Health & Ecological Risk Assessment

Investigating the role of soil mesofauna abundance and biodiversity for organic matter breakdown in arable fields

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Abstract

Intact soil food webs are pivotal to maintaining essential soil functions, such as carbon recycling, sequestering, and biomass production. Although the functional role of micro- (e.g., bacteria and fungi) and macrofauna (e.g., earthworms) is comparatively well established, the importance of the mesofauna community (e.g., abundance and diversity of *Acari* and *Collembola*) in maintaining soil functionality is less clear. We investigated this question in a six-month field experiment in arable soil by actively manipulating mesofauna abundance and biodiversity through the application of two legacy insecticides (lindane and methamidophos) at sufficiently high doses to reduce mesofauna abundance (well above previously registered application rates; 2.5 and 7.5 kg a.s./ha for lindane, and 0.6 and 3 kg a.s./ha for methamidophos) and measure the impact on organic matter degradation. Our results demonstrate that both insecticides had reduced *Collembola* and *Acari* abundances by up to 80% over the study's six-month duration. In addition, we observed less pronounced and more complex changes in mesofauna biodiversity over time. These included insecticide-dependent temporal fluctuations (both reduction and increase) for different estimates (indices) of local (alpha)-diversity over time and no lasting impact for most estimates after six months. Even at these exceptionally high field rates, *Collembola* and *Acari* diversity was observed to generally recover by six months. In contrast, considering organic matter breakdown, we found no evidence of a treatment-related effect. These results suggest that organic matter breakdown in arable soils is likely driven by other trophic levels (e.g., microorganisms or earthworms) with only a limited influence of the mesofauna community. We discuss these findings with regard to their implications for our current understanding of soil food web function and future European soil risk assessments. *Integr Environ Assess Manag* 2022;18:1423–1433. © 2021 The Authors. *Integrated Environmental Assessment and Management* published by Wiley Periodicals LLC on behalf of Society of Environmental Toxicology & Chemistry (SETAC).

KEYWORDS: Biodiversity, Insecticides, Pesticides, Risk assessment, Soil function

INTRODUCTION

In times of an ever-growing human population, combined with the challenges associated with changing climatic conditions, the preservation of soil ecosystem functions is of uttermost importance (Bender et al., 2016). The multitude of pivotal ecosystem services provided by the soil communities range from tangible functions such as biomass production to more subtle processes including organic matter cycling and carbon sequestration (Briones, 2014). Most of these processes, either directly or indirectly, rely on the functional integrity of soil-based food webs (Scheu, 2002).

Underground food webs typically utilize organic matter as the prime energy resource facilitating its liberation, mineralization, and ultimately utilization (Setälä et al., 2005). Traditionally these complex communities are broadly categorized into three groups according to size

and trophic position. Although the functional role of the micro- (e.g., primary decomposers such as bacteria and fungi; Hättenschwiler et al., 2005) and macrofauna (e.g., ecosystem engineers like earthworms; Blouin et al., 2013; Lavelle, 1988) is comparatively well documented, the impact of the mesofauna community (including generalist microarthropod grazers and predators such as *Collembola* and *Acari*) on soil functional traits is less clear (Chauvat et al., 2007; Kampichler & Bruckner, 2009; Setälä et al., 2005). Most viewpoints suggest that a large proportion of the soil microarthropod community is generally characterized by a low degree of feeding specialization, a high degree of functional redundancy at the species level, a limited ability to generate biomass and, consequently, play a limited role in regulating major trophic cycles in the soil (Briones, 2014; Kampichler & Bruckner, 2009; Setälä et al., 2005). Despite the likely restricted overall impact of individual microarthropod species or mesofauna community composition on the large trophic soil cascades, the presence of the mesofauna community can be beneficial to overall soil functions in some habitats (Setälä et al., 2005), but only limited information regarding their role and importance in arable soils and impact on biomass production is available.

Intensively managed landscapes, such as arable fields, rely on functional soils for sustainable crop production, but simultaneously impose high levels of stress on such communities, including unfavorable microclimatic conditions (e.g., drought), frequent mechanical soil disturbance (tillage), fertilizers, and plant protection products (PPP; Alvarez et al., 1999; Brennan et al., 2006; Miller et al., 2017). All of these artificial stressors can have lasting impacts on mesofauna abundance and community composition, often resulting in less diverse but more stress-adapted communities (Chauvat et al., 2007; Marx et al., 2016). However, how changes in abundance and composition of the mesofauna community affect vital soil functional traits including organic matter degradation and nutrient cycling is currently poorly understood. So, it remains unclear how this group should be evaluated in the risk assessment of PPPs aiming to maintain major ecosystem services in arable field soils.

To begin addressing this knowledge gap, we set up a field experiment manipulating the mesofauna abundance and community composition by applying two insecticides (at two different rates) with divergent chemical characteristics and measured the impact on multiple proxies for organic matter degradation. We expected that (1) all insecticide treatments would significantly reduce mesofauna abundance and decrease diversity, (2) the impacts (see 1) on mesofauna abundance and composition would be more pronounced for high application rates and greater insecticide persistence, and (3) these changes would result in a reduction in organic matter degradation in soil. The results of this study are discussed with regard to implications for soil protection and regulation of PPPs.

MATERIALS AND METHODS

Field site and test setup

The test field was an arable field located near Machern in Saxony, Germany (latitude: 51°21′4.73″N, longitude: 12°36′16.14″E); the test was conducted in 2014 by BioChem agrar GmbH. The soil of this arable field was a Luvisol with 43.0% sand, 46.2% silt, and 10.8% clay (loam; USDA). The following additional soil properties were determined: pH ($CaCl_2$) = 6.4, total organic carbon content = 1.04%, maximum water holding capacity = 36.6 g/100 g dry weight soil, and effective cation exchange capacity = 6.2 cmol$^+$/kg. Within three years before the start of the study, *Phacelia tanacetifolia* was grown on the field without the use of any pesticide. In April, the test field was tilled with a cultivator and divided into four blocks (N = 4) containing five 10 × 10 m treatment areas, each separated by a minimum distance of 3 m to avoid cross contamination. Each treatment area within a block was randomly assigned to one of five treatments.

Treatments and application

In this field trial, we used two insecticides (lindane and methamidophos) to manipulate mesofauna community. Lindane and methamidophos were chosen because of known adverse effects on soil microarthropods (Scholz-Starke et al., 2013) and because they strongly differ in their dissipation behavior in soil (lindane: DT_{50} in soil = 42–390 days [EU, 1998]; methamidophos: $DT_{90} < 10$ days [EU, 2006]). These divergent properties allowed us to study short and more extended disturbance effects on the mesofauna community. In all cases, sufficiently high doses for all treatments were chosen to ensure substantial disturbance of the mesofauna community. All treatments were applied in early May 2014, with lindane at application rates of 2.5 kg/ha (lindane low rate) and 7.5 kg/ha (lindane high rate), using an experimental formulation containing 150 g lindane/L. Methamidophos was applied at 0.6 kg/ha (methamidophos low rate) and 3 kg/ha (methamidophos high rate) using a formulation containing 600 g methamidophos/L. These high application rates were intentionally chosen based on the strong effects on soil mesofauna (see Scholz-Starke et al., 2013 and Cristi de Barros et al., 2015) and were clearly greater than formerly registered uses. Neither formulation is commercially available; both were supplied by Bayer AG. Control plots were treated with the same amount of water at the same rate. All applications were achieved using spray booms on bare soil with a water volume of 600 L/ha.

Sampling of Collembola and Acari

Two weeks before the start of the experiment, mesofauna sampling was performed to ensure that mesofauna populations of comparable abundances were present in all plots. After the experiment began, *Collembola* and *Acari* were sampled 0.5, 1, 2, 3, and 6 months after treatment application. Six soil cores (two in the pre-application) were collected at random locations from each plot on each sampling date.

The soil cores were collected using stainless steel tubes with a diameter of 5 cm (sampling area = 19.6 cm²) extracting the top 5 cm of the soil. Soil cores were collected so that the soil pores were not destroyed. Immediately after sampling, the tubes were sealed with caps, labeled, and stored in cooling boxes for transport to the laboratory. The mesofauna were extracted from the soil cores using a MacFadyen high-gradient extractor (heat and light extraction method; MacFadyen, 1961). In short: the tops of the soil cores were covered with a plastic mesh (1 mm mesh size), and the six cores per sampling event were placed into a canister, which was inverted and then placed on the extraction system. Beneath each canister, a funnel was attached to a collecting flask containing 25 mL of a fixing liquid (70% ethanol solution). A temperature gradient was created between the upper part (containing the soil cores) and the lower part of the system (containing the collecting flasks). The temperature gradient was obtained by circulating heated air in the canister area (upper part of the system) and cooled air in the collecting area (lower part of the system). The extraction lasted 9 d at the following heating regimen in the upper part of the setup: 20 °C for 24 h, 25 °C for 24 h, 30 °C for 24 h, 35 °C for 24 h, 40 °C for 24 h, 45 °C for 24 h, 50 °C for 24 h, and 55 °C for 48 h. During this time, the *Collembola* and *Acari* moved down through the soil away from the heat source until they fell from the soil into the funnel and into the collecting flask.

Mesofauna identification (taxonomy)

The *Collembola* and *Acari* were counted and determined to the lowest taxonomic rank, if possible. The taxonomic identification of *Collembola* and *Acari* followed various sources, which are provided in the supplemental information (Table S1).

Biodiversity estimates

Species diversity (alpha diversity) was quantified by calculating three specific Hill numbers (Hill, 1973). Hill numbers provide a continuous range of diversity measures and can be used to highlight specific properties of the diversity of a community. The three Hill numbers that were calculated included ^0D, ^1D, and ^2D (for further details on calculations, see also, e.g., Chao et al., 2014; Jost, 2006). ^0D equals species richness (number of species) and, as such, does not give weight to individual species' abundance. ^1D is an algebraic transformation of the Shannon entropy (i.e., the exponential of Shannon diversity; Chao et al., 2014; Jost, 2006). This gives more weight to more abundant species and is thus a measure for the number of common species in a community ("the number of typical species" following Gotelli & Chao, 2013). ^2D is an algebraic transformation of the Gini–Simpson index (i.e., the inverse of the index) and gives weight to the very abundant species and only marginally considers rare and less abundant species. ^2D is thus a measure of diversity of the very abundant species in an assemblage (see the chapter by Gotelli & Chao in Levin, 2013). Confidence intervals were calculated based on

a bootstrap method described in Appendix S2 of Chao and Jost (2015) with 1000 permutations.

Organic matter breakdown

Decomposition is one key functional parameter of soil food webs and indicates the soil's ability to recycle and improve bioavailability of nutrients to support ecosystem services like biomass production. Numerous methods exist to measure decomposition of different substrates (e.g., litter type), each providing information on different aspects of this fundamental process. For an overview and comparison of the different methods, see Ghaley et al. (2014), Kula and Römbke (1998), and Paulus et al. (1999). The methods used are described below.

Bait-lamina. The bait-lamina method is recommended as a practical method for studying overall biological activity in soil (Kula & Römbke, 1998; van Gestel et al., 2003). We followed the approach of Kratz (1998). In short, 80 plastic strips (length ~25 × 3 cm 0.5 cm width) containing 12 bait-filled holes each (diameter ~1 cm) were laid horizontally within the top soil layer (5 cm depth) in each of the five treatment areas in all four plots (320 per treatment). Bait-lamina holes were filled with a substrate of 70% cellulose powder, 25% finely ground bran flakes, and 5% active coal. At 0.5, 1, 2, 3, and 6 months after treatment, 64 strips per plot (16 per treatment area) were collected and the ratio of filled to empty (eaten) holes recorded. Although this method provides a quick and easy assessment of the overall feeding (decomposition) activity in the soil, a clear disadvantage is the uncertainty regarding the contribution of soil organism groups to the feeding process.

Mini-container. To address the limitations of the bait-lamina method, a more targeted approach is provided by the mini-container method (Eisenbeis, 1994, 1995; Eisenbeis et al., 1999), which gives a better understanding of the contribution of different decomposer groups to the overall degradation performance in soil (Emmerling & Eisenbeis, 1998). In contrast to the bait-lamina system, which allows indiscriminate access of the soil community, the mini-container system uses nets of different mesh sizes to selectively exclude specific groups of the soil community depending on body size. By comparing the decomposition process between different mesh sizes, it is possible to disentangle the contribution of soil mesofauna and soil microorganisms (Marx et al., 2016).

In general, the mini-container system consists of two components: Polyvinyl chloride (PVC) bars (length 38 mm), with 12 holes as carriers, and a polyethylene (PE) mini-container, which contains the feeding substrate (for type of substrate, see below). Each mini-container has a central cylinder (height 16 mm, diameter 11 mm) with both sides being closed by meshes of different sizes. In this experiment, we used two different mesh sizes to investigate the contribution of the mesofauna to the overall decomposition: 20 μm (fine mesh; microbial decomposers only) and 1000 μm (wide mesh;

microbial and mesofauna). Both treatments excluded the macrofauna community (e.g., earthworms).

Decomposition substrate.　One additional factor influencing the decomposition patterns of leaf litter degradation in soil is the quality of the feeding substrate (Fujii & Tekada, 2010; Szauser et al., 2011). In order to attain a more complete picture, two different feeding substances with different C:N ratios were used for the test: lucerne stems, which had a C:N ratio of 37.9, and cereal leaves, which had a C:N ratio of 136.6. The test substrates were dried at 60 °C for 24 h before filling the mini-container. The nodes of the lucerne stems were excluded due to the high mineral content, and only internodes were used.

Mini-container setup.　This test design resulted in four mini-container systems: lucerne (fine and wide mesh) and cereal leaves (fine and wide mesh). For both substrates, four bars containing six mini-containers per mesh size were filled with substrate (0.1 g substrate per mini-container) and horizontally deployed at a depth of 5 cm in each treatment area in all four plots ("set one"). For lucerne, an additional set of mini-containers ("set two") were buried three months after the start of the experiment. This step was necessary because we expected that most of the lucerne substrate would have decomposed after three months, potentially limiting the information gain for the remaining duration of the experiment.

Mini-container sampling.　One bar of the lucerne holding mini-containers was sampled at eight timepoints: 0.5, 1, 2, and 3 months after application ("set one"), and 3.5, 4, 5, and 6 months after the start of the experiment measurements for both mesh sizes ("set two"). One bar of mini-containers holding cereal leaves was sampled 1, 2, 3, and 6 months after application.

Decomposition rate.　After removing the bars from the soil at the end of the exposure period and returning them to the laboratory, the remaining feeding substrate was carefully removed from the mini-container and cleared of larger soil particles and animals. The remaining substrate was dried at 60 °C for 24 h and weighed. The water and mineral content was measured by oven drying (105 °C for 3 h) and ashing (600 °C for 3 h) to obtain data on mineral infiltrations from surrounding soil. The decomposition rate was calculated as the weight loss of the feeding substrate (% of initial weight) of the mini-container for a given mesh size and application rate.

Statistics and analytical rationale

Although it is common practice to fit advanced models (e.g., GLMM) to field data such as this study, the often limited sampling size (plots) do not support such approaches because of model overfitting (Motulsky, 2014). Consequently, we decided to present the results as mean values, nonstandardized effect sizes, standardized effect sizes (Cohan's D), and associated 95% confidence intervals (95% CI), which allows comparable conclusions (Motulsky, 2014). In all cases, we (1)

analyzed effects on total *Collembola* and *Acari* abundance separately; (2) focused on the statistical comparison of the insecticide treatments compared with the control and between the treatments for *Collembola* and *Acari* abundance, as well as organic matter breakdown; and (3) considered a nonoverlap of 95% CI (raw data) between control and treatments or between insecticide treatments as significantly different as well as a nonoverlap with zero (for effect size estimates; Mair et al., 2020).

For abundance and biodiversity estimates (Hill number ^0D, ^1D, and ^2D), standardized effect sizes were presented to make both parameters comparable. In this article, we did not conduct an in-depth analysis of the species level changes because this was outside the article's scope. In case of organic matter breakdown, we chose to present nonstandardized effects sizes (% change compared with the control) to facilitate a more intuitive interpretation of the results. The pretreatment samples were analyzed separately because the sampling regimen differed from the main experiment. All statistics were computed in R v. 3.5.2 (Team, 2013) using its basic function and the ggpubr package (Kassambara, 2017). All calculations for empirical Hill numbers and bootstrapped confidence intervals were conducted using the functions from Appendix S8 of Chao and Jost (2015).

RESULTS

Abundance

Overview. Differences in the mesofauna abundance of the treatment blocks compared with the control plots were not evident before treatment (all 95%CI overlapping; Figure S1). When considering the control plots, we observed pronounced but contrasting seasonal population dynamics (Figures 1 and 2). For *Acari*, we found an increase in abundance during the first three months of the experiment, reaching maximum population abundance in July (three months after application), followed by a decrease in population levels to October (six months after application; see Figure 1). For *Collembola*, we saw an opposing seasonal dynamic with a decrease in the abundance to June (three months after application) and a recovery of the population to October (six months after application; see Figure 2). Further data on single species population counts of *Acari* and *Collembola* are given in the supplemental information (Tables S2–S13).

Treatment effects.
Acari.　The *Acari* population reacted strongly to insecticide exposure with abundance decreasing in all treatments over the entire study duration (all effect size estimates are negative; Figures 1 and S2). Total *Acari* abundance values were significantly reduced in both lindane application rates over the whole study period. The high lindane application rate revealed significantly stronger effects than the low rate three and six months after application (Figure 1). Overall, lindane treatments demonstrated more pronounced effects than methamidophos applications (Figure S2). Total abundance
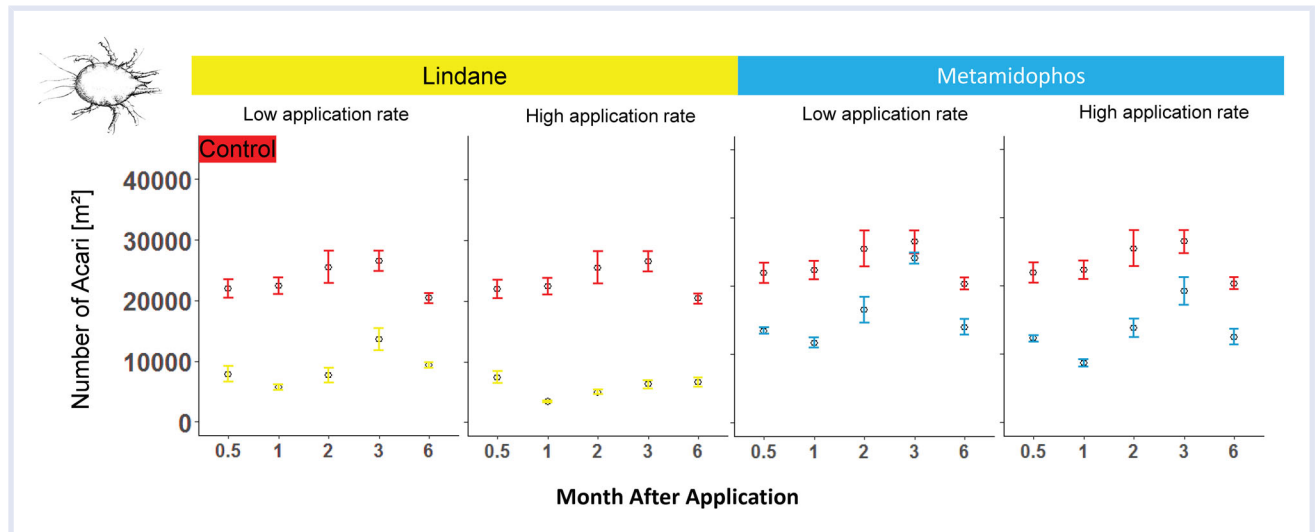
FIGURE 1 Total *Acari* abundance (individuals per m²) over time in the four treatment groups. The effects of the two tested application rates of both lindane (yellow) and methamidophos (blue) are compared with abundance in the control plots (red). We present mean values and associated 95% confidence intervals
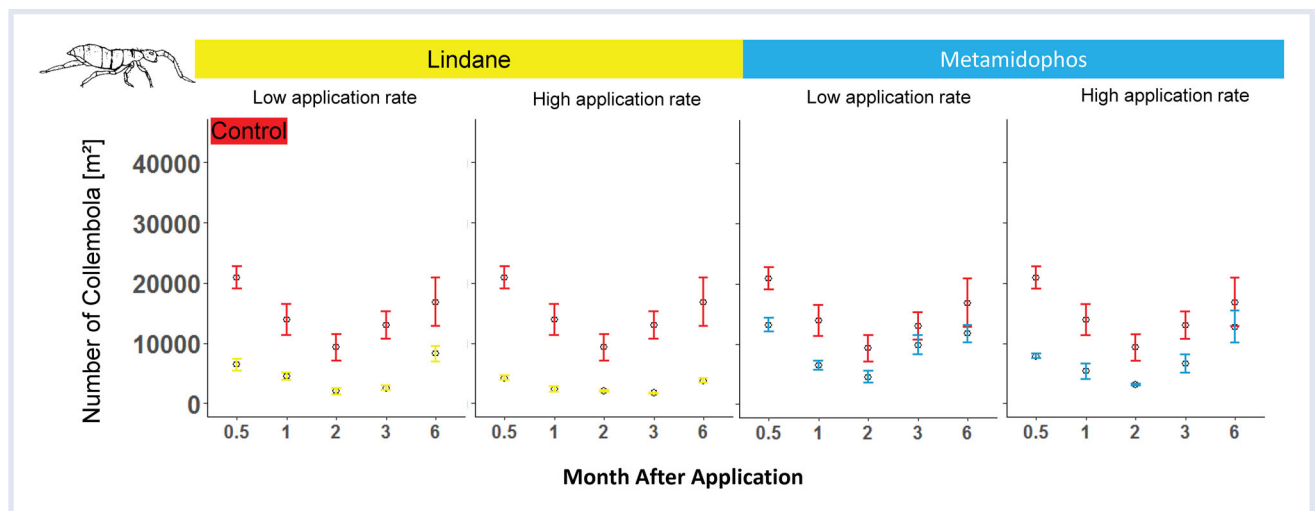

FIGURE 2 Total *Collembola* abundance (individuals per m²) over time in the four treatment groups. The effects of the two tested application rates of both lindane (yellow) and methamidophos (blue) are compared with abundance in the control plots (red). We present mean values and associated 95% confidence intervals

of *Acari* was reduced by up to 87% in the lindane treatments and by up to 67% in the methamidophos treatments. The high dose of methamidophos caused significantly stronger effects on *Acari* than the low dose, one and three months after application (Figure 1). None of the four treatments demonstrated recovery of *Acari* populations six months after application (Figure S2).

Collembola. The total *Collembola* population exhibited clear, but less pronounced, responses to insecticide exposure than *Acari* (Figures 2 and S3). Following applications, we observed strong negative effects of all four insecticide treatments on total *Collembola* abundance, with reduced abundance for the entire duration of the experiment (all effect size estimates were negative; Figure S3). These effects were more pronounced, longer lasting, and dose dependent for the lindane treatments; we saw no sign of

population recovery after six months (Figures 2 and S3). For the methamidophos treatments, the observed effects were dose dependent only 0.5 months after application. Both methamidophos treatments demonstrated a smaller effect on total *Collembola* toward the end of the study starting two months after application. Compared with the control, no significant differences were evident three and six months after application in the low dose and six months after application in the high dose treatment (Figures 1 and S3).

Biodiversity

Overview. When looking at the overall patterns of biodiversity, we found that all insecticide treatments, on at least one sampling date, altered some aspects of *Acari* and *Collembola* biodiversity (see effect size estimates in Figure 3; all raw Hill numbers are presented in the
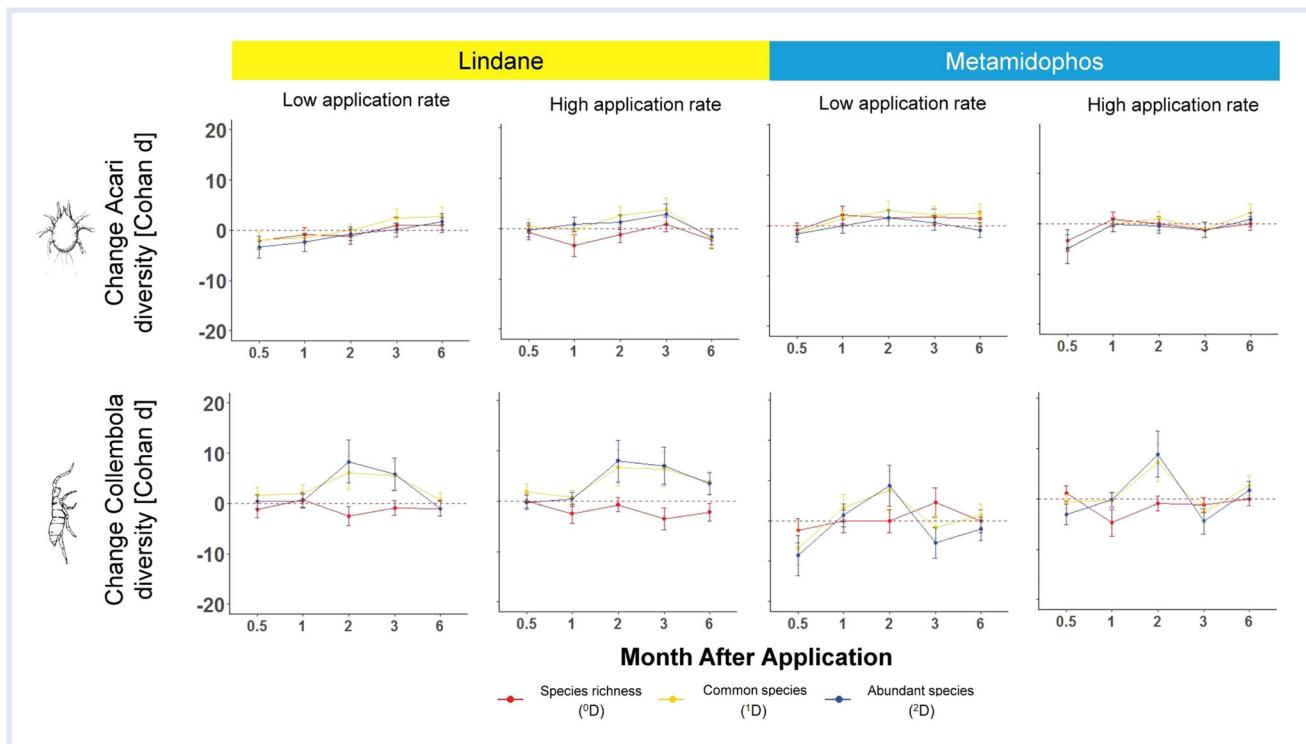
FIGURE 3 Standardized relative changes (standardized effect sizes) over time (month after application) of species richness (^{0}D red), common species (^{1}D yellow), and abundant species (^{2}D blue) in response to insecticide treatments. We present mean estimates and associated 95% confidence intervals

Supporting Information [Figure S5]). Compared with the effects on abundance, the changes to local diversity were less pronounced (see Figures S2, S3, and 3) and in many cases positive, meaning an increase in diversity estimates over the control (Figure 3). Apart from the high application rate of lindane, all diversity estimates indicated comparable or slightly increased diversity six months after application.

Treatment effects.
Acari. For *Acari* populations, no obvious insecticide or rate-specific pattern was evident, with only the lindane high treatment displaying effects six months after application (Figure 3). For lindane, we observed an initial reduction in all diversity indicators for the low, but not for the high, application rate. In the low application rate, this initial reduction was followed by a steady increase in species diversity with an overall increase in the number of common and abundant species six months after application (Figure 3), that is, a more even distribution of individuals among species. In contrast, the high lindane application rate led to a more complex temporal pattern cumulating in significant reduction in diversity six months after application (Figure 3). The high dose of methamidophos had clear initial effects on *Acari* diversity, but only the low application rate resulted in a lasting increase in the number of common species six months after application (Figure 3).

Collembola. *Collembola* diversity reacted more strongly to insecticide application with an apparent substance, but not rate-specific pattern (Figure 3). For lindane, we saw

a decrease in species richness, with an increase in the number of common and abundant species two months after application (Figure 3), that is, a more even distribution of individuals among species. The maximum effects of lindane were comparable between treatments, but in the high application rate, negative effects on diversity lasted to the end of the experiment. For methamidophos, we found inconsistent effects on species richness including both temporal decreases and increases over the experiment (Figure 3). As with the lindane treatment, temporal increases in the number of common and abundant species were observed, but none of the diversity indices differed from the control six months after application.

Organic matter degradation

Mini-container.
Cereal leaves. The results of the decomposition experiment using cereal leaves as a substrate demonstrated a clear and consistent increase in the amount of digested substrate throughout the experiment and clearly increased variation on the final sampling date (Figure 4). This overall pattern was similar for both mesh sizes, with large mesh sizes (mesofauna present) exhibiting slightly elevated (less than 10%) and sometimes significantly elevated decomposition (see Figure S4B). We found no clear treatment effect, with small (less than 10%) occasional deviations in both directions depending on the timepoint (Figure 4). The most pronounced differences between treatment and control (~10%) were observed on the last sampling date. However,
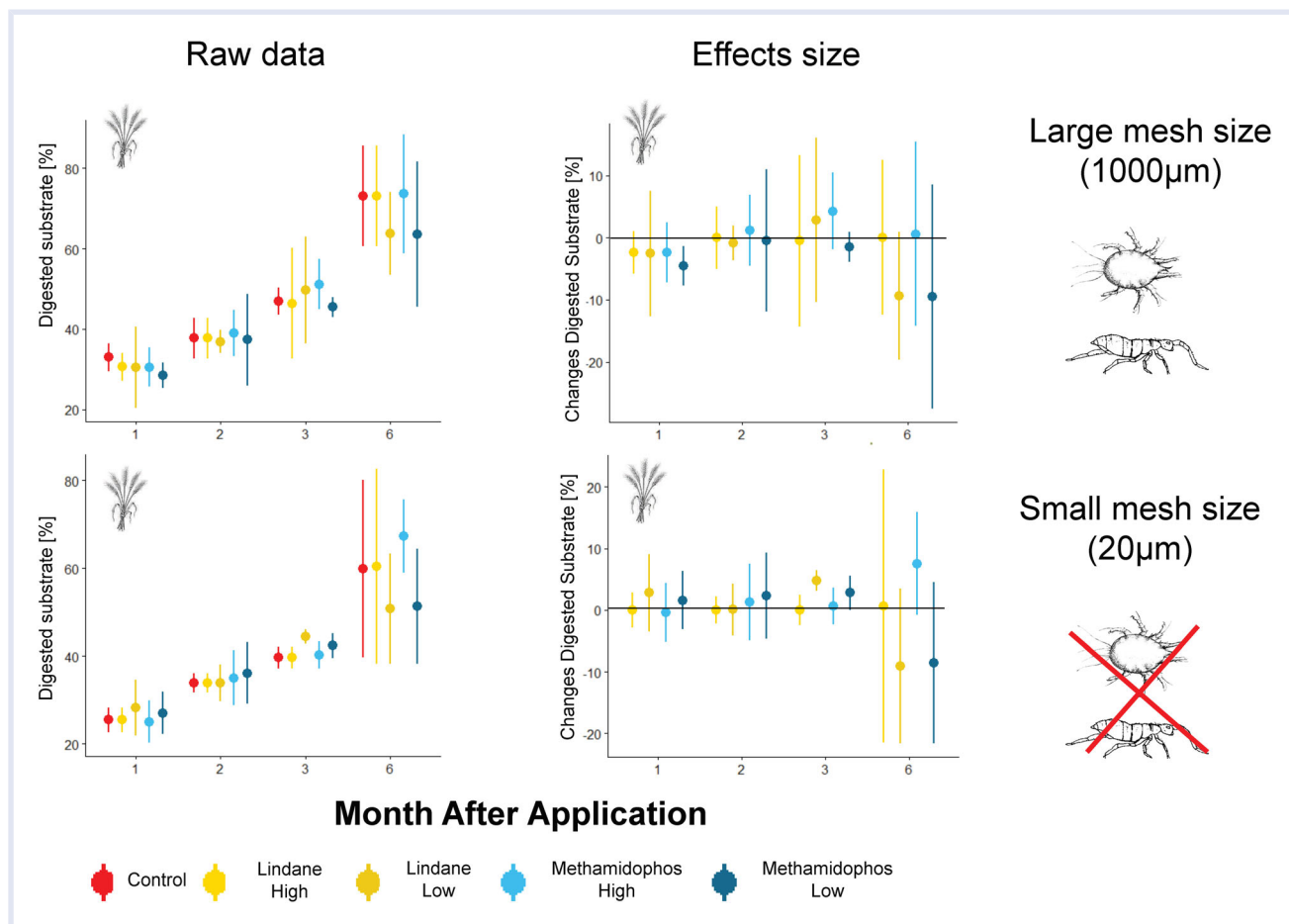
FIGURE 4 Results of cereal leaf degradation experiment over time (month after application) in the presence (upper) and absence (lower) of mesofauna. We present the raw (left) and relative changes (standardized effect sizes) over time for all insecticide treatments. We present mean values and associated 95% confidence intervals

none of the treatment groups differed significantly from the control, and the low treatment rates exhibited larger effects in a dose-independent manner. Most temporal trends were reflected in both mesh sizes (mesofauna present and absent; see Figures 4 and S4B).

Lucerne. The second set of mini-containers demonstrated greater overall litter decomposition and greater variability in measurements for both mesh sizes, in particular, at the end of the last measurement of the respective sets (Figure 5). Comparing the control groups of the two mesh sizes revealed that the decomposition was consistently (less than 10% difference), but not significantly, elevated in the large mesh sizes (Figure S4A). Compared with the control, temporal fluctuating effects were seen in all treatments (Figure 5), with no clear adverse effects of treatment on overall decomposition rates. The largest increases in decomposition rates, compared with control (approximately +10%), were observed in the high methamidophos and high lindane treatments five months after the start of the experiment, whereas the largest decreases (approximately −10%) were observed in both lindane and the high methamidophos treatments, six months after the treatment, with similar patterns in both mesh sizes

(Figure 5). For the last sampling point of the large mesh size mini-container, only one sample was successfully analyzed and, consequently, no confidence interval could be calculated.

Bait-lamina. The control treatment indicated that feeding activity patterns were not uniform throughout the season, but rather followed a stepwise pattern. We observed a high feeding activity between May and June and between July and August with phases of reduced feeding activity in between. This pattern was consistent for all treatment groups, and there were no differences between treatments and the control (Figure 6).

DISCUSSION

In this study, we investigated the contribution of mesofauna abundance and diversity on multiple proxies of organic matter decomposition, in a field experiment in arable soil, over a six-month period, by applying two different insecticides, at two application rates deliberately chosen to cause effects in the mesofauna community. In line with our expectations, (1) we found that all insecticide treatments initially reduced mesofauna abundance significantly and temporarily altered
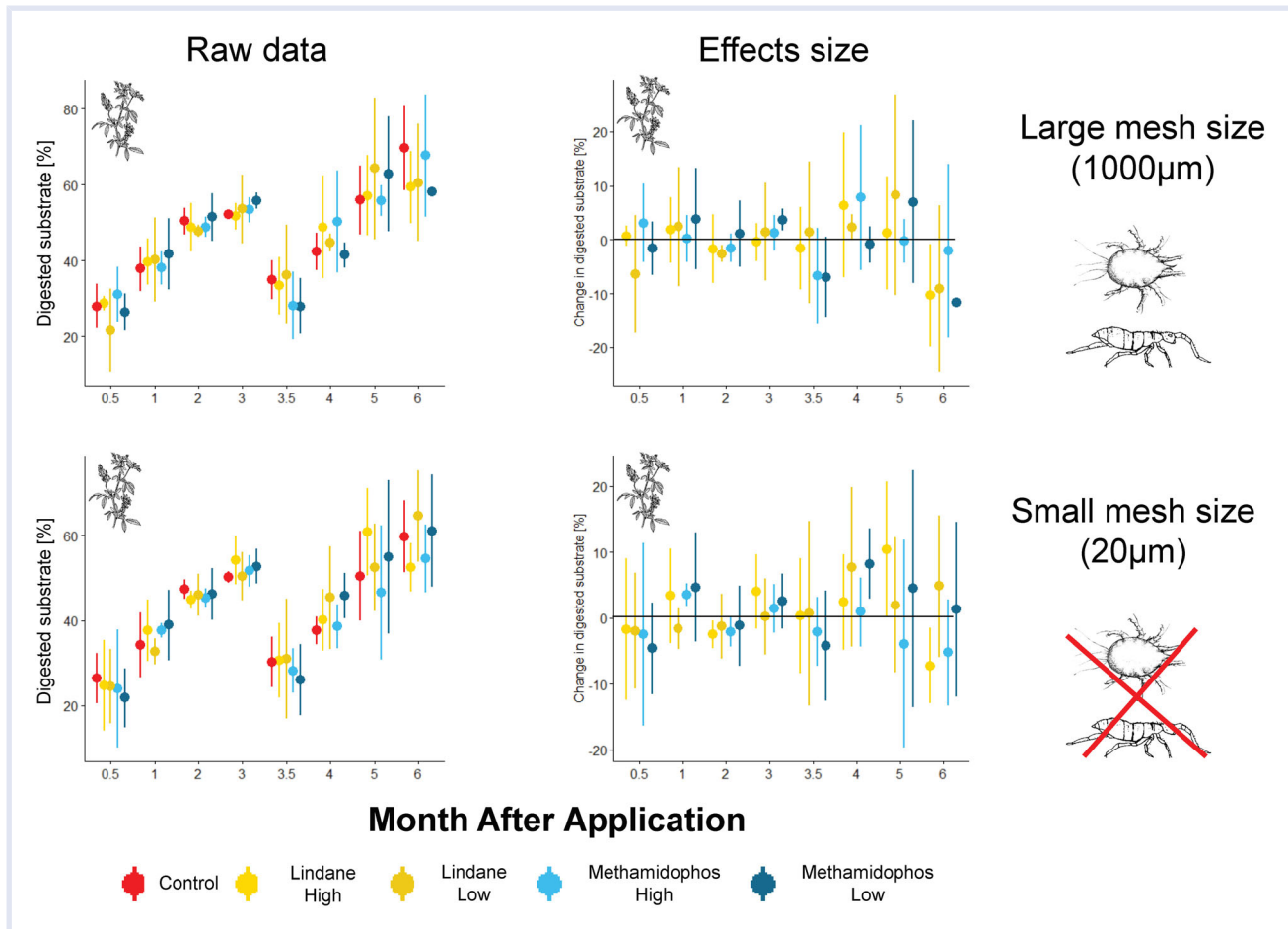
FIGURE 5 Results of the lucerne degradation experiment over time (month after application) in the presence (upper) and absence (lower) of mesofauna. We present the raw data (left) and relative changes compared with control (standardized effect sizes) over time for all insecticide treatments. We present mean values and associated 95% confidence intervals

community composition. In addition, as expected, (2) the more persistent compound (lindane) demonstrated longer lasting effects on abundance. However, dose dependence of mesofauna abundance was not observed on any sampling date. Similarly, a clear dose-dependent effect of the insecticides on diversity (pattern or magnitude) was not evident and most biodiversity proxies were not significantly different from the control six months after application. (3) By measuring organic matter degradation with mini-containers and bait-lamina in the presence and absence of mesofauna, we demonstrated that (1) mesofauna had an overall limited effect (in terms of magnitude) and no-significant effect on organic matter breakdown three and six months after application (see Figure S4); and (2) treatment effects on mesofauna abundance and diversity had a only a small (less than 10%) temporal isolated and dose-independent effect on organic matter degradation in mini-containers. We did not find any significant treatment effect on bait-lamina consumption.

Treatment effects on Acari and Collembola

Our results demonstrate that both insecticides had the intended effects of reducing *Collembola* and *Acari* abundances by up to 80% over the entire study duration. Findings confirmed our initial expectations of significant initial reductions of mesofauna abundance in all treatments with stronger and longer lasting effects of the lindane treatment and some indication of recovery of *Collembola* for both methamidophos application rates starting two months after the application (Figures 1, S2, 2, and S3). Abundances of *Acari* demonstrated stronger effects from insecticide treatments than *Collembola* (i.e., lindane). However, we did not always find clear dose dependence over the study duration (Figures 1, 2, S1, and S2). *Acari* abundance demonstrated a contrasting population development over time compared with *Collembola* in the control, with increasing numbers until three months after application followed by a reduction until the end of the study. *Acari* probably tolerated warmer soil climatic conditions over summer better than *Collembola*, which may have moved to deeper soil layers. The climatic conditions might have caused a shorter exposure duration to the chemical in the upper soil layer and so a smaller effect for *Collembola* than *Acari*.

For mesofauna diversity, the treatments did not result in a consistent reduction, but rather temporal fluctuations with sometimes a lasting increase in some diversity indicators (Figure 3). This demonstrated that the species richness of the mesofauna is surprisingly robust following insecticide
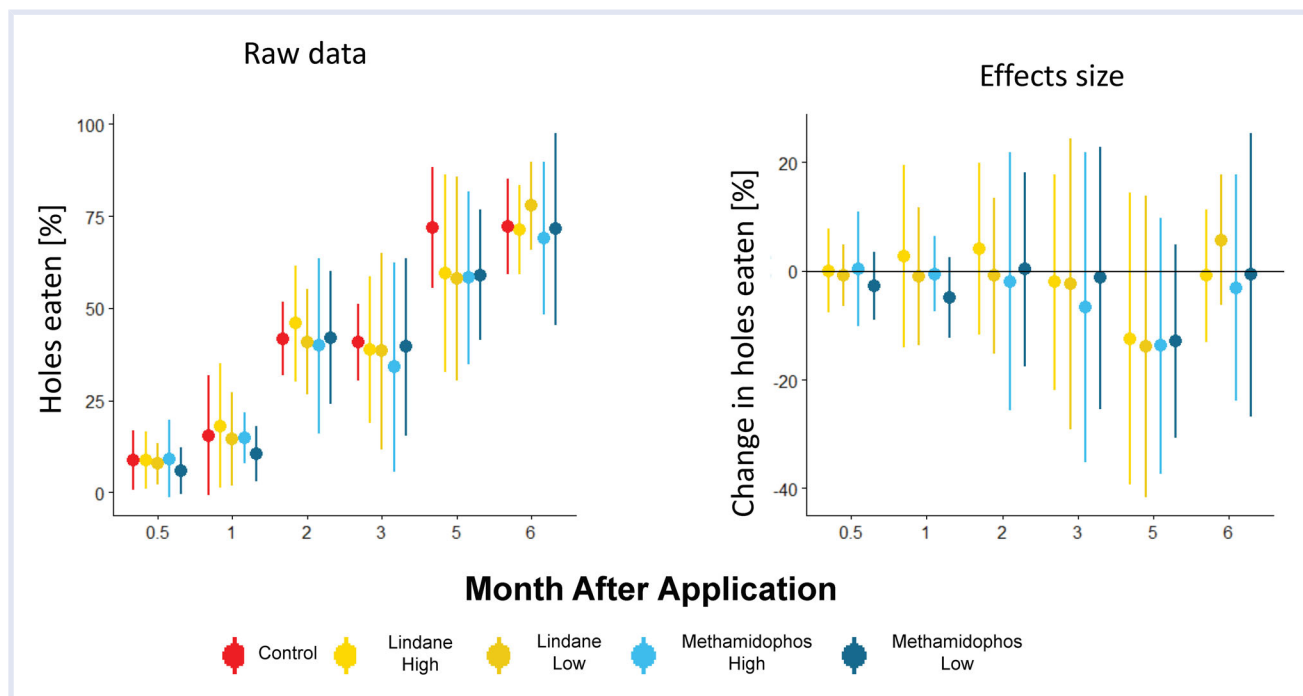
FIGURE 6 Results of bait-lamina experiment over time (month after application). We present the raw (left) and relative changes (standardized effect sizes) of holes eaten (in percent) over time for all insecticide treatments. We present mean values and associated 95% confidence intervals

exposure with only the high dose of the persistent lindane, resulting in clear adverse effects six months after application. In particular, *Collembola* communities responded with a temporal increase in the number of common and abundant species. This might indicate that the previously less common species had increased in number, reducing the skew of the common species distribution.

Litter decomposition

By directly comparing the decomposition rates in the presence (large mesh size) and absence (small mesh size) of mesofauna in untreated soils (control plots), we found that mesofauna presence had limited effects (less than 10%) on litter degradation of both substrates (see Figure S4). Although we did not directly quantify the presence of *Collembola* in the large mesh size treatment, Dunger et al. (2002) have demonstrated that *Collembola* species enter and feed mainly in the large mesh-sized mini-container (0.5 and 2 mm). Our findings are in line with earlier experiments in a variety of other nonagricultural habitats (Briones, 2014; Herletzius, 1983; Kampichler & Bruckner, 2009; Setälä et al., 2005), which provide evidence of the greater importance of soil microorganisms for the overall litter decomposition process.

When looking at all mesofauna relevant proxies for organic matter degradation (bait-lamina and mini-container with lucerne and cereal), we found only a few cases of significant differences (increase and decrease compared with the control), with effect size estimates exhibiting mostly less than a 10% difference and no clear dose dependence of effects (Figures 4–6). This suggests that mesofauna abundance and diversity may have only a limited role in organic

matter breakdown in arable soils and provides support for possible functional redundancy of the mesofauna at the species level (Setälä et al., 2005). Overall, our experiment supports the initial hypothesis that litter decomposition in arable soils is driven mainly by the microbial communities (Frouz et al., 2015; Heisler, 1994; Vreeken-Buijs & Brussaard, 1996; Zangerl et al., 2013) and does not validate the postulated role of microarthropods as key facilitators of the decomposition of recalcitrant litter in arable soils (Kampichler & Bruckner, 2009). Further comparative studies of the quantification of organic matter degradation by soil mesofauna and microbes are recommended to further understand their functional relevance in different agricultural soil ecosystems.

Implication for soil protection

Our results contribute to the discussion on the protection of soil functions in arable fields. To allow sustainable crop production, soil functionality must be protected even when using modern agricultural methodology including PPPs. This requires the preservation of functional soil food webs capable of nutrient cycling. Although this goal is undisputed, recent regulatory developments have moved away from utilizing direct estimates of functional soil parameters, such as degradation (e.g., "litterbag test"; see Guidance Document on Terrestrial Ecotoxicology; EC, 1991, 2002) and instead favor the use of indirect structurally (community) related endpoints (e.g., abundance of individual mesofauna species) that are generated during long-term, semi-field, or field studies (data requirements specified in EC, 2009). Although such studies can provide insights into the direct

community response to specific PPPs, there is no clear evidence that such structural (single morpho-species) endpoints are reliable indicators of soil functions, which need to be protected.

Although the litterbag was an established test system used to directly address functional soil endpoints, it has been criticized as being insensitive in detecting relevant mesofauna associated effects (EFSA, 2017). The mini-container test introduced by Eisenbeis (1994) and used in this study allows us to disentangle the contribution of different soil fauna size groups in organic matter degradation (see Figure S4). Our study reveals that the mini-container test can represent a flexible supplemental tool in the risk assessment of PPP, which could be used to directly address functional soil endpoints, such as decomposition by soil microorganisms and/or mesofauna, and their interactions, if needed.

Organic matter breakdown in arable soils is a complex process involving a wide range of organisms. Quantifying the contribution of soil mesofauna to organic matter degradation is vital to better understand and protect this essential ecosystem service. The observed limited influence of soil mesofauna on organic matter breakdown in this study might indicate a limited functional relevance of structural soil mesofauna endpoints in the risk assessment of PPPs in treated fields. Therefore, the suitability of structural endpoints in this regard might be considered questionable from a soil functional point of view and would add to the ongoing discussion on specific protection goals for the future soil risk assessment of PPPs (EFSA, 2017).

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CONFLICT OF INTEREST

The authors work for chemical companies, industry associations, or contract research institutes. The authors discuss the results of this original study in context of future pesticide regulation for soil organisms.

DATA AVAILABILITY STATEMENT

All data discussed in the manuscript are presented as figures. Raw data on abundances of single *Acari* and *Collembola* species, from which the diversity indices, total *Acari* and *Collembola* abundances, and effect sizes are calculated, are presented in the supporting information.

SUPPORTING INFORMATION

The supporting information contains figures that describe results in more detail as well as raw data on *Collembola* and *Acari* abundances of single species, which were used to calculate the diversity indices and the total abundances.

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