as a Calcic Fluvisol. The treatments included (1) conventional tillage with a mouldboard plough to a depth of 25-30 cm followed by 1-2 cultivator passes to a depth of 15-20 cm and disc harrowing to a depth of 15 cm, and (2) no tillage actions except for the crumbling of sunflower stalks in the minimum tillage plots followed by sowing by direct drilling. For further information see López-Garrido et al. (2011).

### 2.2.2 Soil Collembola

Soil Collembola were sampled using 5 cm diameter split soil corers. Four soil cores were taken from each plot at random to a depth of 30 cm. In the field, the soil cores were separated into 5 cm sections using a knife. Collembola were extracted using high gradient heat extraction (Macfadyen 1961). Animals were collected in a 1:1 diethyleneglycol – water solution. After extraction, the animals were transferred into 70% ethanol and stored until species identification. Prior to statistical analyses, the numbers of individuals in respective depth layers of the four soil cores taken per plot were summed up. Species identification was done using transmitted light microscopy using the keys of Fjellberg (1998, 2007) and Hopkins (2007).

Traits of species were used to assign them to the following ecological groups: epedaphic, hemiedaphic and euedaphic. The traits included length of antenna, length of furca, number of ocelli, coloration and presence or absence of scales and hairs as protection against desiccation. Each of these traits received a score ranging from 0 (well adapted to aboveground live) to 4 (well adapted to belowground live) according to Vandewalle et al. (2010) (see Supplementary Table 2). The scores were added up and ranged between 0 and 18. Species with scores of 0-6 were ascribed as epedaphic, those with scores of 7-12 as hemiedaphic and those with scores of 13-18 as euedaphic (see Supplementary Table 3). In addition, for each plot the abundance of traits were recorded to investigate the effect of tillage treatments on assemblages of Collembola traits.

### 2.2.3 Soil properties

For measuring chemical soil properties about 200 g of soil was taken in 10 cm sections down to a depth of 30 cm. Three replicated samples were taken. For measurement of total carbon ( $C_t$ ) and total nitrogen ( $N_t$ ) soil samples were sieved, dried, milled and analysed using an elemental analyser (Elementar Vario El, Heraeus, Hanau, Germany). Organic carbon ( $C_{org}$ ) was determined by removing inorganic carbon with HCl before repeating the measurement.

For measuring physical soil properties samples of known volume were taken in 10 cm sections down to a depth of 30 cm. The samples were weighed, dried at 105°C for 24 h and weighed again for calculating soil moisture. Soil bulk density was calculated by dividing soil dry weight by soil volume.

### 2.2.4 Statistical analysis

All statistical analyses were done using R (R core Team 2020). The abundance of total Collembola as well as the abundance of ecological groups of Collembola and species richness was analysed using generalized linear mixed effect models using the *glmer.nb* and *glmer* function of the *lme4* package



(Bates et al. 2015). Soil physical and chemical properties were analysed by linear mixed effect models using the *lme* function of the *nlme* package (Pinheiro et al. 2020). Non-independence of abundance data of Collembola at different soil depths per plot was accounted for by including „plotID“ as random factor. The impact of tillage intensity on Collembola abundances was investigated by including „treatment“ as factor (levels: conventional tillage, minimum tillage, direct drilling). If an analysis included all investigated countries to ensure comparability between countries the factor “treatment” was simplified by combining the levels minimum tillage and direct drilling as reduced tillage). To investigate Collembola depth distribution „depth“ was included as ordered factor (with the levels 0-5, 5-10, 10-15, 15-20, 20-25 and 25-30 cm). Models were chosen starting with a full model including the treatment × depth interaction and excluding non-significant factors stepwise. Model quality was checked using the *DHARMA* package (Hartig 2020) checking for overdispersion, model conversion, outliers and zero inflation.

To compare structures of the species and trait community composition of Collembola non-metric multi-dimensional scaling using Bray-Curtis dissimilarity was used (metaMDS, *vegan* package; Oksanen et al. 2019). To identify differences in community composition between treatments multivariate analysis of variance was conducted (*manova*, *stats* package). To account for non-independence of samples within countries, an error term was included. Linear discriminant analysis (*lda*, *MASS* package, Venables and Ripley 2002) in combination with Hotellings T<sup>2</sup>-test (*HotellingsT2*, *ICSNP* package, Nordhausen et al. 2018) were used for pairwise comparisons between treatments and depths within each individual country, and for two dimensional graphical depiction of communities, using the first two dimensions of the NMDS.

## 2.3 Results

### 2.3.1 Abiotic conditions

All soil chemical and physical properties showed larger differences between countries than between treatments or sampling depth (Table 1). Bulk density was lowest in Romania and Sweden, and highest in Germany. Soil moisture,  $C_{org}$  and  $N_t$  were higher in Sweden than in the other countries. Changes in abiotic conditions with soil depth varied in the different countries and with tillage treatments (significant treatment x depth x country interaction; Table 1). Therefore, in the following effects of tillage practices on abiotic conditions in different sampling depths will be presented for each country separately.

In Sweden bulk density increased with sampling depth (Table 2). Soil moisture decreased with sampling depth and was lower in direct seeding than in minimum tillage fields. Both  $C_{org}$  as well as  $N_t$  decreased with sampling depth in minimum tillage and direct seeding fields. In addition,  $N_t$  in direct seeding fields was higher than in conventional tillage fields. In Germany soil moisture increased with sampling depth regardless of treatment (Table 2). By contrast,  $C_{org}$  as well as  $N_t$  in minimum tillage fields decreased with sampling depth in a linear way. In addition,  $N_t$  was higher in minimum tillage than in conventional tillage fields. In France bulk density in minimum tillage fields increased with sampling depth and overall was higher than in conventional tillage fields (Table 2). Similarly, soil moisture was overall higher in minimum tillage fields, but with a strong decrease at medium sampling depth (10-20 cm).  $C_{org}$  decreased in a linear way with sampling depth in both tillage treatments, but the decrease was stronger in minimum tillage fields (significant tillage x depth interaction).  $N_t$  in minimum tillage fields decreased with sampling depth. In Romania bulk density,  $C_{org}$  and  $N_t$  were higher in conventional tillage than in minimum tillage fields. In addition, bulk density increased with sampling depth (Table 2). In Spain soil moisture decreased at medium depth (10-20 cm) (Table 2).  $C_{org}$  and  $N_t$  were higher in direct seeding fields and decreased with sampling depth regardless of tillage treatment.

**Table 1** Linear mixed effect model table on the effect of reduced tillage (RT) and depth on soil physical and chemical properties [bulk density, total nitrogen (N<sub>t</sub>), organic carbon (C<sub>org</sub>), soil moisture] in five European countries [Sweden (SW), Germany (GE), France (FR), Romania (RO), Spain (SP)]; Df = Degrees of freedom; Int = Intercept; L = Linear change with sampling depth; Q = Quadratic change with sampling depth; only significant factors and interactions are shown.

		Int	GE	RO	SP	SW	RT x RO	RT x SW	L x SW	Q x SW	RT x L x SW	RT x L x SP
Bulk density	Df	62	11	11		11			62			62
	t-value	30.10	4.98	-6.21		-2.57			2.15			-2.80
	p-value	<0.01	<0.01	<0.01		0.03			0.04			<0.01
N <sub>t</sub>	Df	62	11	11	11	11	62	62			62	
	t-value	29.13	3.04	14.09	-4.7	16.44	-2.45	-2.45			-3.62	
	p-value	<0.01	0.01	<0.01	<0.01	<0.01	0.02	0.02			<0.01	
C <sub>org</sub>	Df	62	11	11	11	11					62	
	t-value	13.93	2.26	8.82	-2.88	12.52					-4.26	
	p-value	<0.01	0.05	<0.01	0.01	<0.01					<0.01	
Soil moisture	Df	64	9		9	9			64	64		
	t-value	0.72	8.61		28.36	35.15			-8.16	3.85		
	p-value	0.48	<0.01		<0.01	<0.01			<0.01	<0.01		



Table 2 continued

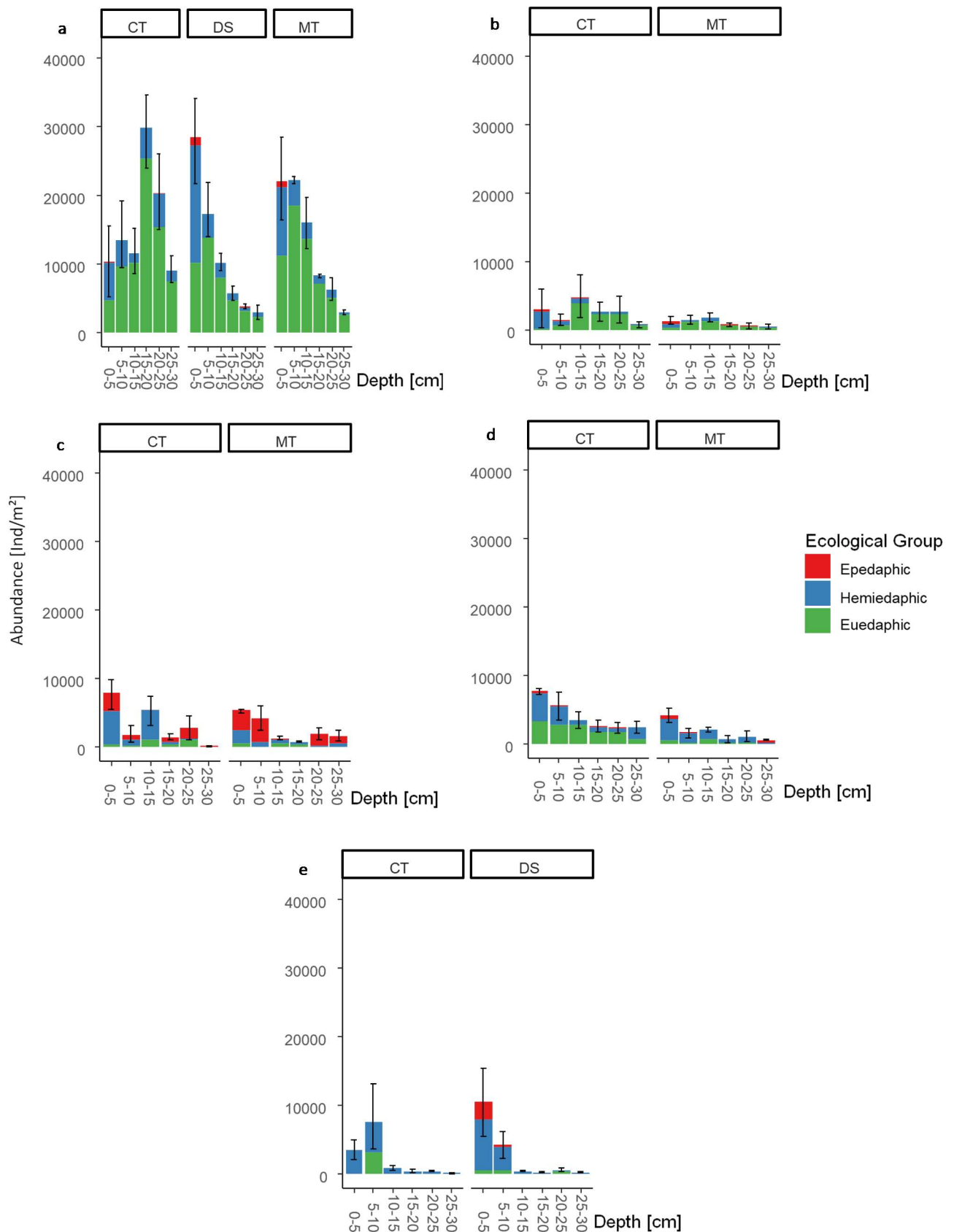
		C <sub>org</sub>					N <sub>t</sub>									
		Int	DS	MT	L	DS x L	MT x L	Int	DS	MT	L	Q	DS x L	MT x L	MT x Q	
FR	Df	10			10		10							10		
	t-value	39.57			-2.32		-2.74		46.93					-2.63		
	p-value	<0.01			0.04		0.02		<0.01					0.03		
GE	Df	15					15		15				15	15	15	
	t-value	21.97					-4.11		75.38				4.14	-3.62	-10.78	4.03
	p-value	<0.01					<0.01		<0.01				<0.01	<0.01	<0.01	
SP	Df	12	12		12				12	12				12		
	t-value	36.25	2.75		-5.37				35.71	2.87				-3.16		
	p-value	<0.01	0.02		<0.01				<0.01	0.01				<0.01		
SW	Df	16				16	16		16	16				16	16	
	t-value	18.13				-5.55	-3.38		31.14	2.75				-6.52	-4.27	
	p-value	<0.01				<0.01	<0.01		<0.01	0.04				<0.01	<0.01	
RO	Df	14		14					14					14		
	t-value	63.09		-4.64					69.53					-3.59		
	p-value	<0.01		<0.01					<0.01					<0.01		

### 2.3.2 Abundance

Generally, Collembola abundances were highest in Sweden ( $13336 \pm 9998$  ind/m<sup>2</sup>; mean  $\pm$  SD) and Romania ( $4668 \pm 3173$  ind/m<sup>2</sup>) and lowest in Germany ( $2234 \pm 2906$  ind/m<sup>2</sup>) and Spain ( $2780 \pm 5455$  ind/m<sup>2</sup>). In France abundances were intermediate ( $3721 \pm 3695$  ind/m<sup>2</sup>). In Sweden the abundance of euedaphic and hemiedaphic Collembola ( $9557 \pm 7034$  ind/m<sup>2</sup> and  $3623 \pm 5135$  ind/m<sup>2</sup>, respectively) was much higher than that of epedaphic Collembola ( $156 \pm 394$  ind/m<sup>2</sup>). The same was true for Romania (respective values of  $1217 \pm 1460$ ,  $1517 \pm 1750$  and  $138 \pm 193$  ind/m<sup>2</sup>). In Germany euedaphic Collembola were most abundant ( $1422 \pm 2276$  ind/m<sup>2</sup>) compared to hemiedaphic ( $597 \pm 1491$  ind/m<sup>2</sup>) and epedaphic Collembola ( $139 \pm 325$  ind/m<sup>2</sup>). In Spain the abundance of hemiedaphic Collembola ( $1796 \pm 3311$  ind/m<sup>2</sup>) was higher than that of epedaphic ( $261 \pm 1192$  ind/m<sup>2</sup>) and euedaphic Collembola ( $389 \pm 1548$  ind/m<sup>2</sup>). In France the abundance of euedaphic Collembola was lowest ( $410 \pm 654$  ind/m<sup>2</sup>) compared to hemiedaphic ( $1188 \pm 2339$  ind/m<sup>2</sup>) and epedaphic Collembola ( $1259 \pm 1983$  ind/m<sup>2</sup>). Changes in abundances of Collembola with soil depth varied in the different countries and with different tillage practices (significant treatment  $\times$  depth  $\times$  country interaction; Table 3). Therefore, effects of tillage practices on Collembola abundance and richness as well as the abundance of the different ecological groups in different depths were analysed for each country separately (Table 4).

Tillage treatment only affected total Collembola abundance in Romania where abundances in conventional tillage fields exceeded those in minimum tillage fields (Table 4). Total Collembola abundance uniformly declined with increasing sampling depth with the exception of conventional tillage fields in Sweden where abundances peaked at medium sampling depth (Figure 1a), in Germany where the decline was not as clearly linear as in the other countries with high abundances down to medium soil depths (Figure 1b), and in France where abundances in minimum tillage fields were lowest at medium sampling depth (Figure 1c). Similarly, hemiedaphic Collembola declined in almost all fields with sampling depth in a linear way, with conventional tillage fields in Sweden being the only exception showing high abundances not only in shallow soil layers (0-10 cm) but also at medium sampling depth (15-25 cm). Epedaphic Collembola declined with sampling depth in Sweden and Germany while in France they were more abundant in shallow as well as deeper soil layers compared to medium sampling depth. In Spain epedaphic Collembola were more abundant in direct seeding fields, while they were absent from conventionally tilled fields (Figure 1e). In Romania the changes in epedaphic Collembola with sampling depth differed between tillage treatments with abundances declining with sampling depth in conventional tillage fields in a linear way, while abundances being high in shallow soil depth as well as in deep soil layers in minimum tillage fields (significant depth  $\times$  tillage interaction; Figure 1d). Euedaphic Collembola in Sweden and Germany declined with sampling depth in minimum tillage as well as direct seeding fields, but were most abundant at medium sampling depth in

conventional tillage fields in both countries (significant tillage x depth interaction). Euedaphic Collembola in Romania were more abundant in conventional than in minimum tillage fields and decreased with sampling depth.



**Figure 1** Abundance of Collembola (ind/m<sup>2</sup>) varying between tillage systems (CT = conventional tillage; MT = minimum tillage; DS = direct seeding) and with soil depth (0-5, 5-10, 10-15, 15-20, 20-25, 25-30 cm) in five European countries. Means with standard deviation. a – Sweden (n = 54), b – Germany (n = 48), c – France (n = 36), d – Romania (n = 36), e – Spain (n = 36).



**Table 3** Generalized linear mixed effect model table on the effect of reduced tillage (RT) and depth on total Collembola abundance in five European countries [Sweden (SW), Germany (GE), France (FR), Romania (RO), Spain (SP)]; Df = Degrees of freedom; Int = Intercept; L = Linear change with sampling depth; Q = Quadratic change with sampling depth; only significant factors and interactions are shown.

	Total Collembola abundance															
	Int	L	Q	RO	SP	SW	RT × Q	RT × RO	L × GE	L × RO	L × SW	RT × L × GE	RT × Q × GE	RT × L × RO	RT × Q × RO	RT × L × SW
Df	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139	139
Z-value	6.00	-5.37	-2.22	2.74	-2.00	4.16	3.36	-3.34	3.45	2.80	4.87	-2.63	-2.13	-2.45	-3.28	-4.87
p-value	<0.01	<0.01	0.03	<0.01	0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.03	0.01	<0.01	<0.01

**Table 4** Generalized linear mixed effect model table on the effect of minimum tillage (MT), direct seeding (DS) and depth on total Collembola abundances, abundances of different ecological groups and species richness in five European countries [Sweden (SW), Germany (GE), France (FR), Romania (RO), Spain (SP)]; Df = Degrees of freedom; Int = Intercept; L = Linear change with sampling depth; Q = Quadratic change with sampling depth; C = Cubic change with sampling depth; only significant factors and interactions are shown.

		Total Collembola abundances							Species richness					
		Int	DS	MT	L	Q	DSxL	MTxL	MTxQ	Int	L	Q	DSxL	MTxL
FR	Df	22			22				22	28	28			
	Z-value	10.38			-4.09				2.74	6.38	-2.10			
	p-value	<0.01			<0.01				<0.01	<0.01	0.04			
GE	Df	39			39	39				39		39		
	Z-value	3.15			-2.62	-2.47				3.21		-2.47		
	p-value	<0.01			<0.01	0.01				<0.01		0.01		
SP	Df	28			28					28	28			
	Z-value	7.55			-6.77					2.15	-3.34			
	p-value	<0.01			<0.01					0.03	<0.01			
SW	Df	34	34	34		34	34	34	34	34			34	34
	Z-value	34.90	-4.72	-2.47		-3.93	-8.18	3.40	2.41	31.53			-2.48	-2.91
	p-value	<0.01	<0.01	0.01		<0.01	<0.01	<0.01	0.02	<0.01			0.01	<0.01
RO	Df	27		27	27					28	28			
	Z-value	22.39		-4.41	-4.65					10.61	-2.27			
	p-value	<0.01		<0.01	<0.01					<0.01	0.02			

Table 4 continued

		Epedaphic Collembola						Hemiedaphic Collembola					Euedaphic Collembola								
		Int	DS	MT	L	Q	MT x L	MT x Q	Int	L	C	DS x L	MT x L	Int	DS	MT	L	Q	DS x L	MT x L	DS x Q
FR	Df	28				28		28	28												
	Z-value	4.32				2.09		3.75	-3.95												
	p-value	<0.01				0.04		<0.01	<0.01												
GE	Df	39			39			39	39					33			33	33		33	
	Z-value	-1.27			-3.18			0.11	-4.14					2.52			2.50	-4.60		-2.74	
	p-value	0.21			0.01			0.91	<0.01					0.01			0.01	<0.01		<0.01	
SP	Df	32	32					28	28												
	Z-value	-2.12	2.21					6.65	-6.58												
	p-value	0.03	0.03					<0.01	<0.01												
SW	Df	46			46	46		34		34	34	34	34	34	34	34	34	34	34	34	34
	Z-value	-3.25			-3.32	2.17		12.65		-2.49	-4.05	-3.50	35.55	-5.34	-2.09	2.96	-5.50	-7.24	-7.23	2.80	
	p-value	0.01			<0.01	0.03		<0.01		0.01	<0.01	<0.01	<0.01	<0.01	0.04	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
RO	Df	22		22	22		22	22	28	28				27		27	27	27			
	Z-value	-10.62		4.15	-8.80		9.97	7.14	10.17	-2.95				7.70		-10.43	-5.87	-2.29			
	p-value	<0.01		<0.01	<0.01		<0.01	<0.01	<0.01	<0.01				<0.01		<0.01	<0.01	0.02			

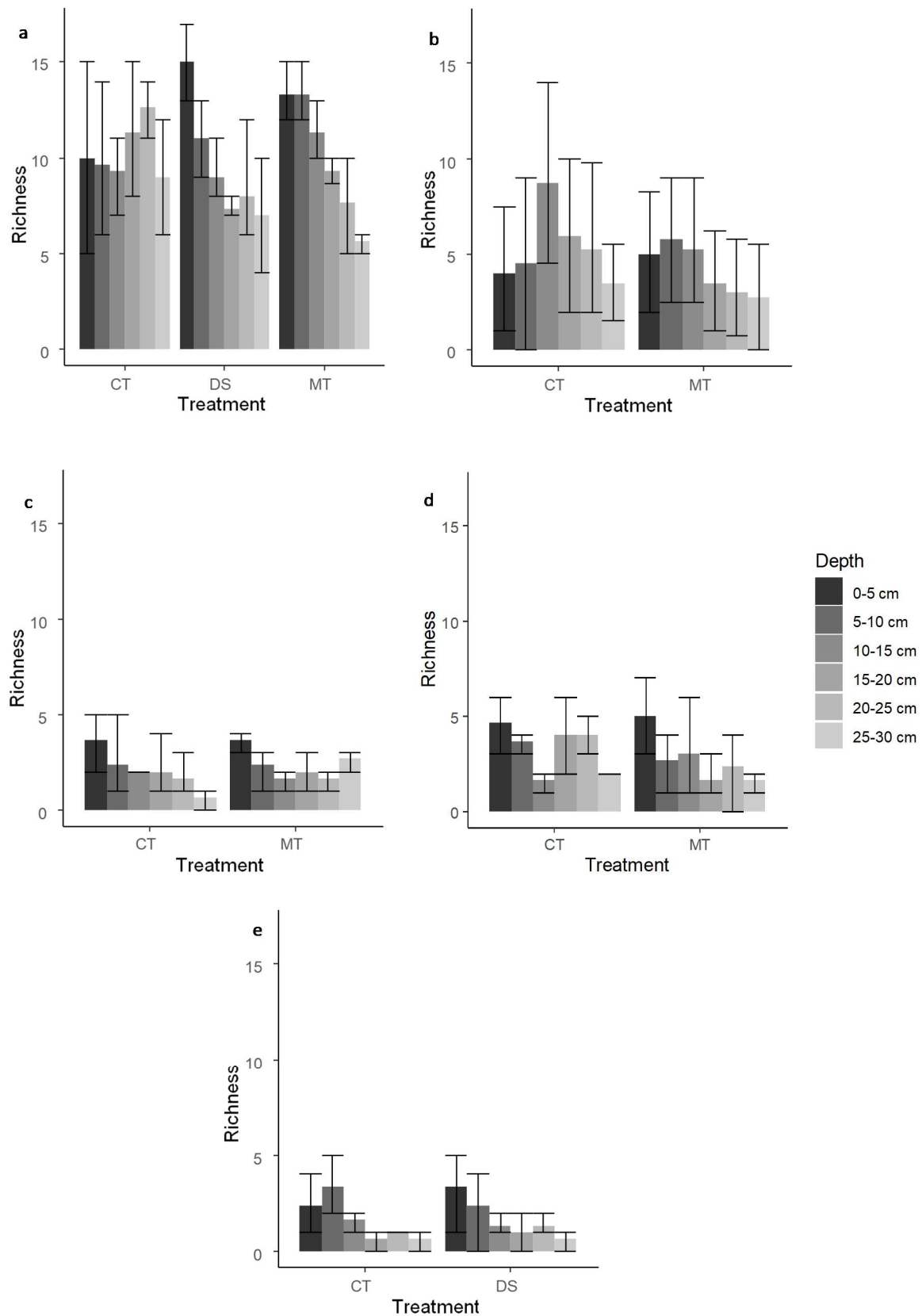
### 2.3.3 Species richness

Species richness of Collembola in Sweden ( $10.0 \pm 3.2$  species/plot and sampling depth; mean  $\pm$  SD) and Germany ( $5.5 \pm 3.7$  species/plot and sampling depth) was higher than in the other countries ( $2.2 \pm 1.3$ ,  $3.0 \pm 1.7$  and  $1.6 \pm 1.4$  species/plot and sampling depth for France, Romania and Spain, respectively). In general, species richness declined with sampling depth, but changes in species richness with soil depth varied between tillage practices and in the different countries (significant treatment  $\times$  depth  $\times$  country interaction; Table 5). Therefore, in the following effects of tillage practices on Collembola species richness in different sampling depths are presented for each country separately.

In Sweden species richness declined with depth in minimum tillage as well as direct seeding fields but not in conventional tillage fields (significant treatment  $\times$  depth interaction; Table 5, Figure 2a). In Germany species richness peaked at medium sampling depths, in minimum tillage fields in 5-10 cm and in conventional tillage fields in 10-15 cm depth (Figure 2b). In France, Romania and Spain species richness declined with increasing sampling depth, but did not vary significantly with tillage treatments (Figure 2c-e).

**Table 5** Generalized linear mixed effect model table on the effect of reduced tillage (RT) and depth on species richness in five European countries [Sweden (SW), Germany (GE), France (FR), Romania (RO), Spain (SP)]; Df = Degrees of freedom; Int = Intercept; L = Linear change with sampling depth; only significant factors and interactions are shown.

	Species Richness								
	Int	L	GE	SW	RT $\times$ L	L $\times$ SW	RT $\times$ L $\times$ GE	RT $\times$ L $\times$ RO	RT $\times$ L $\times$ SW
Df	139	139	139	139	139	139	139	139	139
Z-value	5.89	-3.20	3.15	5.26	2.49	3.01	-2.09	-2.09	-3.75
p-value	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.04	0.04	<0.01



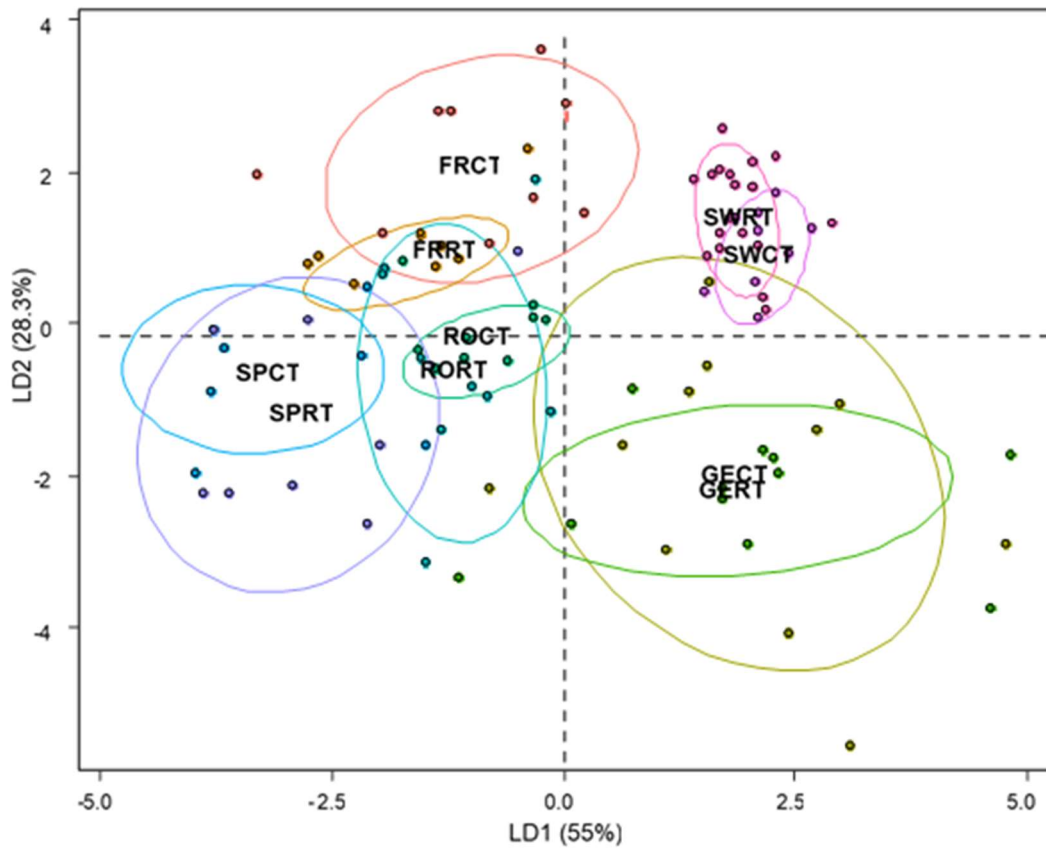
**Figure 2** Collembola species richness varying between tillage systems (CT = conventional tillage; MT = minimum tillage; DS = direct seeding) and with soil depth (0-5, 5-10, 10-15, 15-20, 20-25, 25-30 cm) in five European countries. Means with standard deviation. a – Sweden (n = 54); b – Germany (n = 48); c – France (n = 36); d – Romania (n = 36); e – Spain (n = 36).

### 2.3.4 Species and trait composition

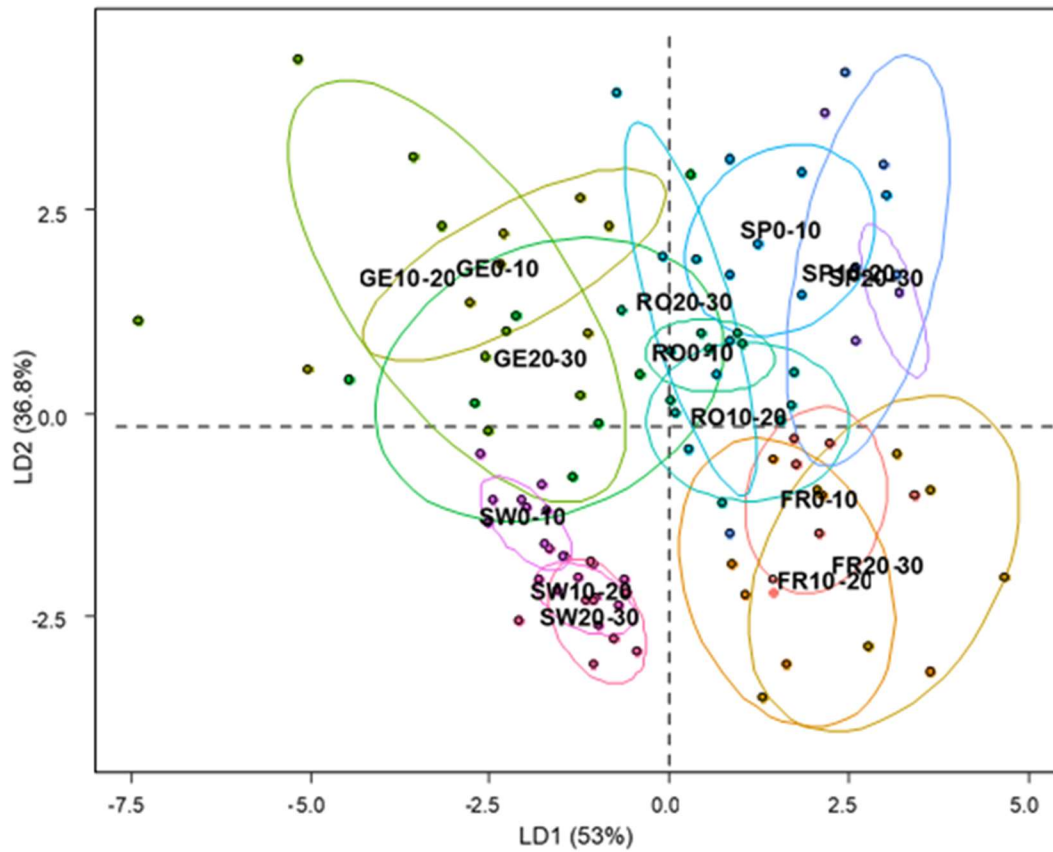
Manova of NMDS scores of species composition (NMDS  $k=4$ ;  $R^2 = 0.99$ , stress = 0.085) and of trait composition (NMDS  $k=3$ ;  $R^2=0.99$ , stress= 0.101) indicated that both, regardless of country, varied significantly with sampling depth ( $F_{8,170} = 4.19$ ,  $p < 0.01$  and  $F_{6,174} = 3.60$ ,  $p < 0.01$ , respectively). In addition, species composition also varied with tillage treatment ( $F_{4,84} = 2.79$ ,  $p = 0.03$ ). However, changes with sampling depth in both species and trait composition varied among countries (significant depth x country interaction;  $F_{32,348} = 2.48$ ,  $p < 0.01$ , and  $F_{24,264} = 2.30$ ,  $p < 0.01$ , respectively). Differences among countries were much larger than differences between treatments or sampling depths within countries (Figure 3 – Figure 6). Nevertheless, as indicated by pairwise comparisons, community and trait compositions between tillage treatments and sampling depths within countries also differed significantly (Table 6). For the tillage treatment this was true for species composition in Sweden and France, and for the trait composition in Romania. For the sampling depth this was true for species composition in Sweden (between 0-10 and 10-20 cm), Germany (between 0-10 and 20-30 cm) and Spain (between 0-10 and 20-30 cm), and for the trait composition in Sweden (between 0-10 and 20-30 cm), France (between 0-10 and 20-30 cm and between 10-20 and 20-30 cm) and Spain (between 0-10 and 20-30 cm).

**Table 6** Mahalanobis distances based on Hotellings  $T^2$  test for pairwise comparisons of Collembola species and trait composition within each country based on Linear Discriminant Analysis. CT = conventional tillage, RT = reduced tillage; SW = Sweden; GE = Germany; FR = France; RO = Romania; SP = Spain; Df1 = numerator degrees of freedom, DF2 = denominator degrees of freedom; Top = 0-10 cm, Mid =10-20 cm, Deep = 20-30 cm.

	Comparison	Df1	Df2	F-value	P-value	Mahalanobis distance
Species composition	FR CT / FR RT	4	13	5.27	0.01	0.60
	SW CT / SW RT	4	22	5.51	<0.01	0.44
	GE Top / GE Deep	3	10	8.67	<0.01	2.24
	SP Top / SP Deep	3	8	13.13	<0.01	1.37
	SW Top / SW Mid	3	14	15.8	<0.01	0.50
Trait composition	RO CT / RO RT	3	14	10.09	<0.01	1.19
	FR Top / FR Deep	3	8	5.05	0.03	1.65
	FR Mid / FR Deep	3	8	6.58	0.02	3.17
	SP Top / SP Deep	3	8	20.51	<0.01	4.14
	SW Top / SW Deep	3	14	4.01	0.03	0.38

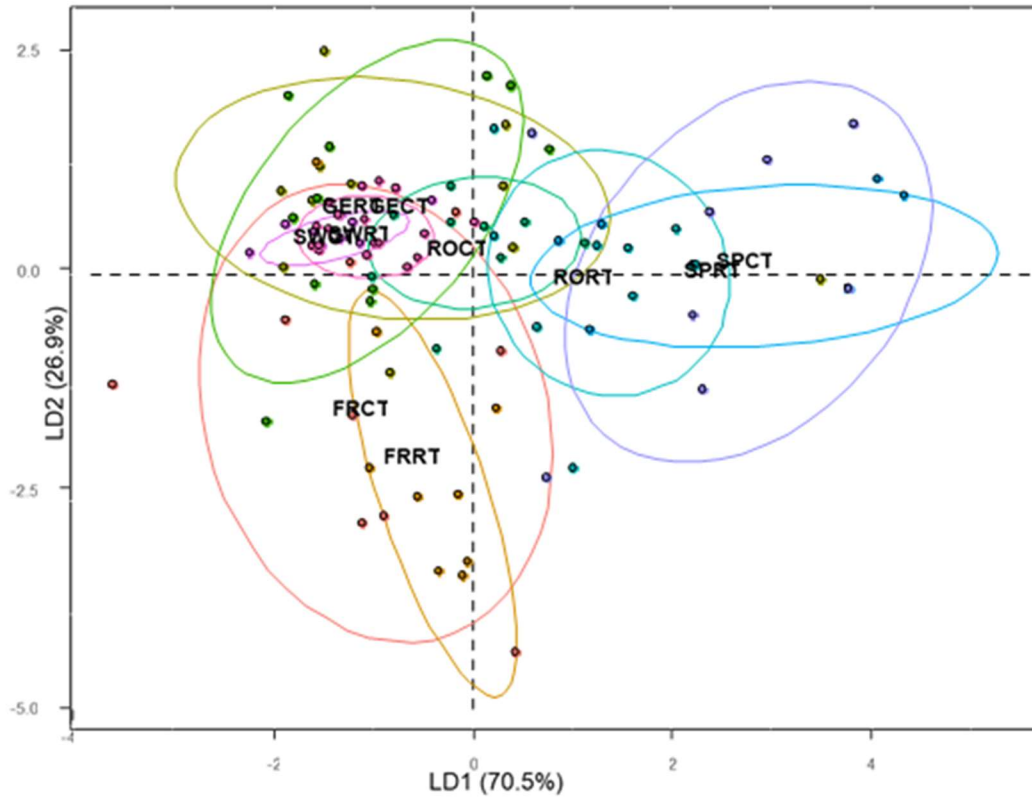


**Figure 3** LDA plot based on NMDS scores of Collembola species composition grouped by tillage treatments in five countries. LD1 accounted for 55.0% and LD2 for 28.3% of the total variance. SW = Sweden; GE = Germany; FR = France; RO = Romania; SP = Spain; CT = Conventional tillage; RT = Reduced tillage.

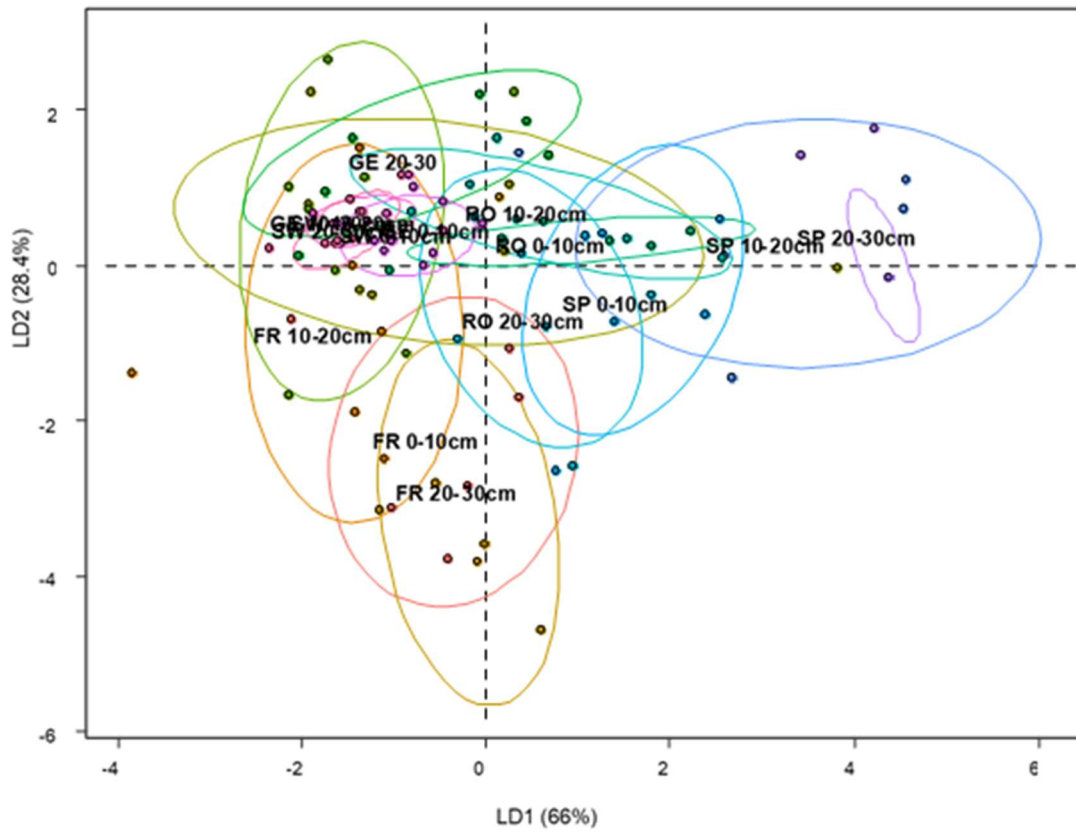


**Figure 4** LDA of NMDS scores of Collembola species composition grouped by different sampling depth in different countries. LD1 accounts for 53% of the variance found and LD2 accounts for 36.8%. SW = Sweden; GE = Germany; FR = France; RO = Romania; SP = Spain; Sampling depth is given in cm.





**Figure 5** LDA of NMDS scores of Collembola trait composition grouped by different tillage treatments in different countries. LD1 accounts for 70.5% of the variance found and LD2 accounts for 26.9%. SW = Sweden; GE = Germany; FR = France; RO = Romania; SP = Spain; CT = Conventional tillage; RT = Reduced tillage.



**Figure 6** LDA of NMDS scores of Collembola trait composition grouped by different sampling depth in different countries. LD1 accounts for 66% of the variance found and LD2 accounts for 28.4%. SW = Sweden; GE = Germany; FR = France; RO = Romania; SP = Spain; Sampling depth is given in cm.

## 2.4 Discussion

We hypothesized conventional tillage to similarly affect Collembola abundance, species richness and depth distribution across different European countries. Furthermore, we hypothesized conventional tillage to differentially affect Collembola of different ecological groups and consequently changing species community as well as trait community composition in a similar way across European countries. Contrary to these hypotheses, Collembola abundance, species richness and depth distribution between tillage systems varied in different countries suggesting that other factors were more important in structuring Collembola communities than the mechanical disturbance by tillage itself. However, ecological groups responded in a similar way to tillage practices across European countries, which was also the case for the trait community composition highlighting the usefulness of trait based approaches for analysing the response of highly variable and diverse communities to environmental changes across regions.

Contrasting our hypothesis 1 conventional tillage neither reduced total Collembola abundance nor species richness compared to reduced tillage practices suggesting that in agricultural fields in Europe the mechanical disturbance caused by tillage is not acting as primary regulatory factor for Collembola communities in soil. The higher abundance in conventionally tilled fields compared to reduced tillage fields in Romania even suggests that the promotion of Collembola by other factors can surpass the negative effects of conventional tillage. Similar to the abundance of Collembola  $C_{org}$  and  $N_t$  concentrations were higher in conventional tillage than in reduced tillage fields in Romania pointing to higher availability of food resources. Possibly, the decline in Collembola abundances caused by conventional tillage reported in previous studies (House and Parmelee 1985; Brennan et al. 2006; Vignozzi et al. 2019) at least in part was due to the fact that only upper soil layers were investigated, whereas we investigated the full soil profile to a depth of 30 cm. Similar to our results, other studies which considered deeper soil layers found higher abundance of Collembola in soil layers below 10 cm compensating for the lower abundance in shallow soil layers (Winter et al. 1990; Petersen 2002; Reeleder et al. 2006). This suggests that for evaluating the impact of tillage practices on Collembola their depth distribution across the soil profile to the ploughpan needs to be considered.

Contrary to our expectations and our hypothesis 2, the abundance of Collembola in conventionally tilled fields was not uniformly at a maximum in deeper soil layers across the European countries studied; rather, this was only the case in Sweden and Germany. In the other countries depth distribution of Collembola followed a linear decline with increasing sampling depth similar to the pattern in reduced tillage fields. This linear decline in Collembola abundance resembles natural habitats such as grasslands and forests, and is related to the distribution of resources (Berg et al. 1998, Berg and Bengtsson 2007). Similarly, in minimum or no tillage agricultural fields resources also decline with soil depth (House and Parmelee 1985;

Vignozzi et al. 2019). Conventional tillage on the other hand homogenizes the soil and integrates plant residues down to the ploughpan, and thereby more evenly distributes resources across soil depth (House and Parmelee 1985). The depth distribution of Collembola in our study generally was closely related to the concentrations of  $C_{org}$  and  $N_t$ . In fact, the depth distribution in conventional tillage fields in Germany and Sweden, which deviated from the general pattern of declining Collembola abundance and species richness with soil depth, was consistent with the depth distributions of  $C_{org}$  and  $N_t$ . Both soil parameters declined in a linear way in reduced tillage fields in these countries, but this was not the case in conventional tillage fields. This suggests that the availability of resources is the main regulating factor for the abundance of Collembola across European countries. On the other hand, bulk density and soil moisture, which often are considered limiting factors for Collembola (Berg et al. 1998; Jucevica and Melecis 2006), did not fit the abundance and depth distribution of Collembola in our study. Bulk density and soil moisture only differed between conventional tillage and reduced tillage fields in France, and therefore cannot be considered crucial factors affecting Collembola abundance in agricultural fields across Europe. The results rather support earlier suggestions that Collembola follow the spatial distribution of their food resources with physical habitat characteristics playing only a minor role (Verhoef and Nagelkerke 1977; Vignozzi et al. 2019).

Our hypothesis 3 in part was confirmed as different ecological groups of Collembola indeed responded differently to tillage practices. However, contrasting our hypothesis in particular epedaphic species were detrimentally affected by conventional tillage. Differential response of ecological groups of Collembola to agricultural management practices have been described before. Van Capelle et al. (2012) found the response of Collembola to tillage practices to depend on the soil texture with in particular euedaphic species being detrimentally affected by reduced tillage in soils with fine texture. Petersen (2002) found especially epedaphic and hemiedaphic species to be detrimentally affected by conventional tillage and assumed them to be translocated to deeper soil layers by ploughing. Other authors, however, found euedaphic species to be little or not affected by tillage practices (Petersen 2002; Brennan et al. 2006). Our results generally support the latter findings as neither hemiedaphic nor euedaphic Collembola were detrimentally affected by conventional tillage. Rather, especially the density of euedaphic species increased in deeper soil layers in conventional tillage fields in Sweden, Germany and Romania. Epedaphic species, on the other hand, were detrimentally affected by conventional tillage practices in Romania and Spain. The differential response of different ecological groups of Collembola to tillage practices across European countries may allow to better understand variations in the response of total Collembola abundance and species richness to tillage practices across large spatial gradients. In fields where the community is mainly composed of euedaphic species, total Collembola abundance and species richness is promoted due to resources being translocated to deeper soil layers, where they benefit from these

resources without being exposed to adverse environmental conditions at the soil surface (Germany and Romania) (Krab et al. 2010). On the other hand, in fields where epedaphic and hemiedaphic species are dominating, Collembola do not benefit from the translocation of resources into deeper soil layers, presumably, as they are unable to exploit these resources as they are not adapted to living in deeper soil (France and Spain) (Krab et al. 2010). Only if the community comprises a diversity of species of each of ep-, hemi- and euedaphic species, which are able to utilize decomposing organic material across all soil depths, Collembola can benefit from organic matter irrespective of the depth it is located in soil (Sweden). This ability, however, is not only depending on the species present, but also on environmental conditions. Presumably, the lower density of Collembola at shallow soil depths in the German field sites compared to that in the other countries studied was due to the very low soil moisture close to the soil surface. Dry conditions are unfavourable for Collembola and their spatial distribution often is related to soil moisture (Verhoef and Nagelkerke 1977; Verhoef and van Selm 1983). The combination of resources being translocated to deeper soil layers, where they are available under favourable soil moisture conditions during dry periods, is especially promoting euedaphic species (Krab et al. 2010). The translocated resources turn deeper soil layers into a more favourable habitat, which likely is particularly important during dry periods (Sjursen et al. 2001). When conditions become benign again, the surviving populations in deep soil layers may function as source for fast recolonization of shallow soil layers, and this may explain the dominance of euedaphic and hemiedaphic species in Sweden and Germany. The often described stratification of resources under reduced tillage practices (House and Parmelee 1985; Vignozzi et al. 2019) may be beneficial for Collembola species colonizing the soil surface (epedaphic species), but detrimental for euedaphic species preferentially colonizing deeper soil layers. By contrast, the more homogenous distribution of plant residues due to tillage (House and Parmelee 1985) likely facilitates the colonization of deeper soil layers by Collembola. This, however, often has been neglected by only investigating upper soil layers and ignoring soil layers beneath 10 cm depth (House and Parmelee 1985; Brennan et al. 2006; Vignozzi et al. 2019). As also stressed in previous studies (Winter et al. 1990; Petersen 2002; Reeleder et al. 2006), differences in the abundance of Collembola between agricultural fields managed in different ways may disappear if deeper soil layers are considered. Our results suggest that the full spatial range where food resources are distributed needs to be considered, highlighting the need for investigating deeper soil layers in particular in arable systems where crop residues are translocated into deeper soil by ploughing.

As expected, species composition between countries differed much more than between treatments within countries hampering general conclusions considering our hypothesis 4. Generally, the communities reflected the geographical location of the different sites, e.g. species composition of Collembola at the sites in Sweden and Germany, located in northern and central Europe, were similar and separated from

those at the sites in France, Romania and Spain, situated in southern Europe, and being similar to each other. Due to the different species composition at the different sites across the countries studied it was not possible to draw conclusions on the effect of the reduction of tillage practices on individual Collembola species across Europe. Trait composition, on the other hand, allowed general insight, although it may also vary due to variations in the abundance of different ecological groups driven by regional differences in climate (see above). At the field sites in Sweden and Germany euedaphic and hemiedaphic species were dominating, and this also shaped the trait composition of Collembola at these sites. By contrast, at the study sites in southern Europe euedaphic species were generally less abundant and this also is reflected in the trait composition of Collembola communities at these sites. In part, however, this varied among tillage practices. Conventional tillage fields in France as well as Romania accommodated more Collembola displaying euedaphic and hemiedaphic traits resembling the sites in Germany and Sweden, whereas in reduced tillage fields in France and Romania the abundance of epedaphic Collembola was higher and the respective traits were more prevalent. Generally, however, the trait composition of Collembola communities showed that conventional tillage favours euedaphic Collembola, but at the same time detrimentally affects epedaphic species.

Overall, using traits instead of species allowed more detailed insight into the effect of agricultural practices on Collembola communities across countries and geographical regions of Europe. The results therefore suggest that agricultural practices have to be adapted to the biota present or dominating in the different geographical regions. In addition, our results underline the importance of food resources in soil, presumably functioning as limiting factor for Collembola of deep soil layers, with their importance surpassing the importance of soil moisture and mechanical disturbances for Collembola communities. In conclusion, agricultural practices cannot be easily adapted to favour all soil organisms as the communities present in different bioclimatic regions may respond differently to the same soil management practices. For developing management practices favouring local soil animal communities and the services they provide, the composition of these communities and the driving factors of community composition need to be considered.

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## References

- Arvidsson, J. (2010). Energy use efficiency in different tillage systems for winter wheat on a clay and silt loam in Sweden. *European Journal of Agronomy*, 33(3), 250-256.
- Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, 515(7528), 505-511.
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). *Fitting Linear Mixed-Effects Models Using lme4*. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.
- Berg, M. P., Kniese, J. P., Bedaux, J. J. M., & Verhoef, H. A. (1998). Dynamics and stratification of functional groups of micro-and mesoarthropods in the organic layer of a Scots pine forest. *Biology and Fertility of Soils*, 26(4), 268-284.
- Berg, M. P., & Bengtsson, J. (2007). Temporal and spatial variability in soil food web structure. *Oikos*, 116(11), 1789-1804.
- Brennan, A., Fortune, T., & Bolger, T. (2006). Collembola abundances and assemblage structures in conventionally tilled and conservation tillage arable systems. *Pedobiologia*, 50(2), 135-145.
- Dubie, T. R., Greenwood, C. M., Godsey, C., & Payton, M. E. (2011). Effects of tillage on soil microarthropods in winter wheat. *Southwestern Entomologist*, 36(1), 11-20.
- Ehlers, W., Werner, D., & Mähner, T. (2000). Wirkung mechanischer Belastung auf Gefüge und Ertragsleistung einer Löss-Parabraunerde mit zwei Bearbeitungssystemen. *Journal of Plant Nutrition and Soil Science*, 163(3), 321-333.
- Fjellberg, A. (1998). *The Collembola of Fennoscandia and Denmark. Part I: Poduromorpha*. Fauna Entomologica Scandinavica.
- Fjellberg, A. (2007). *The Collembola of Fennoscandia and Denmark, Part II: Entomobryomorpha and Symphypleona*. Brill.
- Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., ... & Menta, C. (2009). Soil biodiversity monitoring in Europe: ongoing activities and challenges. *European Journal of Soil Science*, 60(5), 807-819.
- Hartig, R. (2020). *DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. R package version 0.3.2.0. <https://CRAN.R-project.org/package=DHARMA>
- Hopkin, S. P. (2007). *A key to the Collembola (springtails) of Britain and Ireland*. FSC publications.
- House, G. J., & Parmelee, R. W. (1985). Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. *Soil and Tillage Research*, 5(4), 351-360.
- Jucevica, E., & Melecis, V. (2006). Global warming affect Collembola community: A long-term study. *Pedobiologia*, 50(2), 177-184.

- Krab, E. J., Oorsprong, H., Berg, M. P., & Cornelissen, J. H. (2010). Turning northern peatlands upside down: disentangling microclimate and substrate quality effects on vertical distribution of Collembola. *Functional Ecology*, *24*(6), 1362-1369.
- López-Garrido, R., Madejón, E., Murillo, J. M., & Moreno, F. (2011). Short and long-term distribution with depth of soil organic carbon and nutrients under traditional and conservation tillage in a Mediterranean environment (southwest Spain). *Soil use and Management*, *27*(2), 177-185.
- Macfadyen, A. (1961). Improved funnel-type extractors for soil arthropods. *The Journal of Animal Ecology*, 171-184.
- McLaughlin, A., & Mineau, P. (1995). The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment*, *55*(3), 201-212.
- Moretti, M., Dias, A. T., De Bello, F., Altermatt, F., Chown, S. L., Azcarate, F. M., ... & Berg, M. P. (2017). Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, *31*(3), 558-567.
- Nordhausen, K., Sirkia, S., Oja, H., Tyler, D. E. (2018). *ICSNP: Tools for Multivariate Nonparametrics*. R package version 1.1-1. <https://CRAN.R-project.org/package=ICSNP>
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecsand, E., Wagner, H. (2019). *vegan: Community Ecology Package*. R package version 2.5-6. <https://CRAN.R-project.org/package=vegan>
- Parisi, V., Menta, C., Gardi, C., Jacomini, C., & Mozzanica, E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, ecosystems & environment*, *105*(1-2), 323-333.
- Petersen, H. (2002). Effects of non-inverting deep tillage vs. conventional ploughing on collembolan populations in an organic wheat field. *European Journal of Soil Biology*, *38*(2), 177-180.
- Pey, B., Nahmani, J., Auclerc, A., Capowiez, Y., Cluzeau, D., Cortet, J., ... & Hedde, M. (2014). Current use of and future needs for soil invertebrate functional traits in community ecology. *Basic and Applied Ecology*, *15*(3), 194-206.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team (2020). *\_nlme: Linear and Nonlinear Mixed Effects Models\_*. R package version 3.1-145, <URL: <https://CRAN.R-project.org/package=nlme>>.
- Potard, K., Monard, C., Le Garrec, J. L., Caudal, J. P., Le Bris, N., & Binet, F. (2017). Organic amendment practices as possible drivers of biogenic volatile organic compounds emitted by soils in agrosystems. *Agriculture, Ecosystems & Environment*, *250*, 25-36.
- Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Peres, G., & Rutgers, M. (2012). Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches. *Current Opinion in Environmental Sustainability*, *4*(5), 529-538.
- R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Reeleder, R. D., Miller, J. J., Coelho, B. B., & Roy, R. C. (2006). Impacts of tillage, cover crop, and nitrogen on populations of earthworms, microarthropods, and soil fungi in a cultivated fragile soil. *Applied Soil Ecology*, *33*(3), 243-257.



- Rodgers, D., McPhee, J., Aird, P., & Corkrey, R. (2018). Soil arthropod responses to controlled traffic in vegetable production. *Soil and Tillage Research*, *180*, 154-163.
- Salmon, S., Ponge, J. F., Gachet, S., Deharveng, L., Lefebvre, N., & Delabrosse, F. (2014). Linking species, traits and habitat characteristics of Collembola at European scale. *Soil Biology and Biochemistry*, *75*, 73-85.
- Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual review of entomology*, *29*(1), 25-46.
- Sjursen, H., Bayley, M., & Holmstrup, M. (2001). Enhanced drought tolerance of a soil-dwelling springtail by pre-acclimation to a mild drought stress. *Journal of Insect Physiology*, *47*(9), 1021-1027.
- Stinner, B. R., McCartney, D. A., & Van Doren Jr, D. M. (1988). Soil and foliage arthropod communities in conventional, reduced and no-tillage corn (maize, *Zea mays* L.) systems: a comparison after 20 years of continuous cropping. *Soil and Tillage Research*, *11*(2), 147-158.
- Stinner, B. R., & House, G. J. (1990). Arthropods and other invertebrates in conservation-tillage agriculture. *Annual review of entomology*, *35*(1), 299-318.
- Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Doledec, S., Dubs, F., ... & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, *19*(10), 2921-2947.
- van Capelle, C., Schrader, S., & Brunotte, J. (2012). Tillage-induced changes in the functional diversity of soil biota—A review with a focus on German data. *European Journal of Soil Biology*, *50*, 165-181.
- Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S. Fourth Edition*. Springer, New York. ISBN 0-387-95457-0
- Verhoef, H. A., & Nagelkerke, C. J. (1977). Formation and ecological significance of aggregations in Collembola. *Oecologia*, *31*(2), 215-226.
- Verhoef, H. A., & Van Selm, A. J. (1983). Distribution and population dynamics of Collembola in relation to soil moisture. *Ecography*, *6*(4), 387-388.
- Vignozzi, N., Agnelli, A. E., Brandi, G., Gagnarli, E., Goggioli, D., Lagomarsino, A., ... & Gucci, R. (2019). Soil ecosystem functions in a high-density olive orchard managed by different soil conservation practices. *Applied Soil Ecology*, *134*, 64-76.
- Widenfalk, L. A., Bengtsson, J., Berggren, Å., Zwiggelaar, K., Spijkman, E., Huyer-Brugman, F., & Berg, M. P. (2015). Spatially structured environmental filtering of collembolan traits in late successional salt marsh vegetation. *Oecologia*, *179*(2), 537-549.
- Winter, J. P., Voroney, R. P., & Ainsworth, D. A. (1990). Soil microarthropods in long-term no-tillage and conventional tillage corn production. *Canadian Journal of Soil Science*, *70*(4), 641-653.

## Appendix

**Supplementary Table 1** Climatic conditions, fertilization and soil texture of the field sites in Sweden, Germany, France, Romania and Spain.

Country	Fieldsite	Climatic conditions		Fertilization	Soil texture		
		mean annual precipitation [mm]	mean annual temperature [°C]		Sand [%]	Silt [%]	Clay [%]
Sweden	Säby	547	6.7	mineral fertilizer (139 kg N/ha)	25	52	23
Germany	Garte Süd	621	9.5	mineral fertilizer (0-185 kg N/ha)	12	73	15
France	Efele	696	12.1	mineral fertilizer (120 kg N/ha)	16	69	15
Romania	Turda	540	9.0	compound fertilizer (90 kg N/ha)	16	28	56
Spain	La Hampa	496	19.0	compound fertilizer (60 kg N/ha)	58	18	24

**Supplementary Table 2** Collembola traits considered for calculating ecological groups according to Vandewalle et al. (2010).

Trait	Trait scores
Ocelli	0+0 = 4
	1+1 - 2+2 = 3
	3+3 - 4+4 = 2
	5+5 - 6+6 = 1
	7+7 - 8+8 = 0
Antenna length	$0 < X \leq 0.5$ body length = 4
	$0.5$ body length $< X \leq 1$ body length = 2
	$X > 1$ body length = 0
Furca	Absent = 4
	Reduced/short = 2
	Fully developed = 0
Hairs/Scales	Absent = 2
	Present = 0
Pigmentation	Absent (white) = 4
	Coloured but not patterned = 2
	Coloured and patterned = 0

**Supplementary Table 3** Ecological groups ascribed to Collembola species based on trait scores as given in Supplementary Table 2.

Species	Ecological group	Species	Ecological group
<i>Anurida granaria</i>	Euedaphic	<i>Megalothorax minimus</i>	Euedaphic
<i>Archaphorura serratotuberculata</i>	Euedaphic	<i>Mesaphorura hylophila</i>	Euedaphic
<i>Arrhopalites caecus</i>	Hemiedaphic	<i>Mesaphorura macrochaeta</i>	Euedaphic
<i>Arrhopalites pseudoappendices</i>	Hemiedaphic	<i>Mesaphorura</i> sp.	Euedaphic
<i>Ceratophysella denticulata</i>	Hemiedaphic	<i>Mesaphorura sylvatica</i>	Euedaphic
<i>Ceratophysella</i> sp.	Hemiedaphic	<i>Mesaphorura yosiii</i>	Euedaphic
<i>Choreutinula inermis</i>	Hemiedaphic	<i>Micranurida pygmaea</i>	Euedaphic
<i>Cryptopygus thermophilus</i>	Hemiedaphic	<i>Neotullbergia crassiscuspis</i>	Euedaphic
<i>Desoria fennica</i>	Hemiedaphic	<i>Neotullbergia tricuspis</i>	Euedaphic
<i>Deuteraphorura inermis</i>	Euedaphic	<i>Onychiurus</i> sp.	Euedaphic
<i>Deuterosminthurus bicinctus</i>	Epedaphic	<i>Orchesella bifasciata</i>	Epedaphic
<i>Deuterosminthurus pallipes</i>	Epedaphic	<i>Orchesella flavescens</i>	Epedaphic
<i>Entomobrya marginata</i>	Epedaphic	<i>Paratullbergia callypigos</i>	Euedaphic
<i>Folsomia candida</i>	Euedaphic	<i>Paratullbergia macdougalli</i>	Euedaphic
<i>Folsomia dovrensis</i>	Euedaphic	<i>Paratullbergia</i> sp.	Euedaphic
<i>Folsomia fimetaria</i>	Euedaphic	<i>Parisotoma notabilis</i>	Hemiedaphic
<i>Folsomia fimetarioides</i>	Euedaphic	<i>Protaphorura armata</i>	Euedaphic
<i>Folsomia inoculata</i>	Euedaphic	<i>Pseudanurophorus isotoma</i>	Euedaphic
<i>Folsomia quadrioculata</i>	Euedaphic	<i>Pseudisotoma sensibilis</i>	Hemiedaphic
<i>Folsomia</i> sp.	Euedaphic	<i>Pseudosinella alba</i>	Hemiedaphic
<i>Folsomia spinosa</i>	Euedaphic	<i>Pseudosinella decipiens</i>	Hemiedaphic
<i>Folsomides parvulus</i>	Euedaphic	<i>Pseudosinella halophila</i>	Hemiedaphic
<i>Frisea mirabilis</i>	Hemiedaphic	<i>Pseudosinella immaculata</i>	Hemiedaphic
<i>Frisea truncata</i>	Hemiedaphic	<i>Pseudosinella petterseni</i>	Hemiedaphic
<i>Heteromurus nitidus</i>	Hemiedaphic	<i>Sinella coeca</i>	Hemiedaphic
<i>Hypogastrura assimilis</i>	Hemiedaphic	<i>Sminthurides parvulus</i>	Epedaphic
<i>Isotoma anglicana</i>	Hemiedaphic	<i>Sminthurinus aureus</i>	Epedaphic
<i>Isotoma riparia</i>	Hemiedaphic	<i>Sminthurinus concolor</i>	Epedaphic
<i>Isotoma viridis</i>	Hemiedaphic	<i>Sminthurinus elegans</i>	Epedaphic
<i>Isotomiella minor</i>	Euedaphic	<i>Sminthurinus niger</i>	Epedaphic
<i>Isotomurus fucicolus</i>	Epedaphic	<i>Sminthurus viridis</i>	Epedaphic
<i>Isotomurus palustris</i>	Epedaphic	<i>Sphaeridia pumilis</i>	Epedaphic
<i>Lepidocyrtus violaceus</i>	Epedaphic	<i>Stenaphorura denisii</i>	Euedaphic
<i>Lepidocyrtus cyaneus</i>	Epedaphic	<i>Stenaphorura quadrispina</i>	Euedaphic
<i>Lepidocyrtus lanuginosus</i>	Epedaphic	<i>Willemia anophthalma</i>	Euedaphic
<i>Lepidocyrtus lignorum</i>	Epedaphic	<i>Willemia buddenbrocki</i>	Euedaphic
<i>Lepidocyrtus</i> sp.	Epedaphic	<i>Willemia intermedia</i>	Euedaphic
<i>Lipothrix lubbocki</i>	Epedaphic	<i>Willowsia buski</i>	Epedaphic

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

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## **Abstract**

### **Background**

The role of soil mesofauna on 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 (mouldboard ploughing; CT) and minimum tillage (MT). Litterbags filled with maize leaf litter of two mesh sizes (2 mm, 48  $\mu$ m) were used. Litterbags were buried at 23 (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 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 colonized 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.

### 3.1 Introduction

Litter decomposition is based on the activity of soil microorganisms and soil fauna. However, although soil fauna generally accelerates litter decomposition, there are still major knowledge gaps on the role of soil mesofauna on decomposition processes (Kampichler and 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 and 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 and Bruckner, 2009). Although access by mesofauna typically accelerates litter decomposition processes (Yang et al., 2017), it may also reduce decomposition rates via overgrazing of fungal populations (Vreeken-Buijs and Brussaard, 1996). Generally, effects of soil mesofauna on litter decomposition vary with the quality of the litter material and the type of ecosystems (Bokhorst and Wardle, 2013; Gergócs and Hufnagel, 2016).

Decomposition of plant residues is essential for the cycling of elements and the provisioning of nutrients to plants (Casado-Murillo and 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 characterized 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 (Stinner et al. 1988; Dittmer and Schrader 2000). 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 and 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 (Seastedt, 1984; Filser, 2002; Neher and Barbercheck, 2019). Reaching high density also in arable systems, they play a key role for the recycling of plant residues (House and Stinner, 1987; Ke et al., 2005). However, little is known on how tillage practices modify the role of Collembola and mesofauna in general on the decomposition of plant residues (House and Stinner, 1987; Reddy et al., 1994; Kladivko, 2001).

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 and Falca, 2000). Since the  $^{13}\text{C}/^{12}\text{C}$  ratio of consumers changes little compared to their diet it reflects the basal food resources consumed. By contrast, the  $^{15}\text{N}/^{14}\text{N}$  ratio increases in a consistent way per trophic level by 2-4  $\delta$  units, and therefore reflects the trophic position of consumers (Layman et al., 2012; Potapov et al., 2019). By using plant resources of different  $^{13}\text{C}$  signatures, the proportion of these food resources in the diet of consumers can be determined (Scheunemann et al., 2015). Since the  $^{13}\text{C}$  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  $\mu\text{m}$ ) only allowed access of the litter by microorganisms, whereas coarse mesh-size (2 mm) also allowed access by mesofauna.

We hypothesized 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.

## 3.2 Material and Methods

### 3.2.1 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<sup>-1</sup> in spring 2017. Data of soil temperature was 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 was obtained from the station “Wetterwarte Göttingen” (51°30 N 9°57E), which is approximately 2 km away from to the field-site (see Supplementary Figure 1). Soil pH was determined with a soil to solution ratio of 1:2.5 in deionized water. Values were averaged among soil depths and treatments.

### 3.2.2 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 ca 5 cm. Litterbags were buried approximately at the working depth of the machinery to simulate litter dispersal by the respective tillage treatment, i.e. 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).

### 3.2.3 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 diethyleneglycol – water solution. For storage until determination, animals were transferred into 70% ethanol. Collembola species were identified using the keys of Hopkin (2007) and (Fjellberg, 2007, 1998). 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 belowground. Each trait received a score between 0 and 4, with low scores for traits well adapted to aboveground live and high scores for traits well adapted to belowground live (see Supplementary Table 1). 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 Supplementary Table 2).

### 3.2.4 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 and 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 ( $\delta X$ ) was expressed using the  $\delta$  notation with  $\delta X (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1000$ , with X representing the target isotope ( $^{13}\text{C}$  or  $^{15}\text{N}$ ),  $R_{\text{sample}}$  and  $R_{\text{standard}}$  the respective target isotope ratio ( $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ ). Atmospheric nitrogen was used as standard for  $^{15}\text{N}$  and Vienna Peedee Belemnite limestone as standard for  $^{13}\text{C}$ . For internal calibration acetanilide ( $\text{C}_8\text{H}_9\text{NO}$ , 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  $2.0 \pm 0.5 \text{‰}$  and was based on the average difference in  $\delta^{13}\text{C}$  values of Collembola ( $-27.0 \pm 0.9 \text{‰}$ ; mean  $\pm$  SD) and soil organic matter ( $-25.3 \pm 3.1$ ) at our study site ('detrital shift'; Potapov et al., 2019).

### 3.2.5 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  $\delta^{13}\text{C}$  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” (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 generalized linear mixed effects models using the `glmer.nb` and `glmer` function of the `lme4` package (Bates et al., 2015). For the 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 generalized linear mixed effects models. Statistical models calculated with the `lme`, `glmer` or `glmer.nb` functions 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 multi-dimensional 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 and Ripley, 2002) followed by Hotellings  $T^2$ -test (Hotellings $T^2$ , *ICSNP* package) (Nordhausen et al., 2018) were 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.

### 3.3 Results

#### 3.3.1 Litter decomposition

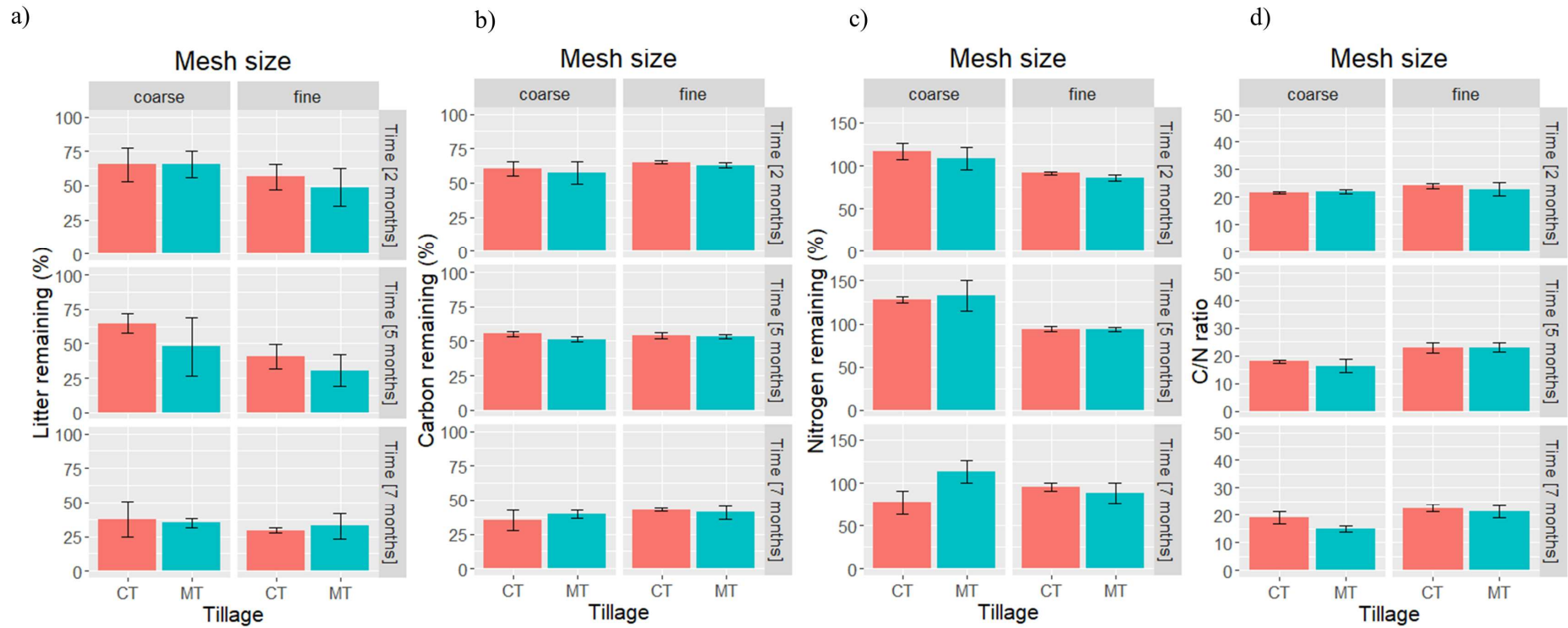
Litter mass remaining in the litterbags varied significantly with Time (linear term only) and Mesh size, but not with Tillage (Table 1, Figure 1a). On average, litter mass remaining decreased in a linear way from  $65.37 \pm 9.54$  % (mean  $\pm$  SD) at the first sampling to  $36.14 \pm 8.29$  % at the last sampling in the coarse mesh size litterbags. Respective values for the fine mesh size litterbags were  $52.52 \pm 10.90$  % and  $30.96 \pm 5.95$  %. The amount of litter remaining was generally lower in litterbags with fine than in those with coarse mesh size (overall means of  $41.74 \pm 13.90$  % and  $50.76 \pm 17.13$  %) 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  $52.79 \pm 11.42$  % and  $48.10 \pm 12.24$  %, respectively). Litter C remaining decreased from  $58.91 \pm 6.02$  % (mean  $\pm$  SD) at the first sampling to  $37.28 \pm 5.42$  % at the last sampling in coarse mesh size litterbags. Respective values for the fine mesh size litterbags were  $63.91 \pm 17.86$  % and  $41.66 \pm 3.21$ %.

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  $88.60 \pm 3.92$  % (mean  $\pm$  SD) at the first sampling to  $91.49 \pm 8.75$ % at the last sampling, but increased in the coarse mesh size litterbags from  $116.52 \pm 8.31$  % and  $108.43 \pm 11.47$ % (for CT and MT treatments, respectively) at the first sampling to  $128.17 \pm 2.86$ % and  $132.61 \pm 15.77$ % at the second sampling before decreasing at the last sampling. This final decrease in N remaining was stronger in the CT ( $77.15 \pm 11.80$ %) than in the MT treatment ( $112.69 \pm 11.19$ %) (significant Tillage x Time x 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  $23.46 \pm 1.67$  (mean  $\pm$  SD) at the

first sampling to  $21.81 \pm 1.79$  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 ( $22.08 \pm 0.70$ ) to the last sampling ( $14.84 \pm 0.89$ ) 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 ( $21.63 \pm 0.20$ ) to the last sampling ( $19.03 \pm 1.83$ ) 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 x 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.



**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  $\pm$  standard deviation; for statistical analysis see Table 1.

**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  $\mu$ m, 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 five 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 Methods); the Tillage  $\times$  Mesh size  $\times$  quadratic term was generally not significant.

		Int	Till	Mesh	L	Q	Till x L	Mesh x L	Mesh x Q	Till x Mesh x L
	df	41		41	41					
Litter mass	t-value	15.63		-4.19	-6.74					
	p-value	<0.01		<0.01	<0.01					
	df	41		41	41	41				
Litter C remaining	t-value	58.92		2.71	-14.98	-2.34				
	p-value	<0.01		<0.01	<0.01	0.02				
	df	33	33	33	33	33	33	33	33	33
Litter N remaining	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
	df	38	38	38	38	38		38	38	
Litter C/N ratio	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	

### 3.3.2 Abundance and community composition of Collembola

Total abundance of Collembola significantly increased with time from  $21.38 \pm 24.53$  individuals/g litter (mean  $\pm$  SD) at the first sampling to  $77.13 \pm 82.69$  individuals/g 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 ( $82.75 \pm 79.68$  and  $15.75 \pm 20.01$  individuals/g litter, respectively; Figure 2).

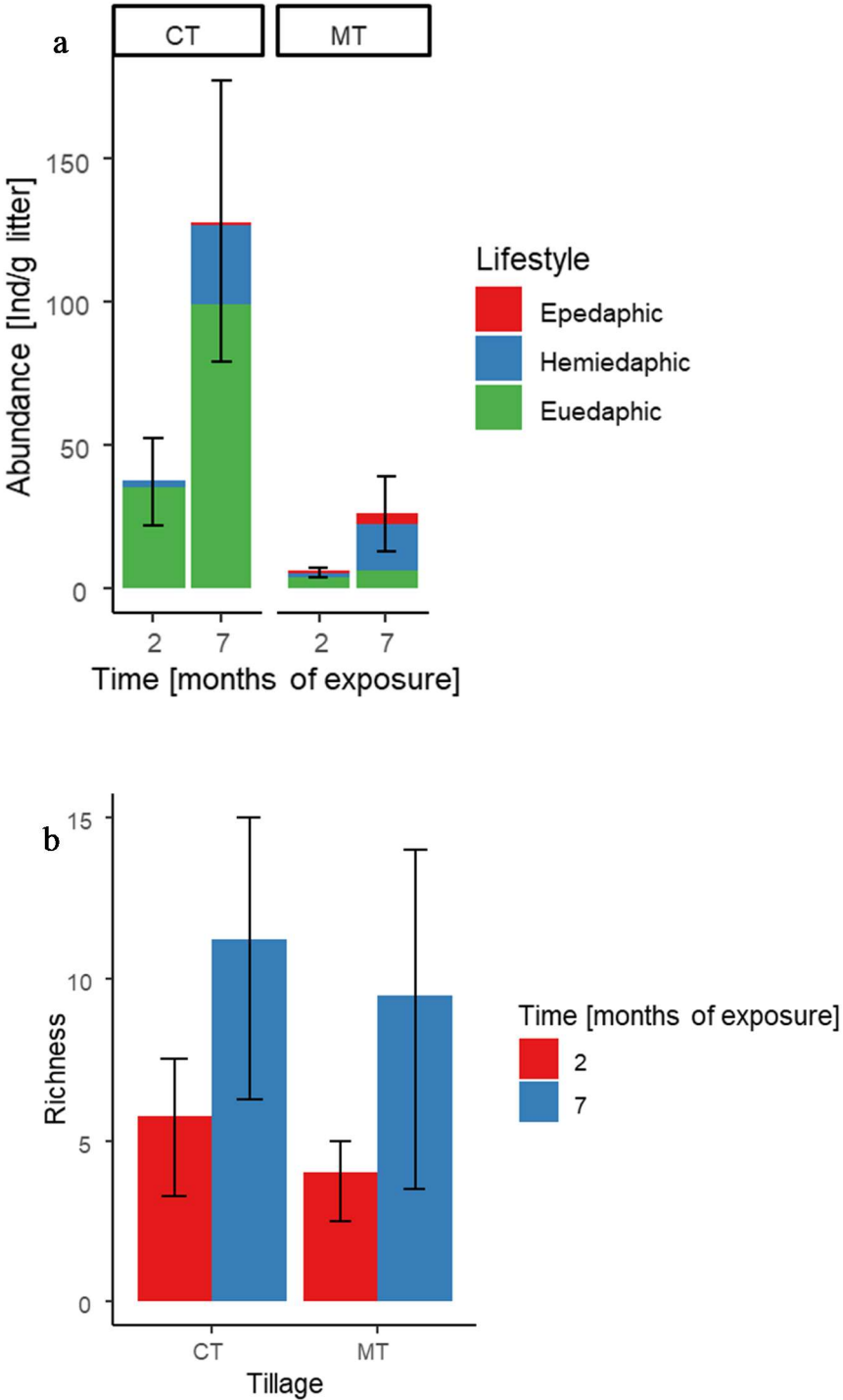
Likewise epedaphic Collembola increased with time from  $0.63 \pm 0.70$  individuals/g litter (mean  $\pm$  SD) at the first to  $2.13 \pm 2.26$  individuals/g litter at the last sampling, but they reacted differently to tillage practices with the abundances in CT being lower than in MT treatments ( $0.63 \pm 1.32$  and  $2.13 \pm 1.96$  individuals/g 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  $1.88 \pm 1.27$  individuals/g litter at the first to  $22.25 \pm 24.31$  individuals/g litter at the last sampling. Euedaphic Collembola followed the same pattern as total Collembola with their abundance increasing from  $19.38 \pm 23.87$  individuals/g litter at the first to  $52.63 \pm 67.37$  individuals/g litter at the last sampling, and being more abundant in CT than MT treatments ( $67.13 \pm 60.93$  and  $4.88 \pm 3.55$  individuals/g litter, respectively; Figure 2).

In contrast to total abundance, Collembola species richness did not differ significantly between tillage treatments, but overall it increased from  $4.88 \pm 2.03$  (mean  $\pm$  SD) at the first to  $10.38 \pm 4.90$  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).

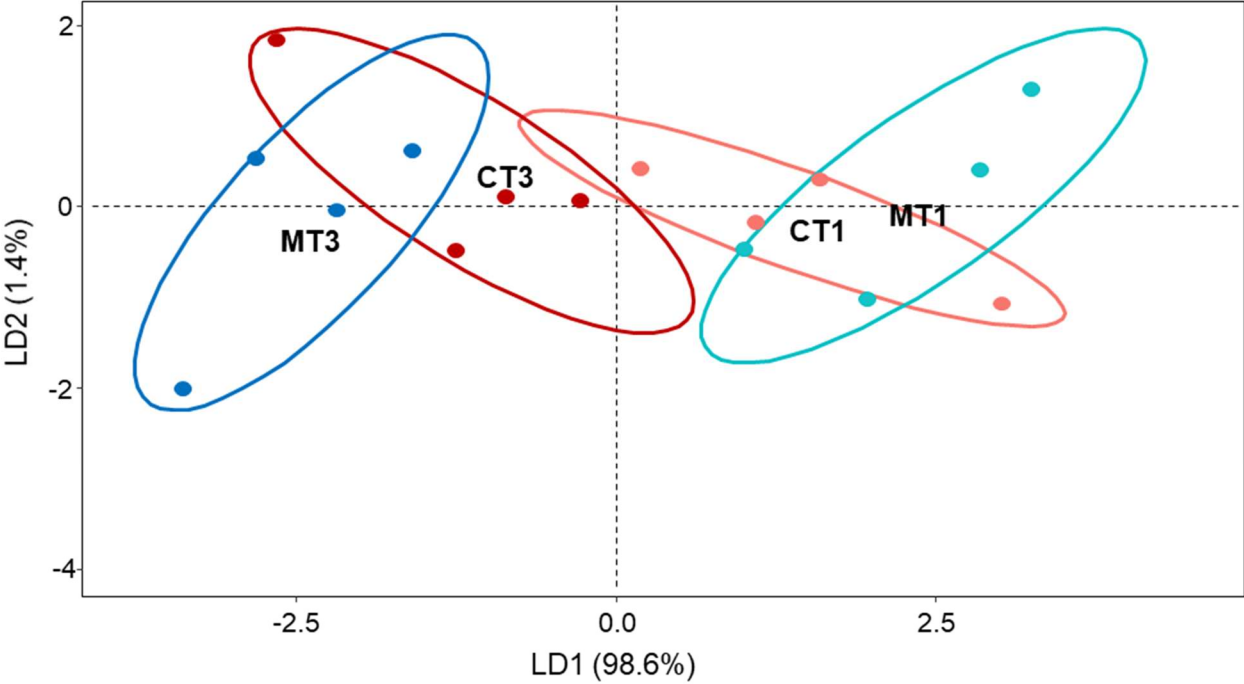


**TABLE 2:** Generalized linear mixed effects model table of Z- and p-values on the effect of tillage (Till; conventional tillage, minimum tillage) and Time (first sampling, third sampling) on total abundance, abundances of different ecological groups and species richness of Collembola; df = degrees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

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



**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  $\pm$  standard deviation.

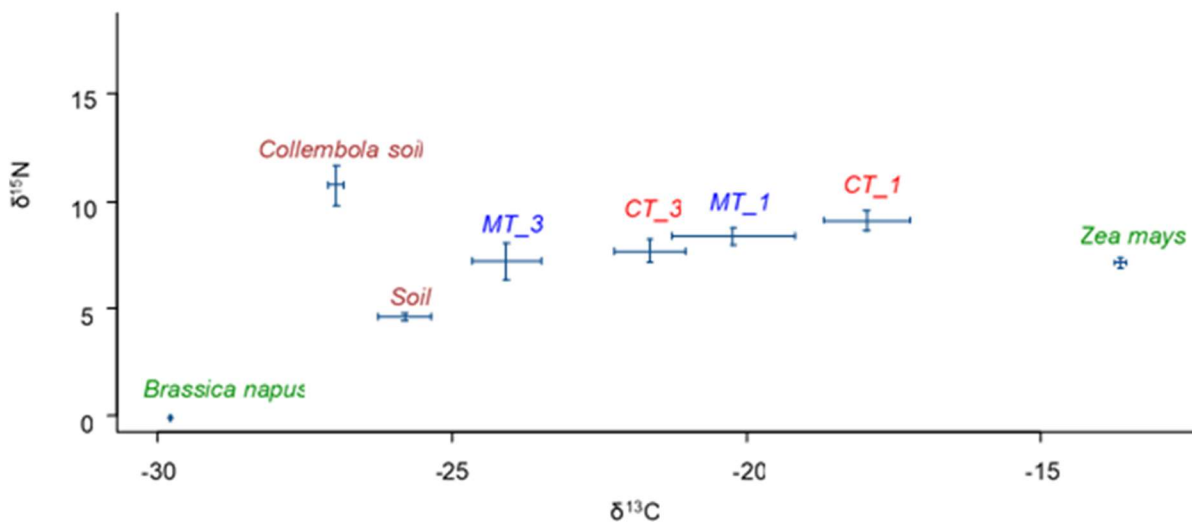


**FIGURE 3:** Collembola community composition in litterbags with maize (*Zea mays*) litter exposed in conventional tillage (CT) and minimum tillage (MT) fields for 2 (CT1 and MT1) and 7 months (CT3 and MT3). LDA plot based on NMDS scores of Collembola species composition grouped by tillage treatments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

### 3.3.3 Incorporation of maize carbon by Collembola

On average, the  $\delta^{13}\text{C}$  signature of Collembola decreased significantly from the first ( $-18.90 \pm 3.08\text{‰}$ ; mean  $\pm$  SD; Figure 4) to the third sampling ( $-22.65 \pm 3.19\text{‰}$ ) regardless of tillage treatment (t-value =  $-5.08$ , df = 64,  $p < 0.01$ ). In addition, the average  $\delta^{13}\text{C}$  signature of Collembola was generally lower in MT ( $-22.75 \pm 3.28\text{‰}$ ) than in CT treatments ( $-20.38 \pm 3.45\text{‰}$ ) 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  $41 \pm 21\%$  (mean  $\pm$  SD). However, the proportion of maize carbon decreased significantly between sampling dates from  $55 \pm 19\%$  at the first to  $34 \pm 18\%$  at the third sampling (t-value =  $-3.74$ , df = 18,  $p < 0.01$ ).



**FIGURE 4:**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (mean  $\pm$  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);  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of rape and maize, the surrounding soil (Soil) and Collembola outside of the litterbags (Collembola soil) are given as control.

### 3.4 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 Supplementary Figure 1). 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 used for correcting mass loss data (see Methods). 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 mobilization 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 (Hättenschwiler et al., 2005; Garcia-Palacios et al., 2013), suggesting that mesofauna facilitates nutrient mobilization from decomposing litter material at later stages of decay, i.e. spring and summer, favouring nutrient capture by plants and thereby crop production. Although mesofauna may accelerate litter decomposition (Seastedt, 1984; Garcia-Palacios et al., 2013) 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 (Parker et al., 1984; Lavelle et al., 1993; Hättenschwiler et al., 2005). Rather, detritivore soil animals may indirectly stimulate microbial litter decay, e.g. by increasing the supply of N (Seastedt, 1984; Lavelle et al., 1993), and this is supported by results of our study. Further, soil mesofauna may stimulate microbial activity by grazing, which may contribute to increased mobilization of nutrients in litter, in particular at later stages of decay (Seastedt, 1984; Parker et al., 1984; Lavelle et al., 1993; Hättenschwiler et al., 2005). Interestingly, our results oppose one of the main criticism of litterbag studies, i.e. increased mechanical loss or active transport of litter out of

the litterbags of larger mesh size (Seastedt, 1984; Kampichler and Bruckner, 2009). 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 colonization of litter by fungi from surrounding soil, thereby transporting N into the litter resulting in lower litter C/N ratio (Berg and 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 (Parker et al., 1984; Lavelle et al., 1993; Zhang et al., 2008; Prescott et al., 2010).

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 and Nagelkerke, 1977; Vignozzi et al., 2018), 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 and 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 colonizing species predominantly feed on the litter material itself, whereas species dominating at later stages of decomposition predominantly feed on bacteria and fungi colonizing 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 colonization 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.

## 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 mobilization of litter N suggesting that it favours nutrient uptake by the crop species in the following year and mesofauna contributes to this mobilization. Overall, the incorporation of crop residues into the soil by both CT and MT fostered Collembola abundance and the mobilization of litter N, and thereby may contribute to the sustainable management of agricultural systems.

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## References

- Albers, D., Schaefer, M. & Scheu, S. (2006). Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology*, *87*, 235–245.
- Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* *67*, 1–48.
- Berg, B. & Staaf, H. (1981) Leaching, accumulation and release of nitrogen in decomposing forest litter. In: Clark, F.E., Rosswall, T. (eds) *Terrestrial Nitrogen Cycles. Ecological Bulletins* (Stockholm), *33*, 163–178.
- Boecklen, W.J., Yarnes, C.T., Cook, B.A. & James, A.C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution, and Systematics*, *42*, 411–440.
- Bokhorst, S. & Wardle, D.A. (2013). Microclimate within litter bags of different mesh size: Implications for the 'arthropod effect' on litter decomposition. *Soil Biology and Biochemistry*, *58*, 147–152.
- Casado-Murillo, N. & Abril, A. (2013). Decomposition and Carbon Dynamics of Crop Residue Mixtures in a Semiarid Long Term No-Till System: Effects on Soil Organic Carbon. *The Open Agriculture Journal*, *7*, 11–21.
- Chassain, J., Vieublé Gonod, L., Chenu, C. & Joimel, S. (2021). Role of different size classes of organisms in cropped soils: What do litterbag experiments tell us? A meta-analysis. *Soil Biology and Biochemistry*, *162*, 108394.
- Climate Data Center, 2018. Deutscher Wetterdienst. <https://www.dwd.de/>.
- Couteaux, M.-M., Bottner, P. & Berg, B. (1995). Litter decomposition, climate and litter quality. *Trends in Ecology & Evolution*, *10*, 63–66.
- Ehlers, W., Werner, D. & Mähner, T., 2000. Wirkung mechanischer Belastung auf Gefüge und Ertragsleistung einer Löss-Parabraunerde mit zwei Bearbeitungssystemen. *Journal of Plant Nutrition and Soil Science*, *163*, 321–333.
- Faust, S., Koch, H.-J., Dyckmans, J. & Joergensen, R.G. (2019). Response of maize leaf decomposition in litterbags and soil bags to different tillage intensities in a long-term field trial. *Applied Soil Ecology*, *141*, 38–44.
- Fernandes, R., Millard, A.R., Brabec, M., Nadeau, M.J. & Grootes, P. (2014). Food reconstruction using isotopic transferred signals (FRUITS): a Bayesian model for diet reconstruction. *PLoS One*, *9*, e87436.
- Filser, J. The role of Collembola in carbon and nitrogen cycling in soil. *Pedobiologia*, *46*, 234–245. 2002.
- Fjellberg, A. (1998). *The Collembola of Fennoscandia and Denmark*. Part I: Poduromorpha. Fauna Entomologica Scandinavica. Brill, Leiden, The Netherlands.
- Fjellberg, A. (2007). *The Collembola of Fennoscandia and Denmark*. Part II: Entomobryomorpha and Symphypleona. Brill, Leiden, The Netherlands.
- Gergócs, V. & Hufnagel, L. (2016). The effect of microarthropods on litter decomposition depends on litter quality. *European Journal of Soil Biology*, *75*, 24–30.
- Giller, P.S. (1996). The diversity of soil communities, the "poor man's tropical rainforest". *Biodiversity and Conservation*, *5*, 135–168.
- Hartig, R. (2020). DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models: R package version 0.3.2.0. <https://CRAN.R-project.org/package=DHARMA>.

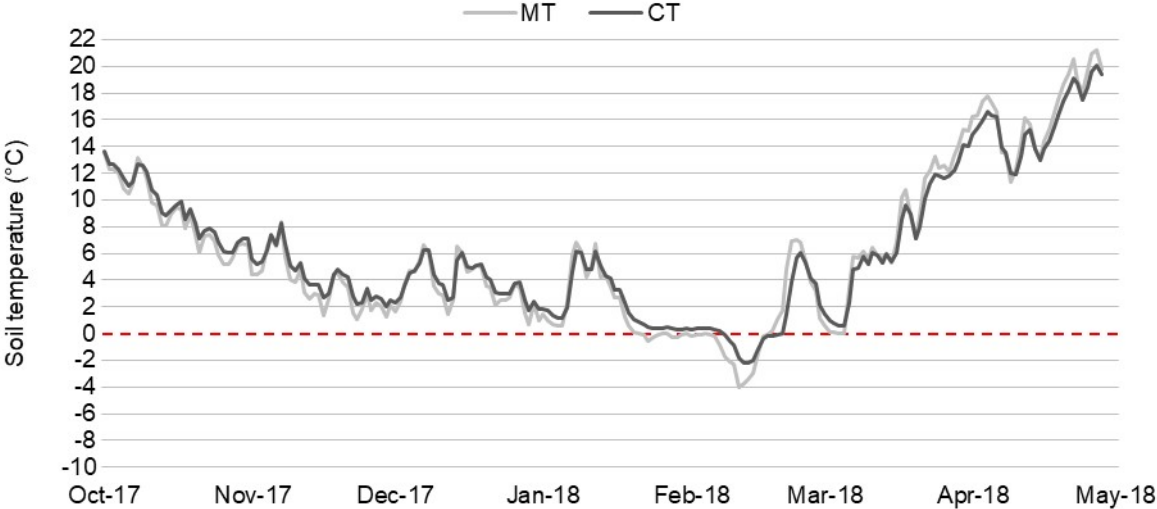
- Hättenschwiler, S., Tiunov, A.V. & Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 191–218.
- Hopkin, S.P. (2007). *A key to the Collembola (springtails) of Britain and Ireland*. FSC Publications, Shrewsbury, UK.
- House, G. J. and Stinner, R. E. (1987). Decomposition of plant residues in no-tillage agroecosystems: influence of litterbag mesh size and soil arthropods. *Pedobiologia*, *30*, 351–360.
- Jacobs, A., Ludwig, B., Schmidt, J.H., Bergstermann, A., Rauber, R. & Joergensen, R.G. (2011). Influence of tillage on degradation kinetics using the litterbag method. *European Journal of Soil Biology*, *47*, 198–204.
- Kampichler, C. & Bruckner, A. (2009). The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews*, *84*, 375–389.
- Ke, X., Winter, K. and Filser, J. Effects of soil mesofauna and farming management on decomposition of clover litter: a microcosm experiment. *Soil Biology and Biochemistry*, *37*, 731–738. 2005.
- Kladivko, E. J. Tillage systems and soil ecology. *Soil and Tillage Research*, *61*, 61–76. 2001.
- Knacker, T., Förster, B., Römbke, J. & Frampton, G.K. (2003). Assessing the effects of plant protection products on organic matter breakdown in arable fields—litter decomposition test systems. *Soil Biology and Biochemistry*, *35*, 1269–1287.
- Langel, R. & Dyckmans, J. (2014). Combined <sup>13</sup>C and <sup>15</sup>N isotope analysis on small samples using a near-conventional elemental analyzer/isotope ratio mass spectrometer setup. *Rapid Communications in Mass Spectrometry*, *28*, 1019–1022.
- Lavelle, P., Blanchart, E., Martin, A., Martin, S. & Spain, A. (1993). A Hierarchical Model for Decomposition in Terrestrial Ecosystems: Application to Soils of the Humid Tropics. *Biotropica*, *25*, 130–150.
- Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M. & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews*, *87*, 545–562.
- Lummer, D., Scheu, S. & Butenschoen, O. (2012). Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. *Oikos*, *121*, 1649–1655.
- Macfadyen, A. (1961). Improved funnel-type extractors for soil arthropods. *The Journal of Animal Ecology*, *30*, 171–184.
- Martínez-García, L.B., Korthals, G.W., Brussaard, L., Mainardi, G. & Deyn, G.B. de (2021). Litter quality drives nitrogen release, and agricultural management (organic vs. conventional) drives carbon loss during litter decomposition in agro-ecosystems. *Soil Biology and Biochemistry*, *153*, 108115.
- McLaughlin, A. & Mineau, P. (1995). The impact of agricultural practices on biodiversity. *Agriculture, Ecosystems & Environment*, *55*, 201–2012.
- Neher, D. A. and Barbercheck, M. E. (2019). Soil microarthropods and soil health: Intersection of decomposition and pest suppression in agroecosystems. *Insects*, *10*, 414. 2019.
- Nordhausen, K., Sirkia, S., Oja, H. & Tyler, D.E. (2018). ICSNP: Tools for Multivariate Nonparametrics: R package version 1.1-1. <https://CRAN.R-project.org/package=ICSNP>.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecsand, E. & Wagner, H. (2019). Community Ecology Package: R package version 2.5-6. <https://CRAN.R-project.org/package=vegan>.

- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2020). nlme: Linear and Nonlinear Mixed Effects Models: R package version 3.1-145. <https://CRAN.R-project.org/package=nlme>.
- Potapov, A.M., Tiunov, A.V. & Scheu, S. (2019). Uncovering trophic positions and food resources of soil animals using bulk natural stable isotope composition. *Biological Reviews*, *94*, 37–59.
- Potthoff, M., Dyckmans, J., Flessa, H., Beese, F. & Joergensen, R.G. (2008). Decomposition of maize residues after manipulation of colonization and its contribution to the soil microbial biomass. *Biology and Fertility of Soils*, *44*, 891–895.
- Potthoff, M. & Loftfield, N. (1998). How to quantify contamination of organic litter bag material with soil? *Pedobiologia*, *42*, 147–153.
- Powers, J.S., Montgomery, R.A., Adair, E.C., Brearley, F.Q., DeWalt, S.J., Castanho, C.T., Chave, J., Deinert, E., Ganzhorn, J.U., Gilbert, M.E., González-Iturbe, J.A., Bunyavejchewin, S., Grau, H.R., Harms, K.E., Hiremath, A., Iriarte-Vivar, S., Manzane, E., Oliveira, A.A. de, Poorter, L., Ramanamanjato, J.-B., Salk, C., Varela, A., Weiblen, G.D. & Lerdau, M.T. (2009). Decomposition in tropical forests: A pan-tropical study of the effects of litter type, litter placement and mesofaunal exclusion across a precipitation gradient. *Journal of Ecology*, *97*, 801–811.
- Prescott, C.E. (2005). Do rates of litter decomposition tell us anything we really need to know? *Forest Ecology and Management*, *220*, 66–74.
- Prescott, C.E. (2010). Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? *Biogeochemistry*, *101*, 133–149.
- Reddy, M. V., Reddy, V. R., Yule, D. F., Cogle, A. L. and George, P. J. (1994). Decomposition of straw in relation to tillage, moisture, and arthropod abundance in a semi-arid tropical Alfisol. *Biology and Fertility of Soils*, *17*, 45–50.
- Rodgers, D., McPhee, J., Aird, P. & Corkrey, R. (2018). Soil arthropod responses to controlled traffic in vegetable production. *Soil and Tillage Research*, *180*, 154–163.
- Scheu, S. & Falca, M. (2000). The soil food web of two beech forests (*Fagus sylvatica*) of contrasting humus type: Stable isotope analysis of a macro- and a mesofauna-dominated community. *Oecologia*, *123*, 285–296.
- Scheunemann, N., Digel, C., Scheu, S. & Butenschoen, O. (2015). Roots rather than shoot residues drive soil arthropod communities of arable fields. *Oecologia*, *179*, 1135–1145.
- Seastedt, T.R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, *29*, 25–46.
- Swift, M.J., Heal, O.W. & Anderson, J.M. (1979). *Decomposition in Terrestrial Ecosystems*. Blackwell Scientific Publications, London, UK.
- van Capelle, C., Schrader & S., Brunotte, J. (2012). Tillage-induced changes in the functional diversity of soil biota – A review with a focus on German data. *European Journal of Soil Biology*, *50*, 165–181.
- Venables, W.N. & Ripley, B.D. (2002). *Modern Applied Statistics with S*. Fourth Edition. Springer, New York, USA.
- Vignozzi, N., Agnelli, A.E., Brandi, G., Gagnarli, E., Goggioli, D., Lagomarsino, A., Pellegrini, S., Simoncini, S., Simono, S., Valbog, G., Caruso, G. & Gucci, R. (2019). Soil ecosystem functions in a high-density olive orchard managed by different soil conservation practices. *Applied Soil Ecology*, *134*, 64–76.
- Vreeken-Buijs, M.J. & Brussaard, L. (1996). Soil mesofauna dynamics, wheat residue decomposition and nitrogen mineralization in buried litterbags. *Biology and Fertility of Soils*, *23*, 374–381.

Yang, B., Zhang, W., Xu, H., Wang, S., Xu, X., Fan, H., Chen, H.Y.H. & Ruan, H. (2017). Effects of soil fauna on leaf litter decomposition under different land uses in eastern coast of China. *Journal of Forestry Research*, 33, 10.

Zhang, D., Hui, D., Luo, Y. & Zhou, G. (2008). Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *Journal of Plant Ecology*, 1, 85–93.

Appendix



**SUPPLEMENTARY FIGURE 1:** Changes in soil temperature at 20 cm (corresponding to conventional tillage (CT) at 23 cm) and 10 cm soil depths (corresponding to minimum tillage (MT) at 5-8 cm) during the time of exposure of litterbags in the field.

**SUPPLEMENTARY TABLE 1:** Collembola traits used for ascribing Collembola species to ecological groups according to Vandewalle et al. (2010).

Trait	Trait scores
Ocelli	0+0 = 4
	1+1 - 2+2 = 3
	3+3 - 4+4 = 2
	5+5 - 6+6 = 1
	7+7 - 8+8 = 0
Antenna length	0 < X ≤ 0.5 body length = 4
	0.5 body length < X ≤ 1 body length = 2
	X > 1 body length = 0
Furca	Absent = 4
	Reduced/short = 2
	Fully developed = 0
Hairs/Scales	Absent = 2
	Present = 0
Pigmentation	Absent (white) = 4
	Coloured but not patterned = 2
	Coloured and patterned = 0

**SUPPLEMENTARY TABLE 2:** Ecological groups ascribed to Collembola species based on trait scores as given in Supplementary Table 1.

Collembola species	Ecological group
<i>Ceratophysella denticulata</i>	Hemiedaphic
<i>Cryptopygus thermophilus</i>	Hemiedaphic
<i>Desoria tigrina</i>	Hemiedaphic
<i>Desoria violacea</i>	Hemiedaphic
<i>Deuteraphorura inermis</i>	Euedaphic
<i>Entomobrya lanuginosa</i>	Epedaphic
<i>Folsomia candida</i>	Euedaphic
<i>Folsomia dovrensis</i>	Euedaphic
<i>Folsomia fimetaria</i>	Euedaphic
<i>Folsomia inoculata</i>	Euedaphic
<i>Folsomia quadrioculata</i>	Euedaphic
<i>Folsomia</i> sp.	Euedaphic
<i>Folsomia spinosa</i>	Euedaphic
<i>Heteromurus nitidus</i>	Hemiedaphic
<i>Isotoma anglicana</i>	Hemiedaphic
<i>Isotoma viridis</i>	Hemiedaphic
<i>Isotomodes productus</i>	Euedaphic
<i>Isotomurus fucicolus</i>	Epedaphic
<i>Lepidocyrtus cyaneus</i>	Epedaphic
<i>Lepidocyrtus lanuginosus</i>	Epedaphic
<i>Megalothorax minimus</i>	Euedaphic
<i>Mesaphorura hylophyla</i>	Euedaphic
<i>Mesaphorura italica</i>	Euedaphic
<i>Mesaphorura krausbaueri</i>	Euedaphic
<i>Mesaphorura macrochaeta</i>	Euedaphic
<i>Mesaphorura peterdassi</i>	Euedaphic
<i>Mesaphorura pongeii</i>	Euedaphic
<i>Mesaphorura</i> sp.	Euedaphic
<i>Mesaphorura sylvatica</i>	Euedaphic
<i>Mesaphorura yosii</i>	Euedaphic
<i>Parisotoma notabilis</i>	Hemiedaphic
<i>Protaphorura armata</i>	Euedaphic
<i>Pseudosinella alba</i>	Hemiedaphic
<i>Pseudosinella immaculata</i>	Hemiedaphic
<i>Sminthurinus aureus</i>	Epedaphic
<i>Sminthurinus bimaculatus</i>	Epedaphic
<i>Sphaeridia pumilis</i>	Epedaphic
<i>Willemia</i> sp.	Euedaphic

# 4 Intercropping of faba bean and winter wheat promotes Collembola diversity and abundance if soil carbon resources are scarce

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## Abstract

The importance of plant species richness for belowground biodiversity is still debated especially in agricultural fields, which are often characterized by monocultures. We investigated the influence of intercropping on Collembola abundance, species richness and community composition at two field sites in Germany. Fields were grown with four different novel faba bean genotypes either in monoculture or intercropped with wheat in alternating rows. Collembola were sampled to a depth of 10 cm using a soil corer. The study sites differed in soil carbon and nitrogen content. The response of Collembola communities to intercropping differed between the field sites. At the low soil carbon site intercropping increased Collembola abundance and Collembola community composition was affected by bean genotype identity, whereas at the high soil carbon site Collembola did not significantly respond to the experimental treatments. The results suggest that intercropping as well as the choice of bean genotypes may promote Collembola communities by the provisioning of additional resources with the effects being restricted to sites with food scarcity, but the diversification of below- and aboveground habitats may also beneficially affect Collembola. The beneficial effects of intercropping at low soil carbon sites presumably are due to increased litter and root resources as well as favourable moisture conditions at the soil surface calling for improved management of arable systems especially at sites poor in organic matter.

## 4.1 Introduction

The increase in the human population combined with the increase in the use of resources is associated with a decrease in biodiversity around the world and altered global environment (Gardi et al. 2009). Humans across the world depend on ecosystem services provided by natural and managed ecosystems (Kibblewhite et al. 2008). For the production of fiber, fuel and food ecosystem services like nutrient cycling, pest control and water regulation are essential (Kibblewhite 2008). Sustainable agriculture aims at increasing crop biomass production while minimizing resource use and maintaining ecosystem services, soil fertility and its physico-chemical properties (Lithourgidis et al. 2011; Rockström et al. 2017).

In general, species richness increases both ecological services and ecological functioning (Loreau and Hector 2001). This not only applies to plants but also holds true for soil fauna (Lemanceau et al. 2014). Soils in agricultural systems provide essential ecosystem functions and for maintaining these functions management practices need to be implemented which foster soil biodiversity (Gardi et al. 2009; Bardgett and van der Putten 2014).

Among the most abundant soil animals in agricultural soils are Collembola, which together with the Acari account for 95 % of total soil arthropods. Detritivore soil mesofauna such as Collembola play a crucial role in litter decomposition and thereby contribute to the fertility and productivity of agricultural systems (Seastedt 1984; Kampichler and Bruckner 2009). Essentially relying on organic matter resources, detritivore animals are closely linked to plants and plant residues (Hooper et al. 2000). In fact, it has been shown that plant diversity promotes detritivore soil animals including Collembola in terms of abundance and diversity (Eisenhauer et al 2010; Eisenhauer et al 2011; Sabais et al 2011). These effects may be based on individual links between plant and animal species, but also by certain plant species favouring a range of detritivore soil animal species (Hooper et al. 2000). Two main effects resulting in higher diversity and abundance of soil fauna have been put forward. First, resources provided by diverse plant communities may comprise a wider range of resources including litter of high quality, but plant diversity may also contribute to a more even provisioning of plant resources in time due to different plant phenologies (Spehn et al. 2000; Sabais et al. 2011). Second, diversification of the habitat by differential root and shoot structure may result in more habitable space (Eisenhauer et al 2011). The combination of both is likely to result in a wider range of ecological niches favouring a more diverse soil fauna (Eisenhauer et al. 2011; Lemanceau et al 2014). However, studies investigating the effect of plant diversity on Collembola found inconsistent results and reported either little effects (Salamon et al. 2004) or positive relationships between Collembola abundance as well as species richness and plant diversity (Sabais et al. 2011). Further, the presence of certain plant functional groups appears to be of critical importance beneficially affecting Collembola (Salamon et al. 2004; Milcu et al. 2006; Eisenhauer et al. 2011). In particular legumes provide resources rich in nitrogen, which is of critical importance as it often limits soil animal abundance and positively affects Collembola as well as earthworms and microorganisms (Milcu et al. 2008; Eisenhauer et al. 2011; Eisenhauer et al. 2012). However, grasses also have been shown to beneficially affect Collembola abundance in plant mixtures (Milcu et al 2006; Eisenhauer et al 2011).

Although the benefits of biodiversity for ecosystem functioning are widely accepted, agricultural systems typically are dominated by monoculture cropping systems since management practices including pesticides and synthetic fertilizers allow efficient management (Horwith 1985; Machado 2009). However, such high input management systems adversely impact the environment by detrimentally affecting the quality of soil, water and air (Matson et al. 1997, Stoate et al. 2001) as well as threatening biodiversity (Kleijn et al., 2006). As alternative to high input monoculture cropping systems mixed cropping systems are receiving increased attention. Mixed cropping systems are based on growing different crops at the same field at the same time (Vandermeer 1992; Lithourgidis et al. 2011). They bare the potential to simultaneously increase yield and resource use (Hauggaard-Nielsen et al. 2008; Jensen et al. 2010). Complementarity and facilitation are the main mechanisms responsible

for the advantages of mixed cropping systems (Duchene et al. 2017). Complementarity results from the utilization of different resources, or the same resources from different locations or at different times, which enables the plants to exploit the available resources more efficiently thereby reducing competition (Hooper and Vitousek 1997; Duchene et al. 2017). Facilitation, on the other hand, is based on positive effects exerted by one organism to another (Michalet and Pugnaire 2016). Regarding mixed cropping systems, the most prominent example may be the facilitative effect of legumes on non-legume plants based on nitrogen fixed by legumes also beneficially affecting non-legume plants (Temperton et al. 2007). Both complementarity and facilitation may result in increased productivity of mixtures compared to monocultures known as transgressive overyielding (Nyfeler et al. 2009). In agricultural systems for example, the yield of mixed stands of faba bean and cereals exceeds that of the respective monocultures (Pristeri et al. 2007). This may not only apply to aboveground plant compartments but also roots as observed for mixed cropping systems of faba bean and maize (Li et al. 2006; Xia et al. 2013). In addition, when intercropped, yield stability of faba bean is increased (Hauggaard-Nielsen et al. 2008). To optimize the beneficial effects of intercropping crop species in mixtures are chosen to maximize both complementarity and facilitation, and to minimize niche overlap (Brooker et al. 2015; Litrico and Violle 2015). This not only applies to crop species but also to different genotypes of the same species as morphological as well as physiological traits may differ between genotypes (Collins et al. 2003; Annicchiarico et al. 2010). In addition, genetic improvement of individual species may enhance the compatibility of plant species in mixture thereby stabilizing the productivity of mixed cropping systems (Annicchiarico et al. 2010).

The aim of the present study was to investigate the effect of intercropping of four novel faba bean genotypes with wheat on the Collembola community in the field. To independently assess the effect of intercropping and bean genotype we sampled Collembola in monocultures of each bean genotype as well as in mixed stands with winter wheat. We hypothesized (1) Collembola abundance and species richness to be increased in mixed cropping systems, (2) Collembola abundances and species richness to vary between bean genotypes, and (3) Collembola community as well as trait composition to differ between monocultures and mixed cropping systems as well as between bean genotypes.

## 4.2 Material and Methods

### 4.2.1 Study sites and experimental design

Samples were taken in May 2015 from field experiments established in 2014 at two sites in the vicinity of the city of Göttingen, Germany: The experimental field sites Reinshof (51°29'N, 9°55'E, 157 m asl) and Deppoldshausen (51°34'N, 9°58'E, 342 m asl). The climate at the field sites is temperate with a

mean annual temperature of 9.5°C and a mean annual precipitation of 621 mm for the last 30 years (1989-2018; DWD, 2019). According to the FAO classification system the soil at Reinshof is Gleyic Fluvisol (WRB), with a high water storage capacity and a mean pH of 6.7. The soil in Deppoldshausen is Calcaric Leptosol (WRB), which is characterized by low water holding capacity and a mean pH of 7.6. Four winter faba bean (*Vicia faba* L.; genotypes: 1, 2, 3, 4) genotypes and winter wheat (Genius; *Triticum aestivum* L.) were grown in different cropping systems, as monoculture and intercropped as mixture of bean and wheat in alternating rows. The faba bean genotypes were provided by the Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, Germany, and from the breeding program of the Department for Crop Sciences of the University of Göttingen (Link and Arbaoui 2005). The wheat cultivar was provided by NORDSAAT Saatzeit GmbH, Langenstein, Germany (Bundessortenamt 2017). In bean monoculture 40 seeds and in wheat monoculture 320 seeds were sown per square metre. In mixtures, each species was sown at 50% density of its monoculture and a row width of 22.5 cm. The experiment was set up in a fully randomized split-plot design with four replications (blocks) at each of the two sites and eight nested main plots per block. Crops were sown in October 2014, not irrigated and grown without fertilizer addition.

#### 4.2.2 Soil mesofauna

Soil mesofauna was sampled using a steel corer of a diameter of 20 cm. Soil cores were taken from each plot at random to a depth of 10 cm. Mesofauna was extracted using high gradient heat extraction (Macfadyen 1961). Animals were collected in a 1:1 diethyleneglycol – water solution. Until species identification the animals were stored in 70% ethanol. Collembola species identification was done using the keys of Fjellberg (1998, 2007) and Hopkins (2007) and transmitted light microscopy.

To assign Collembola to the ecological groups epedaphic, hemiedaphic and euedaphic, morphological traits were used. Length of antenna, length of furca, number of ocelli, coloration and presence of scales or hairs as protection against desiccation were included to evaluate the adaptation of species to belowground live. Each of these traits received a score ranging from 0 (best adapted to aboveground live) to 4 (best adapted to belowground live) (Vandewalle et al. 2010; see Supplementary Table 1). Adding up the scores of all traits results in scores ranging between 0 and 18. Epedaphic species have scores of 0-6, hemiedaphic species scores of 7-12 and euedaphic species scores of 13-18 (see Supplementary Table 2). To investigate the effect of intercropping on assemblages of Collembola traits for each plot the abundance of traits was recorded.

### 4.2.3 Soil nitrogen and carbon

For measuring soil total carbon ( $C_t$ ) and total nitrogen ( $N_t$ ) three soil cores with a diameter of 5 cm to a depth of 10 cm were taken in each plot. Two soil cores were taken within plant rows and one between plant rows. The samples were pooled and sieved. Soil samples were analysed using an elemental analyzer (Vario EL III, elemental, Hanau, Germany).

### 4.2.4 Statistical analysis

Statistical analyses were performed using R (R core Team 2020). Abundance of total Collembola as well as abundance of ecological groups and species richness was analysed using generalized linear mixed effect models using the *glmer.nb* and *glmer* function of the *lme4* package (Bates et al. 2015).  $C_t$ ,  $N_t$  and C/N ratio were analysed by linear mixed effect models using the *lme* function of the *nlme* package (Pinheiro et al. 2020). Non-independence of data collected at different blocks was accounted for by including „blockID“ as random factor. To assess differences between study sites „Site“ was included as factor (levels: Deppoldshausen, Reinshof). The impact of intercropping on Collembola abundances was investigated by including „Cropping system“ as factor (levels: Intercropped, Monoculture). To investigate the impact of bean genotypes on Collembola abundances „Bean genotype“ was included as factor (with the levels 1, 2, 3, 4). Models were chosen starting with a full model including the Cropping system  $\times$  Bean genotype  $\times$  Site interaction and excluding non-significant factors stepwise. Model quality was checked using the *DHARMA* package (Hartig 2020).

Structures of the species and trait community composition of Collembola were compared using non-metric multi-dimensional scaling using Bray-Curtis dissimilarity (metaMDS, *vegan* package; Oksanen et al. 2019). To identify differences in community composition between cropping systems and bean genotypes multivariate analysis of variance was conducted (manova, *stats* package). To account for non-independence of samples within experimental field sites, an error term was included. Linear discriminant analysis (lda, *MASS* package; Venables and Ripley 2002) followed by Hotellings  $T^2$ -test (HotellingsT2, *ICSNP* package; Nordhausen et al. 2018) were used for pairwise comparisons between cropping systems and bean genotypes within the field sites, and for two dimensional graphical depiction of communities, using the first two dimensions of the NMDS.

## 4.3 Results

### 4.3.1 Soil nitrogen and carbon

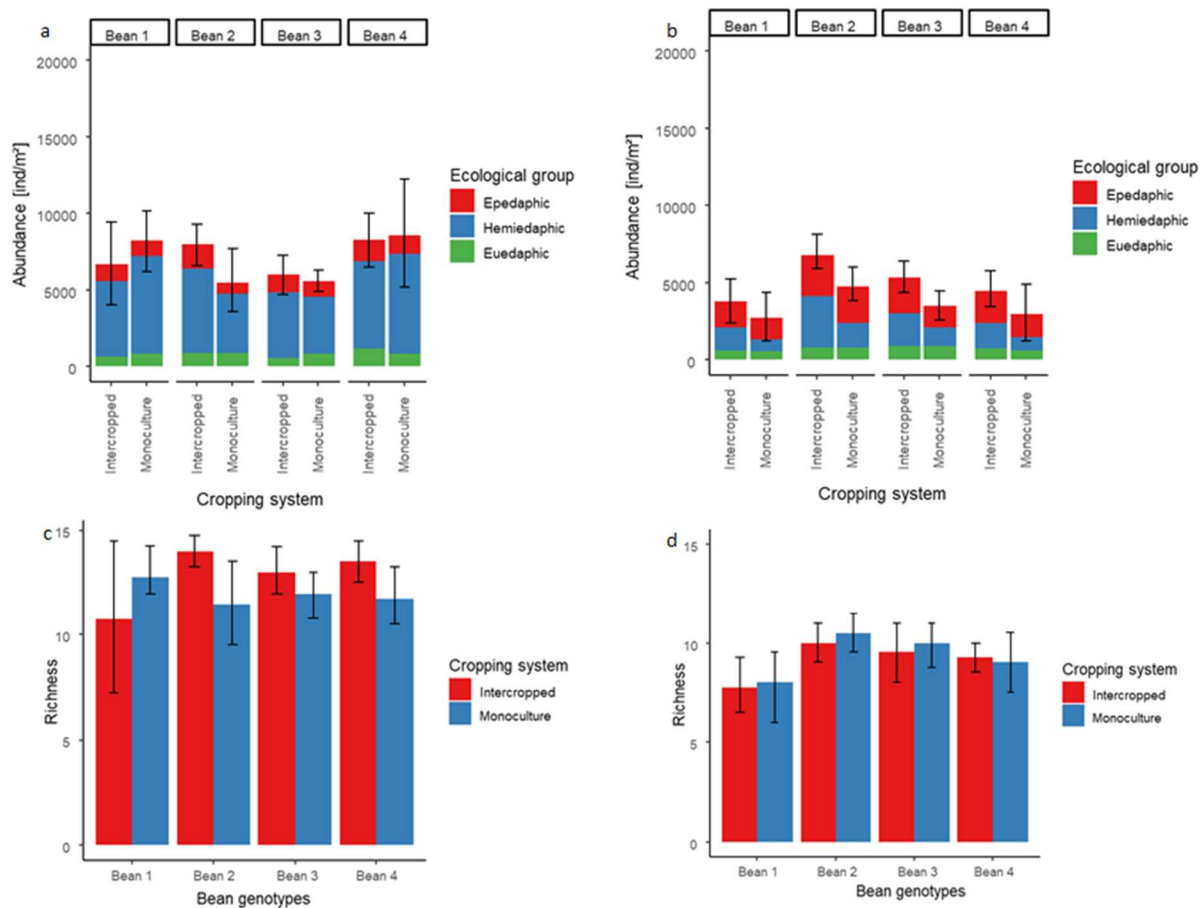
Across the study sites soil  $N_t$  averaged  $0.20 \pm 0.03$  % (mean  $\pm$  SD); it neither differed between study sites nor between cropping systems or bean genotypes. By contrast, soil  $C_t$  was significantly higher (t-value = 3.88, df = 6,  $p < 0.01$ ) at Deppoldshausen ( $3.52 \pm 1.00$  %) compared to Reinshof ( $1.85 \pm 0.27$  %). However, similar to  $N_t$ ,  $C_t$  neither differed between cropping systems nor between bean genotypes. Consequently, the C/N ratio at Deppoldshausen ( $16.96 \pm 5.13$ ) was significantly higher (t-value = 3.86, df = 6,  $p < 0.01$ ) compared to Reinshof ( $9.61 \pm 1.07$ ), but again neither differed between cropping systems nor between bean genotypes.

### 4.3.2 Abundance

Overall, total Collembola abundance was significantly higher at Deppoldshausen ( $7103 \pm 3925$  ind/m<sup>2</sup>; mean  $\pm$  SD) than at Reinshof ( $4205 \pm 1983$  ind/m<sup>2</sup>). However, total Collembola abundance depended on cropping system and study site (significant cropping system  $\times$  site interaction; Table 1, Figure 1), but on average across both sites it was higher in intercropped fields ( $6154 \pm 3049$  ind/m<sup>2</sup>) than in monocultures ( $5154 \pm 3707$  ind/m<sup>2</sup>). Further, total Collembola abundance was affected by bean genotype, but the effect varied between study sites (significant genotype  $\times$  site interaction; see separate analysis of the two study sites below). In contrast to total Collembola, the abundance of epedaphic Collembola was significantly higher at Reinshof ( $1934 \pm 904$  ind/m<sup>2</sup>) than at Deppoldshausen ( $1163 \pm 559$  ind/m<sup>2</sup>), whereas the abundance of hemiedaphic Collembola followed that of total Collembola with significantly higher abundance at Deppoldshausen ( $5131 \pm 3418$  ind/m<sup>2</sup>) than at Reinshof ( $1647 \pm 1023$  ind/m<sup>2</sup>). Across both study sites the abundance of both epedaphic and hemiedaphic Collembola was significantly higher in intercropped fields ( $1757 \pm 910$  and  $3644 \pm 2593$  ind/m<sup>2</sup>; respectively) than in monocultures ( $1340 \pm 715$  and  $3134 \pm 3457$  ind/m<sup>2</sup>; respectively). However, differences in the abundance of hemiedaphic Collembola depended on bean genotype (significant genotype  $\times$  site interaction) as well as on cropping system (significant genotype  $\times$  site interactions; see separate analysis of the two study sites below). In contrast to total, epedaphic and hemiedaphic Collembola, the abundance of euedaphic Collembola did not differ significantly between study sites nor between cropping systems.

Due to the differential response of total and hemiedaphic Collembola to cropping system and bean genotype at the two study sites (Table 1), the two sites were analysed separately. At Deppoldshausen total Collembola abundance did not differ significantly between intercropped and monoculture fields or between different genotypes of bean. Further, the abundance of epedaphic, hemiedaphic as well

as euedaphic Collembola were neither influenced by cropping system nor by bean genotype. By contrast, in Reinshof total Collembola abundance significantly varied between cropping systems ( $F_{1,25} = 19.61$ ,  $p < 0.01$ ) as well as between bean genotypes ( $F_{3,25} = 7.93$ ,  $p < 0.01$ ). Total abundance of Collembola in intercropped fields ( $5060 \pm 2040$  ind/m<sup>2</sup>) was higher than in monoculture fields ( $3350 \pm 1498$  ind/m<sup>2</sup>) independent of bean genotype (Figure 1). In addition, total Collembola abundance was highest in fields with bean genotype 2 ( $5688 \pm 2026$  ind/m<sup>2</sup>) and lowest in fields with bean genotype 1 ( $3072 \pm 1067$  ind/m<sup>2</sup>), while it was intermediate in fields with bean genotypes 4 ( $3436 \pm 1032$  ind/m<sup>2</sup>) and 3 ( $4624 \pm 2276$  ind/m<sup>2</sup>). Hemiedaphic Collembola showed a very similar pattern to total Collembola being more abundant in intercropped ( $2160 \pm 1105$  ind/m<sup>2</sup>) than in monoculture fields ( $1134 \pm 589$  ind/m<sup>2</sup>) independent of bean genotype ( $F_{1,25} = 24.82$ ,  $p < 0.01$ ). Further, the response of the abundance of hemiedaphic Collembola to bean genotypes resembled that of total Collembola abundance being highest in fields with bean genotype 2 ( $2496 \pm 1331$  ind/m<sup>2</sup>), lowest in fields with bean genotype 1 ( $1164 \pm 628$  ind/m<sup>2</sup>) and intermediate in fields with bean genotype 3 ( $1696 \pm 835$  ind/m<sup>2</sup>) and bean genotype 4 ( $1232 \pm 443$  ind/m<sup>2</sup>) ( $F_{3,25} = 6.60$ ;  $p < 0.01$ ).



**Figure 1** Abundance and species richness of Collembola in Deppoldshausen (a, c) and Reinshof (b, d) as affected by cropping system and bean genotype.

**Table 1** Generalized linear mixed effect model and linear mixed effect model table of F- and p-values on the effect of field site (Site: Reinshof, Deppoldshausen), cropping system (Cropping: Intercropping, Monoculture) and bean genotypes (Bean: 1, 2, 3, 4) on total abundance, abundance of different ecological groups and species richness of Collembola in both field sites; df = degrees of freedom; non-significant effects were excluded in a stepwise procedure to obtain reduced models with only significant effects (see Methods).

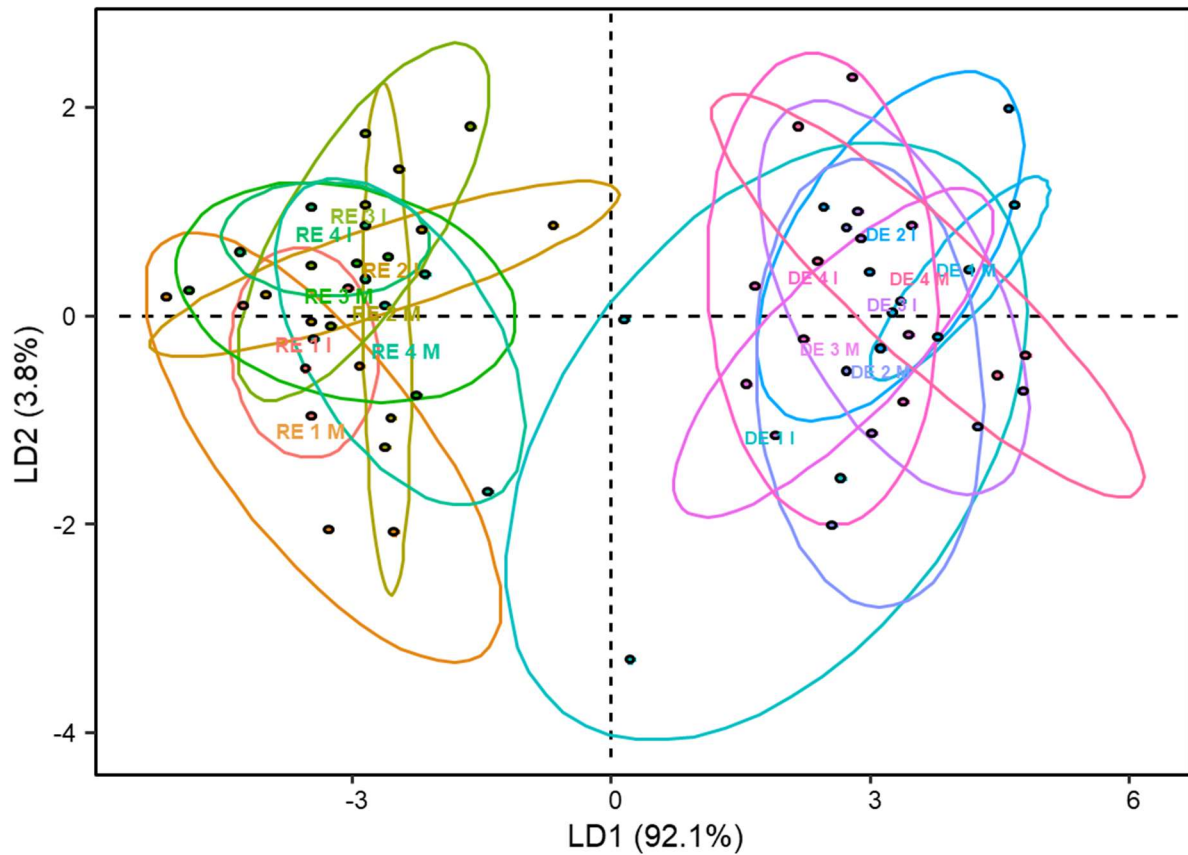
		Cropping	Bean	Site	Cropping x Site	Bean x Site
	df	1		1	1	3
<b>Total Collembola</b>	F-value	9.19		4.73	4.00	13.62
	p-value	<0.01		0.03	0.05	<0.01
	df	1		1		
<b>Epedaphic</b>	F-value	6.42		6.44		
	p-value	0.01		0.01		
	df	1		1	1	3
<b>Hemiedaphic</b>	F-value	13.53		12.99	6.77	4.80
	p-value	<0.01		<0.01	<0.01	<0.01
	df					
<b>Euedaphic</b>	F-value					
	p-value					
	df			1		
<b>Richness</b>	F-value			14.62		
	p-value			<0.01		

#### 4.3.3 Species and trait composition

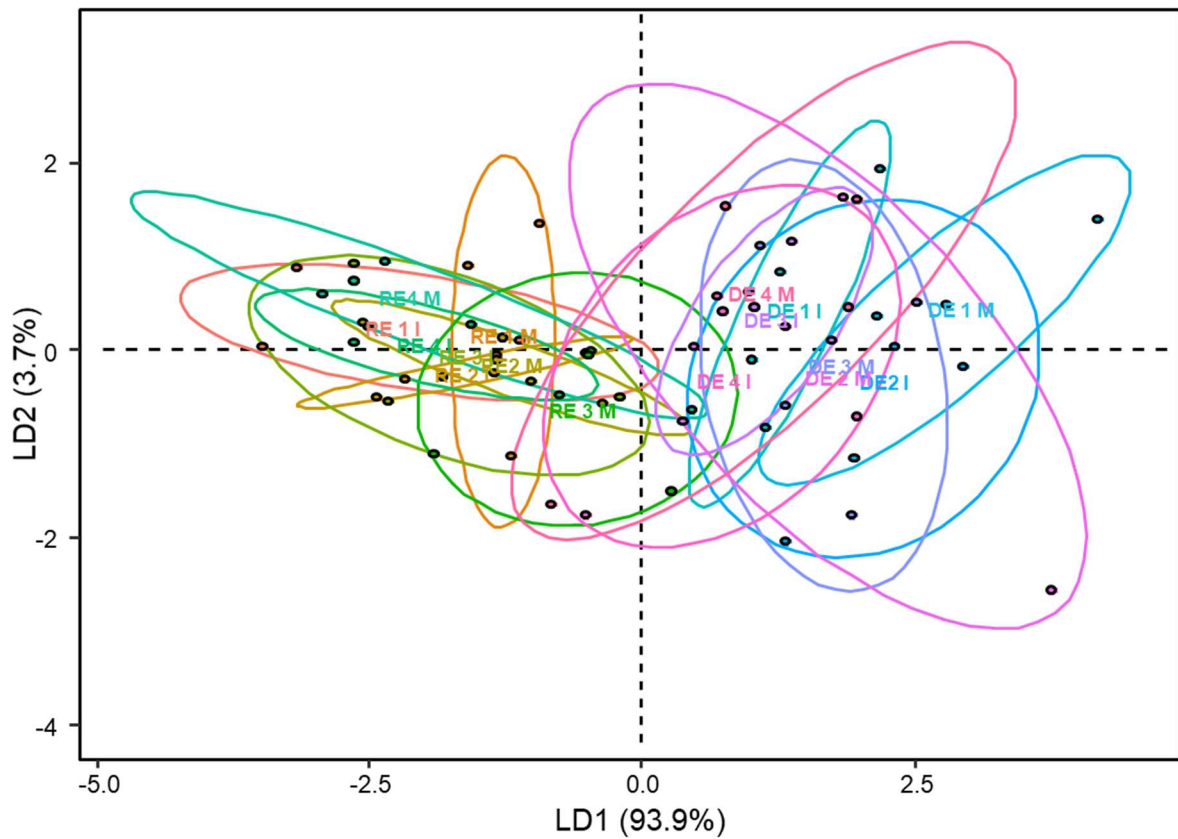
Species richness of Collembola at Deppoldshausen was higher ( $12.4 \pm 2.1$  species/plot; mean  $\pm$  SD) than at Reinshof ( $9.3 \pm 1.7$  species/plot; Table 1, Figure 1). Species composition (NMDS  $k = 5$ ;  $R^2 = 0.99$ ; stress = 0.088) as well as trait composition (NMDS  $k = 3$ ;  $R^2 = 0.99$ ; stress = 0.086) varied significantly between sampling sites ( $F_{5,44} = 104.93$ ,  $p < 0.01$  and  $F_{3,46} = 50.18$ ,  $p < 0.01$ , respectively), but neither between cropping systems nor between bean genotypes as indicated by Manova based on NMDS scores. Differences between field sites for both species composition (Figure 2) as well as trait composition (Figure 3) were much larger than between cropping systems or bean genotypes. Nevertheless, pairwise comparisons of species composition at Reinshof showed differences between the two cropping systems independent of bean genotypes ( $F_{3,28} = 3.52$ ,  $p = 0.03$ ; Supplementary Table 3). In addition, at Reinshof species composition differed significantly between fields with bean genotype 1 and those with other bean genotypes independent of cropping system ( $F_{5,10} = 3.36$ ,  $p = 0.05$ ,  $F_{5,10} = 4.06$ ,  $p = 0.03$  and  $F_{5,10} = 3.46$ ,  $p = 0.04$  for differences between genotype 1 and genotype



2, 3 and 4, respectively). Pairwise comparisons of trait composition within field sites between both cropping systems and bean genotypes were not significantly different (Supplementary Table 4).



**Figure 2** Collembola community composition at Reinshof (RE) and Deppoldshausen (DE) in fields with different bean genotypes (1-4) grown as monoculture (M) or intercropped with winter wheat (I). LDA plot based on NMDS scores of Collembola species composition grouped by sampling sites, bean genotypes and cropping systems. LD1 accounted for 92.1% and LD2 for 3.8% of the total variance.



**Figure 3** Collembola trait composition at Reinshof (RE) and Deppoldshausen (DE) in fields with different bean genotypes (1-4) grown as monoculture (M) or intercropped with winter wheat (I). LDA plot based on NMDS scores of Collembola trait composition grouped by sampling sites, bean genotypes and cropping systems. LD1 accounted for 93.9% and LD2 for 3.7% of the total variance.

#### 4.4 Discussion

We hypothesized intercropping to beneficially affect Collembola abundance as well as species richness. Furthermore, we hypothesized Collembola abundance and species richness to be affected by bean genotypes. Consequently, we hypothesized species community and trait community composition in intercropped systems to differ from that in monocultures as well as between different bean genotypes. Conform to our hypotheses Collembola abundances in intercropped fields exceeded those in monoculture, but contrary to our hypotheses bean genotypes neither affected total Collembola abundance and species richness nor Collembola species and trait community composition. However, Collembola abundance and species richness as well as species and trait community compositions differed strongly between the field sites Deppoldshausen and Reinshof. Further, at the field site Reinshof abundances as well as species and trait community composition of Collembola differed between bean genotypes and cropping systems, while this was not the case at the field site Deppoldshausen. The different effects of intercropping and bean genotypes on Collembola communities at the two field sites indicate that their response depends on site conditions driving their sensitivity to our experimental treatments.

Intercropping increased total Collembola abundance as well as the abundances of epedaphic and hemiedaphic Collembola conform to our first hypothesis. However, contradicting our first hypothesis species richness was not affected by cropping system. Separately analysing the abundance of total Collembola and of both of these ecological groups for the two sites revealed that at Deppoldshausen total Collembola as well as these two ecological groups were not affected by intercropping. By contrast, at Reinshof the abundance of total Collembola, as well as hemiedaphic Collembola were increased by intercropping. Therefore, the overall higher abundance of Collembola due to intercropping was mainly due to differences at the field site in Reinshof.

Similar to cropping system, bean genotypes also affected Collembola abundance mainly at the field site Reinshof, partially confirming our second hypothesis. Especially bean genotype 2 increased the abundance of total Collembola as well as hemiedaphic Collembola. Furthermore, the abundance of total Collembola and the abundance of hemiedaphic Collembola were lowest in fields with bean genotype 1. This suggests that bean genotype 2 beneficially affects Collembola, whereas effects of bean genotype 1 are detrimental.

Our third hypothesis also was only confirmed partially. As differences between Collembola community and trait composition between sites were much bigger than differences between genotypes or cropping systems the communities within each field site were analysed separately. Collembola trait composition was not affected by intercropping or bean genotype at any site. Similarly, at

Deppoldshausen neither intercropping nor bean genotype affected Collembola species composition. By contrast, at Reinshof Collembola species composition was affected by intercropping and bean genotype. Here, Collembola species composition in fields with bean genotype 1 significantly differed from that of the other three bean genotypes.

As Collembola abundance, species richness as well as species composition differed between the two field sites, the field sites likely differ in critical environmental conditions for Collembola. The availability of food resources and moisture conditions are considered crucial factors structuring Collembola communities (Verhoef and Nagelkerke 1977; Berg et al. 1998; Jucevica and Melecis 2006; Vignozzi et al. 2018). Especially euedaphic Collembola, which lack the presence of hairs or scales as protection against desiccation, are sensitive to dry conditions (Krab et al. 2010). However, as differences in abundances were confined to hemiedaphic species it is unlikely that different moisture conditions deeper in soil (the habitat of euedaphic species) between the two sites contributed to the different structure of Collembola communities. Other abiotic conditions or soil moisture at the soil surface therefore likely were responsible for hemiedaphic Collembola being dominant in the field sites at Deppoldshausen and epedaphic Collembola being more abundant at Reinshof.

Collembola are known to react to changes in litter quality (Berg and Bengtsson 2007). Nitrogen is considered the limiting factor in agricultural soils not only for plants but also for soil animals (Seastedt 1984). A notable difference between the two study sites was that soil carbon concentration at Reinshof was lower than at Deppoldshausen. As nitrogen concentrations in soil were similar at both sites the increased carbon concentration resulted in higher C/N ratio of soil organic matter at Deppoldshausen. Litter material of low C/N ratio serves as high quality substrate favouring plant nutrition (Hättenschwiler et al. 2005; Garcia-Palacios et al. 2013) and this also applies to detritivore soil animals (Seastedt 1984). Differences in the quality of food resources therefore likely contributed to the differences in Collembola abundance and community structure between the two study sites. Similarly, intercropping leads to overyielding resulting in increased root and shoot biomass (Pristeri et al. 2007; Li et al. 2006; Nyfeler et al. 2009; Xia et al. 2013), likely also affecting the amount and quality of resources for the decomposer system. In fact, root as well as shoot biomass was higher in intercropping than in monoculture systems at our field sites (Streit et al. 2019). The combination of increased nitrogen availability due to low soil C/N ratio and increased resource input due to overyielding at Reinshof likely contributed to increased quantity and quality of food resources for detritivore animals including Collembola (Seastedt 1984; Nyfeler et al. 2009). As Collembola benefit from both resources provided by plant shoots and roots, the increased plant growth in intercropping systems at Reinshof presumably increased overall resource availability for both epedaphic and hemiedaphic Collembola (Scheunemann et al. 2015; Li et al. 2021). Root derived resources may be particularly important for detritivore animals in agricultural fields as typically most of the aboveground plant compartments are

removed by harvesting the crops (Scheunemann et al. 2015). Furthermore, increased shoot biomass increases shading likely resulting in more favourable moisture conditions at the soil surface and minimizing desiccation risk for Collembola. In addition, more complex structure caused by the differential root and shoot architecture leads to more inhabitable space (Eisenhauer et al. 2011). The assumed beneficial effect of increased root biomass on Collembola is further supported by the increased root biomass of bean genotype 2 in our experiment (Streit et al. 2019a), which also promoted Collembola abundance and species richness. Here, the functional group of plants presumably was of critical importance since overall root biomass of bean and wheat combined was not highest in intercropping systems with bean genotype 2. Rather, the proportion of bean roots compared to the proportion of wheat roots in mixtures was highest in intercropping systems with bean genotype 2 and lowest in intercropping systems with bean genotype 1. Similarly, the proportion of wheat shoot biomass was highest in intercropping systems with bean genotype 1 and lower in intercropping systems with bean genotype 2 (Streit et al. 2019a). This suggests that especially the roots of legumes increase Collembola abundance. Further, the effects of bean genotypes may be attributed to other morphological as well as physiological differences as the bean genotypes used differed in various traits (Streit et al. 2019b; Z. Wang, pers. comm.). For example, the C/N ratio of shoots of bean genotype 2 was lower than that of bean genotype 3 indicating differences in litter quality (Z. Wang, pers. comm.). This difference is attributed to preferential allocation of resources to shoots in bean genotype 2, while bean genotype 3 allocates resources predominantly to roots (Z. Wang, pers. comm.). When comparing morphology of the bean genotypes as described by Streit et al. (2019b) it is apparent that bean genotype 2 with the combination of short shoots and high tillering increases shading and thereby improves moisture conditions for soil arthropods at the soil surface, while the medium size bean genotype 1 with low tillering is leaving the soil surface more unprotected from sun and desiccation. Overall, the results suggest that positive effects of additional carbon provided by increased plant biomass come to full effect at sites with low soil C/N ratio associated with high availability of nitrogen promoting fungal growth. Presumably, additional carbon provided by plant roots therefore enables the Collembola community to fully exploit the available nitrogen and fungal resources especially when moisture conditions at the soil surface are optimized by shading caused by increased shoot biomass.

In conclusion, our study demonstrated that intercropping and the choice of bean genotypes affects Collembola communities in particular at low soil carbon sites, i.e. if food resources are scarce. To optimize beneficial effects of bean genotypes on detritivores in soil, morphological as well as physiological traits of the plants / genotypes need to be taken into account. Our results suggest that in particular genotypes characterized by high tillering and short shoots (bean genotype 2) beneficially affect Collembola. Morphological traits such as high tillering mainly act on abiotic conditions by

improving moisture conditions at the soil surface, while physiological differences such as preferential resource allocation into roots (bean genotype 3) or shoots (bean genotype 2) improve the availability and quality of resources. Overall, at low carbon sites Collembola profit from intercropping with bean genotypes increasing resource availability and habitable space thereby promoting in particular hemiedaphic species.

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### References

- Annicchiarico, P., & Proietti, S. (2010). White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science*, 65(3), 318-324.
- Bardgett, R. D., & Van Der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, 515(7528), 505-511.
- Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). *Fitting Linear Mixed-Effects Models Using lme4*. Journal of Statistical Software, 67(1), 1-48. doi:10.18637/jss.v067.i01.
- Berg, M. P., Kniese, J. P., Bedaux, J. J. M., & Verhoef, H. A. (1998). Dynamics and stratification of functional groups of micro-and mesoarthropods in the organic layer of a Scots pine forest. *Biology and Fertility of Soils*, 26(4), 268-284.
- Berg, M. P., & Bengtsson, J. (2007). Temporal and spatial variability in soil food web structure. *Oikos*, 116(11), 1789-1804.
- Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., ... & White, P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, 206(1), 107-117.
- Bundessortenamt (2017) Beschreibende Sortenliste. Getreide, Mais, Öl- und Faserpflanzen, Leguminosen, Rüben, Zwischenfrüchte, Hannover
- Climate Data Center (CDC), 2019. Deutscher Wetterdienst. <https://www.dwd.de/>.
- Collins, R. P., Fothergill, M., Macduff, J. H., & Puzio, S. (2003). Morphological compatibility of white clover and perennial ryegrass cultivars grown under two nitrate levels in flowing solution culture. *Annals of Botany*, 92(2), 247-258.
- Duchene, O., Vian, J. F., & Celette, F. (2017). Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. *Agriculture, Ecosystems & Environment*, 240, 148-161.

- Eisenhauer, N., Beßler, H., Engels, C., Gleixner, G., Habekost, M., Milcu, A., ... & Scheu, S. (2010). Plant diversity effects on soil microorganisms support the singular hypothesis. *Ecology*, *91*(2), 485-496.
- Eisenhauer, N., Milcu, A., Sabais, A. C., Bessler, H., Brenner, J., Engels, C., ... & Scheu, S. (2011). Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. *PloS one*, *6*(1), e16055.
- Eisenhauer, N., Reich, P. B., & Scheu, S. (2012). Increasing plant diversity effects on productivity with time due to delayed soil biota effects on plants. *Basic and Applied Ecology*, *13*(7), 571-578.
- Fjellberg, A. (1998). *The Collembola of Fennoscandia and Denmark. Part I: Poduromorpha*. Fauna Entomologica Scandinavica.
- Fjellberg, A. (2007). *The Collembola of Fennoscandia and Denmark, Part II: Entomobryomorpha and Symphypleona*. Brill.
- García-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology letters*, *16*(8), 1045-1053.
- Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., ... & Menta, C. (2009). Soil biodiversity monitoring in Europe: ongoing activities and challenges. *European Journal of Soil Science*, *60*(5), 807-819.
- Hartig, R. (2020). *DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models*. R package version 0.3.2.0. <https://CRAN.R-project.org/package=DHARMA>
- Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J., & Jensen, E. S. (2008). Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renewable Agriculture and Food Systems*, *23*(1), 3-12.
- Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics* *36*, 191–218.
- Hooper, D. U., & Vitousek, P. M. (1997). The effects of plant composition and diversity on ecosystem processes. *Science*, *277*(5330), 1302-1305.
- Hooper, D. U., Bignell, D. E., Brown, V. K., Brussard, L., Dangerfield, J. M., Wall, D. H., ... & Wolters, V. (2000). Interactions between Aboveground and Belowground Biodiversity in Terrestrial Ecosystems: Patterns, Mechanisms, and Feedbacks: We assess the evidence for correlation between aboveground and belowground diversity and conclude that a variety of mechanisms could lead to positive, negative, or no relationship—depending on the strength and type of interactions among species. *Bioscience*, *50*(12), 1049-1061.
- Hopkin, S. P. (2007). *A key to the Collembola (springtails) of Britain and Ireland*. FSC publications.
- Horwith, B. (1985). A role for intercropping in modern agriculture. *BioScience*, *35*(5), 286-291.
- Jensen, E. S., Peoples, M. B., & Hauggaard-Nielsen, H. (2010). Faba bean in cropping systems. *Field crops research*, *115*(3), 203-216.
- Jucevica, E., & Melecis, V. (2006). Global warming affect Collembola community: A long-term study. *Pedobiologia*, *50*(2), 177-184.

- Kampichler, C., Bruckner, A., 2009. The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews* 84 (3), 375–389. 10.1111/j.1469-185X.2009.00078.x.
- Kibblewhite, M. G., Ritz, K., & Swift, M. J. (2008). Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1492), 685-701.
- Lemanceau et al. 2014
- Kleijn, D., Baquero, R. A., Clough, Y., Díaz, M., De Esteban, J., Fernández, F., ... & Yela, J. L. (2006). Mixed biodiversity benefits of agri-environment schemes in five European countries. *Ecology letters*, 9(3), 243-254.
- Krab, E. J., Oorsprong, H., Berg, M. P., & Cornelissen, J. H. (2010). Turning northern peatlands upside down: disentangling microclimate and substrate quality effects on vertical distribution of Collembola. *Functional Ecology*, 24(6), 1362-1369.
- Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. A., & Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia*, 147(2), 280-290.
- Li, Z., Shi, L., Kuzyakov, Y., Pausch, J., Scheu, S., & Pollierer, M. M. (2021). The flux of root-derived carbon via fungi and bacteria into soil microarthropods (Collembola) differs markedly between cropping systems. *Soil Biology and Biochemistry*, 160, 108336.
- Link W, Arbaoui M (2005) Neues von der Göttinger Winter-Ackerbohne. In: HBLFA Raumberg-Gumpenstein (ed), Bericht über die 56. Tagung 2005 der Vereinigung der Pflanzzüchter und Saatgutkaufleute Österreichs. Irdning, pp 1–8.
- Lithourgidis, A. S., Vlachostergios, D. N., Dordas, C. A., & Damalas, C. A. (2011). Dry matter yield, nitrogen content, and competition in pea–cereal intercropping systems. *European Journal of agronomy*, 34(4), 287-294.
- Litrico, I., & Violle, C. (2015). Diversity in plant breeding: a new conceptual framework. *Trends in Plant Science*, 20(10), 604-613.
- Loreau, M., & Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature*, 412(6842), 72-76.
- Macfadyen, A. (1961). Improved funnel-type extractors for soil arthropods. *The Journal of Animal Ecology*, 171-184.
- Machado, S. (2009). Does intercropping have a role in modern agriculture? *Journal of Soil and Water Conservation*, 64(2), 55A-57A.
- Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J. (1997). Agricultural intensification and ecosystem properties. *Science*, 277(5325), 504-509.
- Michalet, R., & Pugnaire, F. I. (2016). Facilitation in communities. *Functional Ecology*, 30(1), 3-9.
- Milcu, A., Partsch, S., Scherber, C., Weisser, W. W., & Scheu, S. (2008). Earthworms and legumes control litter decomposition in a plant diversity gradient. *Ecology*, 89(7), 1872-1882.
- Nordhausen, K., Sirkia, S., Oja, H., Tyler, D. E. (2018). *ICSNP: Tools for Multivariate Nonparametrics*. R package version 1.1-1. <https://CRAN.R-project.org/package=ICSNP>
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, 46(3), 683-691.



- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecsand, E., Wagner, H. (2019). *vegan: Community Ecology Package*. R package version 2.5-6. <https://CRAN.R-project.org/package=vegan>
- Partsch, S., Milcu, A., & Scheu, S. (2006). Decomposers (Lumbricidae, Collembola) affect plant performance in model grasslands of different diversity. *Ecology*, *87*(10), 2548-2558.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team (2020). *\_nlme: Linear and Nonlinear Mixed Effects Models\_*. R package version 3.1-145, <URL: <https://CRAN.R-project.org/package=nlme>>.
- Pristeri, A., Dahlmann, C., von Fragstein, P., Gooding, M. J., Hauggaard Nielsen, H., Kasyanova, E., & Monti, M. (2007). Yield performance of Faba bean–Wheat intercropping on spring and winter sowing in European organic farming system. In *Danish Research Centre for Organic Food and Farming, DARCOF Proceedings* (pp. 294-295).
- R Core Team (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rockström, J., Williams, J., Daily, G., Noble, A., Matthews, N., Gordon, L., ... & Smith, J. (2017). Sustainable intensification of agriculture for human prosperity and global sustainability. *Ambio*, *46*(1), 4-17.
- Sabais, A. C., Scheu, S., & Eisenhauer, N. (2011). Plant species richness drives the density and diversity of Collembola in temperate grassland. *Acta Oecologica*, *37*(3), 195-202.
- Salamon, J. A., Schaefer, M., Alpehi, J., Schmid, B., & Scheu, S. (2004). Effects of plant diversity on Collembola in an experimental grassland ecosystem. *Oikos*, *106*(1), 51-60.
- Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O., 2015. Roots rather than shoot residues drive soil arthropod communities of arable fields. *Oecologia* *179*(4), 1135–1145.
- Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual review of entomology*, *29*(1), 25-46.
- Spehn, E. M., Joshi, J., Schmid, B., Alpehi, J., & Körner, C. (2000). Plant diversity effects on soil heterotrophic activity in experimental grassland ecosystems. *Plant and Soil*, *224*(2), 217-230.
- Stoate, C., Boatman, N. D., Borralho, R. J., Carvalho, C. R., De Snoo, G. R., & Eden, P. (2001). Ecological impacts of arable intensification in Europe. *Journal of environmental management*, *63*(4), 337-365.
- Streit, J., Meinen, C., Nelson, W. C. D., Siebrecht-Schöll, D. J., & Rauber, R. (2019a). Above-and belowground biomass in a mixed cropping system with eight novel winter faba bean genotypes and winter wheat using FTIR spectroscopy for root species discrimination. *Plant and Soil*, *436*(1), 141-158.
- Streit, J., Meinen, C., & Rauber, R. (2019b). Intercropping effects on root distribution of eight novel winter faba bean genotypes mixed with winter wheat. *Field Crops Research*, *235*, 1-10.
- Temperton, V. M., Mwangi, P. N., Scherer-Lorenzen, M., Schmid, B., & Buchmann, N. (2007). Positive interactions between nitrogen-fixing legumes and four different neighbouring species in a biodiversity experiment. *Oecologia*, *151*(2), 190-205.
- Vandermeer, J. H. (1992). *The ecology of intercropping*. Cambridge University Press.
- Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Doledec, S., Dubs, F., ... & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, *19*(10), 2921-2947.

Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S. Fourth Edition*. Springer, New York. ISBN 0-387-95457-0

Verhoef, H. A., & Nagelkerke, C. J. (1977). Formation and ecological significance of aggregations in Collembola. *Oecologia*, 31(2), 215-226.

Vignozzi, N., Agnelli, A. E., Brandi, G., Gagnarli, E., Goggioli, D., Lagomarsino, A., ... & Gucci, R. (2019). Soil ecosystem functions in a high-density olive orchard managed by different soil conservation practices. *Applied Soil Ecology*, 134, 64-76.

Xia, H. Y., Zhao, J. H., Sun, J. H., Bao, X. G., Christie, P., Zhang, F. S., & Li, L. (2013). Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. *Field Crops Research*, 150, 52-62.

**Appendix****Supplementary Table 1** Collembola traits considered for calculating ecological groups according to Vandewalle et al. (2010).

Trait	Trait scores
Ocelli	0+0 = 4
	1+1 - 2+2 = 3
	3+3 - 4+4 = 2
	5+5 - 6+6 = 1
	7+7 - 8+8 = 0
Antenna length	0 < X ≤ 0.5 body length = 4
	0.5 body length < X ≤ 1 body length = 2
	X > 1 body length = 0
Furca	Absent = 4
	Reduced/short = 2
	Fully developed = 0
Hairs/Scales	Absent = 2
	Present = 0
Pigmentation	Absent (white) = 4
	Coloured but not patterned = 2
	Coloured and patterned = 0

**Supplementary Table 2** Collembola species found during the study, with calculated species trait scores according to Vandewalle et al. (2010) and assigned ecological groups.

Species	Score	Ecological group
<i>Arrhopalithes caecus</i>	9	Hemiedaphic
<i>Ceratophysella denticulata</i>	10	Hemiedaphic
<i>Deuteraphorura inermis</i>	18	Euedaphic
<i>Deuterosminthurus pallipes</i>	6	Epedaphic
<i>Entomobrya lanuginosa</i>	4	Epedaphic
<i>Folsomia quadrioculata</i>	13	Euedaphic
<i>Folsomia spinosa</i>	16	Euedaphic
<i>Folsomides parvulus</i>	15	Euedaphic
<i>Heteromurus nitidus</i>	11	Hemiedaphic
<i>Hypogastrura manubrialis</i>	10	Hemiedaphic
<i>Isotoma viridis</i>	8	Hemiedaphic
<i>Isotomiella minor</i>	14	Euedaphic
<i>Lepidocyrtus cyaneus</i>	6	Epedaphic
<i>Lepidocyrtus lanuginosus</i>	6	Epedaphic
<i>Megalothorax minimus</i>	16	Euedaphic
<i>Mesaphorura macrochaeta</i>	18	Euedaphic
<i>Orchesella villosa</i>	2	Epedaphic
<i>Parisotoma notabilis</i>	10	Hemiedaphic
<i>Protaphorura armata</i>	18	Euedaphic
<i>Pseudosinella alba</i>	11	Hemiedaphic
<i>Sminthurinus aureus</i>	2	Epedaphic
<i>Sminthurinus elegans</i>	2	Epedaphic
<i>Sphaeridia pumilis</i>	4	Epedaphic
<i>Stenaphorura quadrispina</i>	18	Euedaphic
<i>Symphyleona</i> juvenile	2	Epedaphic
<i>Willemia</i> sp.	18	Euedaphic

**Supplementary Table 3** Mahalanobis distances based on Hotellings  $T^2$  test for pairwise comparisons of Collembola species composition within each field site (RE = Reinshof, DE = Deppoldshausen) based on Linear Discriminant Analysis. I = Intercropped, M = Monoculture; Df1 = numerator degrees of freedom, Df2 = denominator degrees of freedom; Bean Genotype 1 = 1; Bean Genotype 2 = 2; Bean Genotype 3 = 3; Bean Genotype 4 = 4.

Comparison	F-value	Df1	Df2	p-value	Mahalanobis distance
RE I / RE M	3.52	3	28	0.03	0.87
RE 1 / RE 2	3.36	5	10	0.05	1.50
RE 1 / RE 3	4.06	5	10	0.03	2.17
RE 1 / RE 4	3.46	5	10	0.04	0.96
RE 2 / RE 3	0.30	5	10	0.90	0.42
RE 2 / RE 4	0.41	5	10	0.83	0.34
RE 3 / RE 4	0.23	5	10	0.94	0.27
DE I / DE M	0.98	3	28	0.41	0.08
DE 1 / DE 2	0.32	5	10	0.89	0.67
DE 1 / DE 3	1.35	5	10	0.32	0.82
DE 1 / DE 4	0.99	5	10	0.47	1.58
DE 2 / DE 3	0.28	5	10	0.91	0.27
DE 2 / DE 4	0.38	5	10	0.85	0.95
DE 3 / DE 4	0.36	5	10	0.86	0.52
RE 1 I / RE 1 M	0.51	5	2	0.77	1.89
RE 2 I / RE 2 M	0.50	5	2	0.77	1.51
RE 3 I / RE 3 M	0.72	5	2	0.67	2.14
RE 4 I / RE 4 M	18.05	5	2	0.05	1.56
DE 1 I / DE 1 M	1.11	5	2	0.54	2.84
DE 2 I / DE 2 M	0.96	5	2	0.58	4.78
DE 3 I / DE 3 M	0.63	5	2	0.71	0.23
DE 4 I / DE 4 M	0.33	5	2	0.86	0.36

**Supplementary Table 4** Mahalanobis distances based on Hotellings  $T^2$  test for pairwise comparisons of Collembola trait composition within each field site (RE = Reinshof, DE = Deppoldshausen) based on Linear Discriminant Analysis. I = Intercropped, M = Monoculture; Df1 = numerator degrees of freedom, Df2 = denominator degrees of freedom; Bean Genotype 1 = 1; Bean Genotype 2 = 2; Bean Genotype 3 = 3; Bean Genotype 4 = 4

Comparison	F-value	Df1	Df2	p- value	Mahalanobis distance
RE I / RE M	1.33	3	28	0.29	0.22
RE 1 / RE 2	0.79	3	12	0.52	0.46
RE 1 / RE 3	1.25	3	12	0.34	0.97
RE 1 / RE 4	0.15	3	12	0.93	0.21
RE 2 / RE 3	0.2	3	12	0.90	0.10
RE 2 / RE 4	0.97	3	12	0.44	0.23
RE 3 / RE 4	0.98	3	12	0.43	0.59
DE I / DE M	0.76	3	28	0.52	0.10
DE 1 / DE 2	0.49	3	12	0.69	0.59
DE 1 / DE 3	0.86	3	12	0.49	0.29
DE 1 / DE 4	1.92	3	12	0.18	0.61
DE 2 / DE 3	0.17	3	12	0.91	0.15
DE 2 / DE 4	1.9	3	12	0.18	0.69
DE 3 / DE 4	0.85	3	12	0.49	0.38
RE 1 I / RE 1 M	0.59	3	4	0.65	0.67
RE 2 I / RE 2 M	0.25	3	4	0.86	0.06
RE 3 I / RE 3 M	0.71	3	4	0.60	0.99
RE 4 I / RE 4 M	1.04	3	4	0.47	0.17
DE 1 I / DE 1 M	4.39	3	4	0.09	0.60
DE 2 I / DE 2 M	0.08	3	4	0.96	0.24
DE 3 I / DE 3 M	0.29	3	4	0.83	0.39
DE 4 I / DE 4 M	0.27	3	4	0.85	0.86

## 5 General Discussion

Despite the widely accepted importance of soil Collembola for ecosystem functioning of agricultural soils, the effects of conventional management practices on Collembola remain little understood. Effects vary depending on ecological group affiliation and abiotic conditions. However, as stressed increasingly agroecosystems need to foster biodiversity to assure the health of agricultural soils for future generations. Therefore, management of agricultural systems in a sustainable way is mandatory for future human wellbeing. The present study evaluated the response of soil Collembola communities to reduced tillage practices and mixed cropping systems as sustainable alternatives to conventional ploughing and monoculture plantations. Doing that I focussed on identifying mechanisms explaining the differential response of Collembola communities reported in previous studies. The effect of tillage reduction on Collembola communities was assessed in two experiments. In the first experiment we expected to find beneficial effects of reduced tillage intensity on Collembola communities across a large geographical gradient reaching from Spain to Sweden. Contrasting these expectations and our general hypothesis 1, we found effects to depend on community compositions and especially on dominating ecological groups (Chapter 2). In the second experiment we expected faster decomposition in reduced tillage fields with increased abundances of Collembola. Contrasting these expectations and our general hypothesis 2, abundances of Collembola in conventional tillage fields exceeded abundances in reduced tillage fields. Nevertheless, transformation of litter into high quality resources was faster in reduced tillage fields and enhanced by the presence of Collembola regardless of tillage intensity (Chapter 3). A third experiment was established at two field sites to investigate the effect of mixed cropping and different bean genotypes on soil Collembola. We expected Collembola to be promoted by mixed cropping in comparison to monocultures, while being differentially affected by bean genotypes (general hypothesis 3). In fact, Collembola communities benefitted from mixed cropping and differentially responded to bean genotypes in one field site, while they were not affected by cropping system or bean genotype in the other (Chapter 4). Further, ecological groups of Collembola responded in opposite ways. The basis of each of the three studies was the analysis of species communities, but for a deeper understanding of mechanisms it was followed by trait based approaches focussing on ecological groups of Collembola. In the following I will discuss the underlying changes in abiotic conditions caused by changes in the applied management practices. Furthermore, the usefulness of a standardized method for assignment of Collembola to ecological groups will be discussed as well as the usefulness of trait composition. Finally, the overall usefulness of Collembola communities and ecological groups as indicators for soil quality is evaluated.

In order to investigate the response of Collembola communities to reduced tillage practices we investigated Collembola communities in five long-term experiments across Europe applying conventional tillage in comparison to reduced tillage treatments (Chapter 2). We sampled Collembola down to a soil depth of 30 cm to include the whole volume of soil where litter is translocated by tillage. A number of previous studies reported Collembola abundance to decrease due to conventional tillage in comparison to reduced tillage practices. However, in these studies Collembola sampling typically was limited to a depth of 10 cm or less (House and Parmelee 1985; Brennan et al. 2006; Vignozzi et al. 2019). By contrast, our experiment showed increased abundances of Collembola in deep soil layers below 10 cm in conventional tillage fields. The increased abundances in deeper soil layers resulted in the overall abundance surpassing that in the reduced tillage fields. Interestingly, however, this was not consistent in all the countries studied, but was restricted to countries where euedaphic species reached high abundances, i.e. Germany and Sweden. At sites dominated by ep- and hemiedaphic species the increase in the abundance of Collembola in deeper soil layers of conventional tillage fields was less pronounced and overall Collembola abundance did not surpass that in reduced tillage fields. Our findings are in line with results of earlier studies reporting increased abundance of Collembola in deeper soil layers in conventional tillage fields compensating for decreased abundances in shallow soil depth (Winter et al. 1990; Petersen 2002; Reeleder et al. 2006). Similarly, differential responses of ecological groups of Collembola to tillage practices had been described before with epedaphic and hemiedaphic species being detrimentally affected by conventional tillage while euedaphic species remained unaffected (Petersen 2002; Brennan et al. 2006; van Capelle et al. 2012), which again resembles results of our study. The differential responses of ecological groups was also mirrored in the differences in effects on Collembola communities in different countries across bioclimatic regions in our experiment which has not been reported before. Across countries dominating ecological groups differed leading to varying effects. Therefore our general hypothesis 1 has to be rejected as reduced tillage practices did not promote Collembola communities in general. Rather, our results indicate that Collembola communities show differential responses to tillage practices depending on the dominating ecological groups present. Further, they suggest that agricultural practices cannot easily be adapted to favour all soil biota. Communities can only profit from resources deeper in the soil if euedaphic species form a prominent component of the community present and can exploit these resources. Therefore, as communities varied across bioclimatic regions management practices have to be adapted to the local soil animal communities in order to sustain the services they provide.

The promotion of euedaphic species in conventional tillage fields and the accompanying decrease of epedaphic species was also reflected by Collembola trait composition. In conventional tillage fields the trait composition was shifted towards euedaphic traits compared to reduced tillage fields at the respective site. Analysing the depth distributions of Collembola in comparison to abiotic conditions we



identified  $C_{org}$  and  $N_t$  as major factors driving the increased abundances in deeper soil layers. The spatial distribution of Collembola therefore is linked to the distribution of their food resources in the soil as previously suggested (Verhoef and Nagelkerke 1977; Berg and Bengtsson 2007; Vignozzi et al. 2019). The stratified distribution of resources in reduced tillage fields is promoting epedaphic Collembola close to the soil surface (House and Parmelee 1985; Vignozzi et al. 2019). In contrast, the homogeneous distribution caused by conventional tillage is promoting euedaphic species in deeper soil layers (House and Parmelee 1985). Soil moisture and bulk density on the other hand did not correlate with the depth distribution of Collembola although they are often considered limiting factors (Winter et al. 1990; Jucevica and Melecis 2006; Dubie et al. 2011). However, soil moisture may play an important role at very dry conditions and result in more spatially heterogeneous distribution (Verhoef and Nagelkerke 1977; Verhoef and van Selm 1983). Overall, the results suggest that the translocation of resources to deeper soil layers by conventional tillage makes them available to Collembola at favourable soil moisture conditions and thereby increases Collembola abundance, especially that of euedaphic species vulnerable to desiccation (Krab et al. 2010).

The impact of Collembola on litter decomposition is still under debate (Kampichler and Bruckner 2009). Typically, litter decomposition is accelerated by the presence of mesofauna (Yang et al. 2017), but overgrazing of fungal populations may also reduce decomposition rates (Vreeken-Buijs and Brussard 1996). Further, effects on litter decomposition of mesofauna vary with litter quality and between different ecosystems (Bokhorst and Wardle 2013; Gergócs and Hufnagel 2016). In the second experiment (Chapter 3) litterbags filled with maize litter were buried in a long-term field experiment with two tillage treatments in order to quantify the effect of Collembola on litter decomposition and the dependency of soil Collembola on litter integrated into the soil by tillage. The litterbags were buried at the depth the plant residues are deposited by the respective management practice, i.e. at approximately 30 cm in conventional tillage fields and approximately 10 cm in reduced tillage fields. To quantify the importance of plant residues integrated into the soil as a food resource for Collembola the  $^{13}C$  signatures of the maize litter from the litterbags was traced using stable isotope analysis of Collembola retrieved from the litterbags. Further, the effect of Collembola on decomposition was analysed by investigating their effect on litter mass, C and N loss. Surprisingly, Collembola did not affect litter mass loss, but accelerated the transformation of the litter into a higher quality resource as indicated by the faster decrease in C/N ratio caused by faster losses of C and transport of material rich in N into the litter, presumably comprising mainly faeces (Seastedt 1984; Lavelle et al. 1993; Frouz et al. 2015). Reduced tillage affected decomposition in a similar way as Collembola (mesofauna) by decreasing the litter C/N ratio, while litter mass and C loss remained unaffected. Presumably, reduced tillage favoured the colonization of the litter material by fungi, which transported N into the litter. Unexpectedly, Collembola abundance in litterbags in conventional tillage fields exceeded that in

litterbags in reduced tillage fields. Our general hypothesis 2 therefore has to be rejected as Collembola were less abundant in reduced tillage fields but nevertheless exerted a positive effect on decomposition. As in the study presented in Chapter 2, the differences in abundance were due to the differential response of ecological groups. Epedaphic species showed higher abundances in reduced tillage fields compared to conventional tillage fields. Euedaphic species displayed the opposite pattern being more abundant in conventional tillage fields and in addition showed generally higher abundances than epedaphic Collembola. The increasing abundance and species richness in litterbags over time indicates the importance of litter resources for Collembola in agricultural fields and was presumably caused by migration of additional species into the litterbags as indicated by shifts in species composition. Collembola fed more intensely on maize litter in conventional tillage fields suggesting that other resources are scarce particularly in deeper soil layers reflecting the dependency of Collembola in agricultural fields on litter material incorporated into the soil (Krab et al. 2010). In reduced tillage fields, other resources are more readily available including fungi and bacteria as well as root exudates being available in particular at shallow soil depth (Parker et al. 1984; Seastedt 1984; Scheunemann et al. 2015). Our results support earlier studies suggesting that the spatial distribution of Collembola depends on the distribution of food resources (Verhoef and Nagelkerke 1977; Vignozzi et al. 2019). However, soil moisture conditions in deeper soil layers might also be important and may have contributed to the increase in euedaphic species (Berg et al. 1998; Jucevica and Melecis 2006). Overall, therefore, the observed patterns in the colonization of litterbags in our study are likely to be driven by resource supply to euedaphic species in deeper soil layers in conventional tillage fields, while in reduced tillage fields the increased resource availability at shallow soil favoured in particular epedaphic species.

The third experiment (Chapter 4) investigated the response of Collembola to mixed cropping systems of faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) in comparison to monocultures of faba bean. Notably, four different bean genotypes were investigated allowing to investigate to what extent variability within plant species affects Collembola in soil. At two field sites in the vicinity of Göttingen intercropped stands and monocultures were established. As expected, mixed cropping increased overall Collembola abundances. However, the beneficial effects of mixed cropping were mainly limited to one of the field sites indicating that the two sites differ in critical environmental conditions. Furthermore, the increase was limited to hemiedaphic Collembola while eu- and epedaphic Collembola remained unaffected. Similarly, bean genotypes only affected hemiedaphic Collembola, with abundances being increased by one genotype characterized by high tillering and short shoots, but maximum root biomass (Streit et al. 2019a, b). Conclusively, our general hypotheses 3 is confirmed by our results. Interestingly, one of the most important factors differing between the two field sites was soil C concentrations and associated soil C/N ratio. The soil C/N ratio reflects the quality of soil organic

matter resources for Collembola (Berg and Bengtsson 2007). Therefore, more high quality resources were available for Collembola at the field site of Reinshof with lower C/N ratio including plant residues as well as fungi (Seastedt 1984; Hättenschwiler et al. 2005; Garcia-Palacios et al. 2013). The results indicate that the availability of high quality resources at this field site enabled Collembola to profit from the additional C resources provided by increased root and shoot biomass through overyielding in mixed cropping systems (Pristeri et al. 2007; Li et al. 2006; Nyfeler et al. 2009; Xia et al. 2013). Differences in morphology of the bean genotypes may have contributed to the increased Collembola abundance as well. While supplying complementary resources by root exudates (Scheunemann et al. 2015) and optimizing moisture conditions at the soil surface they further provide additional habitat through habitat diversification (Eisenhauer et al. 2011). Variability between genotypes or varieties and its importance for mixed cropping systems is largely neglected in agriculture. Cropping systems usually rely on highest producing monocultures (Litrice and Violle 2015; Barot et al. 2017). Consequently, breeding programs do not focus on plant traits which might be beneficial in mixtures with other species or varieties (Litrice and Violle 2015; Barot et al. 2017). However, diversity within agroecosystems might not only enhance yield, but also may increase resilience against adverse environmental conditions or pest species, while further promoting belowground biodiversity critical for ecosystem services (Litrice and Violle 2015; Barot et al. 2017).

### 5.1 Changes in abiotic conditions by management practices as driving factor for Collembola

Each of the studies presented in Chapters 2, 3 and 4 highlighted the importance of C and N resources for Collembola, but also indicated that soil moisture functions as critical regulating factor for Collembola communities. As Collembola are considered generalist feeders C and N resources may directly promote the abundance of species feeding on decaying organic matter or indirectly by promoting fungi, which also serve as food source for Collembola (Seastedt 1984; Hättenschwiler et al. 2005; Garcia-Palacios et al. 2013). However, our results indicate that Collembola only profit from additional resources if soil moisture conditions are favourable (Verhoef and Nagelkerke 1977; Verhoef and van Selm 1983). By displacing plant residues to a depth of 30 cm tillage turns deeper soil layers into additional habitable space where otherwise resources would be lacking (House and Parmelee 1985; Krab et al. 2010). Results of the study presented in Chapter 2 and Chapter 3 suggest that Collembola deeper in soil heavily rely on litter derived resources and this applies in particular to euedaphic species. Presumably, at favourable moisture conditions deeper in soil the litter is rapidly colonized and exploited allowing them to increase in numbers. On the other hand the litter material translocated deeper into the soil is deprived from epedaphic species which are in turn diminished (Krab et al. 2010). Similarly, additional resources due to overyielding in mixed cropping systems (Li et al.

2006; Pristeri et al. 2007; Nyfeler et al. 2009; Xia et al. 2013) likely promoted the abundance of Collembola in the study presented in Chapter 4. Presumably, the increased root and shoot biomass in mixed cropping systems provided additional resources for Collembola with root-derived resources being particularly important (Scheunemann et al. 2015; Li et al. 2021). In addition, habitat diversification above- and belowground resulting in increased habitable space in mixed cropping systems likely beneficially affected Collembola (Eisenhauer et al. 2011). Beneficial moisture conditions close to the soil surface by shading of bean genotypes with high tillering and short shoots promoted in particular hemiedaphic Collembola. Overall, these results indicate resource availability as critically important factor limiting Collembola abundances, but resource exploitation to also critically depend on favourable soil moisture conditions.

## 5.2 On the use of ecological groups and traits

Designation of ecological groups based on literature or expert opinion requires knowledge of the ecology of the species but still may be ambiguous. For example, *Isotoma viridis* was assigned to epedaphic (Potapov et al. 2018) or hemiedaphic (Malcicka et al. 2017) Collembola in previous studies. Although both classifications can be argued for the ambiguity, which applies to many species, hamper firm conclusions. Mathematical approaches may help in dealing with such ambiguity. The method used in this study for delineating ecological groups was based on easy to determine characters. Our results support the validity of this procedure since species within each of the ecological groups responded in a similar way to the experimental treatments studied. Using trait composition instead of species composition helped in particular in comparing effects of changes in tillage practices across a large spatial gradient as analysed in Chapter 2. The studied tillage practices affected the species composition of Collembola (Chapter 2) and Collembola species composition was also affected by mixed cropping and bean genotypes (Chapter 4). However, species identity and community composition of Collembola varied among the studied European countries hampering comparison of the effects of tillage practices across countries. By contrast, using traits instead of species standardized the communities and allowed straightforward comparison of communities across the large spatial gradient studied. Thereby, using traits allowed general insights into the response of Collembola to variations in tillage treatments across European countries (Moretti et al. 2017). However, the trait composition of Collembola communities is also shaped by regional constraints and therefore has to be interpreted carefully. The prevalence of certain traits in certain climatic regions has to be considered, e.g. by analysing relative changes in trait compositions. We found similar relative changes caused by conventional tillage towards higher abundances of traits related to euedaphic species, which fitted well to our general findings (Chapter 2). Previous approaches using traits in order to assess soil quality have been applied successfully (Parisi

et al. 2005; Vandewalle et al. 2010; Menta et al. 2018). We generally utilized the approach of Vandewalle et al. (2010) and used it to ascribe Collembola to the ecological groups eu-, hemi- and epedaphic. Doing that allowed to link them to abundances as crucial component to evaluate the suitability of the habitat to carry large populations of Collembola as indication for high soil quality. This allowed us to evaluate the usefulness of Collembola as indicator for soil quality of their preferred habitat considering each of the three ecological groups.

### 5.3 Collembola as indicators for soil quality

The results of the present study allowed us to evaluate the usefulness of Collembola as indicators of soil quality. In order to be considered suitable indicators soil organisms have to fulfill five criteria as defined by Doran and Zeiss (2000). In each of the three experiments presented in Chapters 2, 3 and 4 Collembola sensitively responded to changes in management practices. Further, the sensitivity could be proven at the ecological group level increasing the resolution of the conclusions which can be drawn from our approach. We were able to show their correlation to litter decomposition and their usefulness for elucidating this ecosystem process as well as the changes in abiotic conditions caused by the investigated management practices. By using a standardized trait based approach to assign species to ecological groups, Collembola may serve as easy and cheap indicator for assessing agricultural management practices. Therefore, Collembola assessed by using a trait based approach fulfill all the proposed criteria for soil biota to be used as indicator for soil quality (Doran and Zeiss 2000).

### 5.4 Conclusions

Collembola are an important component of the soil fauna involved in the provisioning of ecosystem services of agricultural systems crucial for human wellbeing and in addition are useful indicators of soil quality. Although Collembola affected litter decomposition only little they are likely to speed up litter breakdown by facilitating nitrogen transfer into plant residues thereby decreasing litter C/N ratio and this is likely to contribute to the productivity of agricultural systems. Collembola sensitively respond to agricultural management practices affecting the distribution of litter resources in soil as well as abiotic conditions such as soil moisture. Our results underline the importance of food resources in soil as critical driving factor for Collembola abundance and community composition. In particular, the results highlight the dependency of Collembola on litter material incorporated into deeper soil layers by tillage, which may overcompensate for the detrimental effects caused by disturbance due to tillage.

Furthermore, our study demonstrated that additional resources and habitat provided in mixed cropping systems beneficially affect Collembola communities if resources are scarce. Importantly, using traits in addition to species allowed more detailed insight into the effect of agricultural practices on Collembola communities across large spatial scales. Future research needs to focus on the development of standardized sampling and evaluation protocols taking into account the sphere of influence of the investigated management practice and refining evaluation standards to match the importance of each ecological group of Collembola. Management practices aiming at promotion of soil Collembola have to be adapted to habitat conditions as well as the biota present at each agricultural site.

## References

- Barot, S., Allard, V., Cantarel, A., Enjalbert, J., Gauffreteau, A., Goldringer, I., ... & Porcher, E. (2017). Designing mixtures of varieties for multifunctional agriculture with the help of ecology. A review. *Agronomy for Sustainable Development*, 37(2), 1-20.
- Berg, M. P., Kniese, J. P., Bedaux, J. J. M., & Verhoef, H. A. (1998). Dynamics and stratification of functional groups of micro-and mesoarthropods in the organic layer of a Scots pine forest. *Biology and Fertility of Soils*, 26(4), 268-284.
- Berg, M. P., & Bengtsson, J. (2007). Temporal and spatial variability in soil food web structure. *Oikos*, 116(11), 1789-1804.
- Bokhorst, S., Wardle, D.A., 2013. Microclimate within litter bags of different mesh size: Implications for the 'arthropod effect' on litter decomposition. *Soil Biology and Biochemistry*, 58, 147–152. 10.1016/j.soilbio.2012.12.001.
- Brennan, A., Fortune, T., & Bolger, T. (2006). Collembola abundances and assemblage structures in conventionally tilled and conservation tillage arable systems. *Pedobiologia*, 50(2), 135-145.
- Doran, J. W., & Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology*, 15(1), 3-11.
- Dubie, T. R., Greenwood, C. M., Godsey, C., & Payton, M. E. (2011). Effects of tillage on soil microarthropods in winter wheat. *Southwestern Entomologist*, 36(1), 11-20.
- Eisenhauer, N., Milcu, A., Sabais, A. C., Bessler, H., Brenner, J., Engels, C., ... & Scheu, S. (2011). Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. *PLoS ONE*, 6(1), e16055.
- Frouz, J., Roubíčková, A., Heděnc, P., & Tajovský, K. (2015). Do soil fauna really hasten litter decomposition? A meta-analysis of enclosure studies. *European Journal of Soil Biology*, 68, 18-24.
- García-Palacios, P., Maestre, F. T., Kattge, J., & Wall, D. H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecology letters*, 16(8), 1045-1053.

- Gergócs, V., Hufnagel, L., 2016. The effect of microarthropods on litter decomposition depends on litter quality. *European Journal of Soil Biology* 75, 24–30. 10.1016/j.ejsobi.2016.04.008.
- Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. *Annual Review of Ecology, Evolution, and Systematics* 36, 191–218.
- House, G. J., & Parmelee, R. W. (1985). Comparison of soil arthropods and earthworms from conventional and no-tillage agroecosystems. *Soil and Tillage Research*, 5(4), 351-360.
- Jucevica, E., & Melecis, V. (2006). Global warming affect Collembola community: A long-term study. *Pedobiologia*, 50(2), 177-184.
- Kampichler, C., Bruckner, A. (2009). The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews*, 84 (3), 375–389. 10.1111/j.1469-185X.2009.00078.x.
- Krab, E. J., Oorsprong, H., Berg, M. P., & Cornelissen, J. H. (2010). Turning northern peatlands upside down: disentangling microclimate and substrate quality effects on vertical distribution of Collembola. *Functional Ecology*, 24(6), 1362-1369.
- Lavelle, P., Blanchart, E., Martin, A., Martin, S., Spain, A. (1993). A Hierarchical Model for Decomposition in Terrestrial Ecosystems: Application to Soils of the Humid Tropics. *Biotropica* 25, 130–150.
- Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F. A., & Smith, S. E. (2006). Root distribution and interactions between intercropped species. *Oecologia*, 147(2), 280-290.
- Li, Z., Shi, L., Kuzyakov, Y., Pausch, J., Scheu, S., & Pollierer, M. M. (2021). The flux of root-derived carbon via fungi and bacteria into soil microarthropods (Collembola) differs markedly between cropping systems. *Soil Biology and Biochemistry*, 160, 108336.
- Litrico, I., & Violle, C. (2015). Diversity in plant breeding: a new conceptual framework. *Trends in Plant Science*, 20(10), 604-613.
- Malcicka, M., Berg, M. P., & Eilers, J. (2017). Ecomorphological adaptations in Collembola in relation to feeding strategies and microhabitat. *European Journal of Soil Biology*, 78, 82-91.
- Menta, C., Conti, F. D., Pinto, S., & Bodini, A. (2018). Soil Biological Quality index (QBS-ar): 15 years of application at global scale. *Ecological Indicators*, 85, 773-780.
- Moretti, M., Dias, A. T., De Bello, F., Altermatt, F., Chown, S. L., Azcárate, F. M., ... & Berg, M. P. (2017). Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, 31(3), 558-567.
- Nyfelner, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J., & Lüscher, A. (2009). Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, 46(3), 683-691.
- Parisi, V., Menta, C., Gardi, C., Jacomini, C., & Mozzanica, E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: a new approach in Italy. *Agriculture, Ecosystems & Environment*, 105(1-2), 323-333.
- Parker, L. W., Santos, P. F., Phillips, J., & Whitford, W. G. (1984). Carbon and nitrogen dynamics during the decomposition of litter and roots of a Chihuahuan desert annual, *Lepidium lasiocarpum*. *Ecological Monographs*, 54(3), 339-360.

- Petersen, H. (2002). Effects of non-inverting deep tillage vs. conventional ploughing on collembolan populations in an organic wheat field. *European Journal of Soil Biology*, 38(2), 177-180.
- Potapov, A. M., Korotkevich, A. Y., & Tiunov, A. V. (2018). Non-vascular plants as a food source for litter-dwelling Collembola: Field evidence. *Pedobiologia*, 66, 11-17.
- Pristeri, A., Dahlmann, C., von Fragstein, P., Gooding, M. J., Hauggaard Nielsen, H., Kasyanova, E., & Monti, M. (2007). Yield performance of Faba bean–Wheat intercropping on spring and winter sowing in European organic farming system. In *Danish Research Centre for Organic Food and Farming, DARCOF Proceedings* (pp. 294-295).
- Reeleder, R. D., Miller, J. J., Coelho, B. B., & Roy, R. C. (2006). Impacts of tillage, cover crop, and nitrogen on populations of earthworms, microarthropods, and soil fungi in a cultivated fragile soil. *Applied Soil Ecology*, 33(3), 243-257.
- Scheunemann, N., Digel, C., Scheu, S., Butenschoen, O. (2015). Roots rather than shoot residues drive soil arthropod communities of arable fields. *Oecologia* 179 (4), 1135–1145.
- Seastedt, T. R. (1984). The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology*, 29(1), 25-46.
- Streit, J., Meinen, C., Nelson, W. C. D., Siebrecht-Schöll, D. J., & Rauber, R. (2019a). Above-and belowground biomass in a mixed cropping system with eight novel winter faba bean genotypes and winter wheat using FTIR spectroscopy for root species discrimination. *Plant and Soil*, 436(1), 141-158.
- Streit, J., Meinen, C., & Rauber, R. (2019b). Intercropping effects on root distribution of eight novel winter faba bean genotypes mixed with winter wheat. *Field Crops Research*, 235, 1-10.
- van Capelle, C., Schrader, S., & Brunotte, J. (2012). Tillage-induced changes in the functional diversity of soil biota—A review with a focus on German data. *European Journal of Soil Biology*, 50, 165-181.
- Vandewalle, M., De Bello, F., Berg, M. P., Bolger, T., Doledec, S., Dubs, F., ... & Woodcock, B. A. (2010). Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. *Biodiversity and Conservation*, 19(10), 2921-2947.
- Verhoef, H. A., & Nagelkerke, C. J. (1977). Formation and ecological significance of aggregations in Collembola. *Oecologia*, 31(2), 215-226.
- Verhoef, H. A., & Van Selm, A. J. (1983). Distribution and population dynamics of Collembola in relation to soil moisture. *Ecography*, 6(4), 387-388.
- Vignozzi, N., Agnelli, A. E., Brandi, G., Gagnarli, E., Goggioli, D., Lagomarsino, A., ... & Gucci, R. (2019). Soil ecosystem functions in a high-density olive orchard managed by different soil conservation practices. *Applied Soil Ecology*, 134, 64-76.
- Vreeken-Buijs, M.J., Brussaard, L. (1996). Soil mesofauna dynamics, wheat residue decomposition and nitrogen mineralization in buried litterbags. *Biol Fertil Soils*, 23, 374–381.
- Winter, J. P., Voroney, R. P., & Ainsworth, D. A. (1990). Soil microarthropods in long-term no-tillage and conventional tillage corn production. *Canadian Journal of Soil Science*, 70(4), 641-653.
- Xia, H. Y., Zhao, J. H., Sun, J. H., Bao, X. G., Christie, P., Zhang, F. S., & Li, L. (2013). Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. *Field Crops Research*, 150, 52-62.



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Yang, B., Zhang, W., Xu, H., Wang, S., Xu, X., Fan, H., Chen, H.Y.H., Ruan, H. (2017). Effects of soil fauna on leaf litter decomposition under different land uses in eastern coast of China. *J. For. Res.*, 33, 10. 10.1007/s11676-017-0521-5.

# List of Publications

## Peer-reviewed articles

**Hanisch, J.**, Engell, I., Linsler, D., Scheu, S., & Potthoff, M. (2022). The role of Collembola for litter decomposition under minimum and conventional tillage. *Journal of Plant Nutrition and Soil Science*, doi: 10.1002/jpln.202200077.

Hoeffner, K., Beylich, A., Chabbi, A., Cluzeau, D., Dascalu, D., Graefe, U., Guzmán, G., Hallaire, V., **Hanisch, J.**,... & Pérès, G. (2021). Legacy effects of temporary grassland in annual crop rotation on soil ecosystem services. *Science of the Total Environment*, 780, 146140.

## Articles in preparation

**Hanisch, J.**, Taylor, A., Stefan, S., Displacement of resources in agricultural fields by tillage practices differentially affects ecological groups of Collembola across Europe, *in preparation* (2022).

**Hanisch, J.**, Götsch, P., Scheu, S. Intercropping of faba bean and winter wheat promotes Collembola diversity and abundance if soil carbon resources are scarce, *in preparation* (2022).

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# Thesis Declarations

## **Declarations of the author's own contribution to manuscripts with multiple authors**

**Chapter 2:** Displacement of resources in agricultural fields by tillage practices differentially affects ecological groups of Collembola across Europe

Jörg Hanisch, Astrid Taylor, Stefan Scheu

I am the first author of the paper. I was involved in data collection. I analysed the data and wrote the manuscript. Astrid Taylor was involved in data collection and contributed to the conceptualization of the experiment. Stefan Scheu contributed to the conceptualization of the experiment, data analysis and finalizing the manuscript.

**Chapter 3:** The role of Collembola for litter decomposition under minimum and conventional tillage

Jörg Hanisch, Ilka Engell, Deborah Linsler, Stefan Scheu, Martin Potthoff

Ilka Engell and I share the first authorship of this paper. I was involved in data collection and analysed the soil fauna and stable isotope data. Ilka Engell was involved in data collection and analysed and prepared the decomposition data for statistical analysis. I performed statistical analysis in consultation with Ilka Engell. Ilka Engell and I wrote the manuscript together in equal parts with me focussing on soil fauna and stable isotope data and Ilka Engell focussing on decomposition data. Deborah Linsler was involved in data collection. All authors contributed to the conceptualization of the experiment. Deborah Linsler, Stefan Scheu and Martin Potthoff were involved in finalizing the manuscript. Ilka Engell was the corresponding author during the submission progress.

**Chapter 4:** Intercropping of faba bean and winter wheat promotes Collembola diversity and abundance if soil carbon resources are scarce

Jörg Hanisch, Paul Götsch, Stefan Scheu

I am the first author of the paper. I was involved in data collection. I analysed the data and wrote the manuscript. Paul Götsch was involved in data collection and finalizing the manuscript. Stefan Scheu contributed to the conceptualization of the experiment, data analysis and finalizing the manuscript.

## **Plagiarism Declaration**

I hereby declare that I have written this thesis independently. I did not submit this thesis in parts or as a whole at any other university or institution for any degree and I will not do so in the future. I named all contributions to the manuscript by other persons. By clear cross-referencing, I acknowledged all information acquired from other author's work.

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## Veröffentlichungen

**Hanisch, J.**, Engell, I., Linsler, D., Scheu, S., & Potthoff, M. (2022). The role of Collembola for litter decomposition under minimum and conventional tillage. *Journal of Plant Nutrition and Soil Science*, doi: 10.1002/jpln.202200077.

Hoeffner, K., Beylich, A., Chabbi, A., Cluzeau, D., Dascalu, D., Graefe, U., Guzmán, G., Hallaire, V., **Hanisch, J.**,... & Pérès, G. (2021). Legacy effects of temporary grassland in annual crop rotation on soil ecosystem services. *Science of the Total Environment*, 780, 146140.

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