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Role of different size classes of organisms in cropped soils: what do litterbag experiments tell us? A meta-analysis.

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## Abstract

Soil organisms are essential for the functioning of agrosystems, especially in the process of litter decomposition. Litterbags constitute one common way to assess litter decomposition and to investigate the role of the different groups of soil organisms in the decay activity. However, there is currently no standardized litterbag protocol to measure the effects of soil organisms on litter decomposition. Furthermore, litterbag studies remain scarce in agrosystems and little information is available about the influence of different groups of soil organisms depending on agricultural practices. The development of cropping systems that rely on high soil biodiversity and fertility however requires a detailed understanding of these processes. In order to address this need and to have an overview of the existing protocols, we conducted a review on litterbag experiments in annually cropped soils. We collected information on the experimental design (e.g. duration, number of replicates), litterbags (e.g. size, mesh), climate, soil type, standing crop, enclosed litter (e.g. litter type, quality) and methods used to characterize organic matter decomposition and soil organisms. The effects of soil organisms of different size classes (meso- and macrofauna) was assessed with a meta-analysis performed on studies using litterbags of different mesh sizes. The general effect size of soil macrofauna, mesofauna (in addition to soil microorganisms) and of their combination was

assessed with a three-level random-effect model accounting for the random effect at the study level. This effect was compared for subgroups based on climate, soil, standing crop, agricultural system and litter type as categorical factors, and for depth of bags, duration of the experiment and size of litter pieces as continuous factors. Macrofauna, mesofauna and the combination of both were found to significantly increase litter decomposition. Surprisingly, meso- and macrofauna contributed equally to litter decomposition and their effects were not additive (when comparing the role of meso- and macrofauna independently and simultaneously). These effects tend to be influenced by various factors: climate, soil, standing crop and agricultural system for macrofauna; standing crop and litter type for mesofauna; and soil, standing crop and litter type for their combined effects. Multi-mesh litterbag experiments showed that even in soils with high disturbances, soil organisms of several size classes have a significant impact on organic matter decomposition. While this study showed that both soil macrofauna and mesofauna increased litter decomposition in annual cropping systems, there are still numerous gaps in our knowledge of the impacts of the agronomic (e.g. cropping system, practices, crop type) and environmental contexts (e.g. climates, soils). Forecasting future studies, we provide guidelines to develop a standard litterbag protocol adapted to the specificities of annually cropped soils.

Keywords: litterbag, litter decomposition, soil macrofauna, soil mesofauna, agrosystems, organic matter

## 1. Introduction

Organic matter plays a key role in soil functioning and in particular on soil fertility through the release of nutrients during decomposition (Tiessen et al., 1994; Carter, 2002). In cropping systems, organic matter comes from the restitution of crop residues to the soil, inputs of organic waste products (e.g. livestock manure or compost) or burial of cover crops. Its distribution in soil depends

on agricultural practices. In particular, soil tillage leads to a more or less homogenous soil-organic matter mixture over variable depths (Salinas-Garcia et al., 1997; Kay and VandenBygaart, 2002).

Soil organisms are essential drivers of the decomposition of organic matter. According to their size, microorganisms (< 0.1 mm), mesofauna (0.1 - 2 mm) and macrofauna (> 2 mm) (Swift et al., 1979) can act at different spatial and temporal scales. However, the contribution and context specific effects of different groups of soil organisms during decomposition is still not well known. In addition, the density and diversity of soil organisms are generally lower in agrosystems than in any other ecosystem (Tsiafouli et al., 2015; Joimel et al., 2017). Intensive management of cropping systems affects soil quality in the short and long term by modifying physical, chemical and biological soil characteristics, with the intensity of changes depending on pedoclimatic conditions and farming practices (Hati et al., 2007; Mazzoncini et al., 2010). These changes can strongly affect decomposition, thus threatening core ecosystem functions such as nutrient cycling (Bradford et al., 2002; Chen et al., 2020). A detailed understanding of the role of soil organisms during organic matter decomposition is fundamental to develop agroecology and to implement systems that will rely on soil biodiversity and fertility.

To address the gaps on the role of soil organisms on decomposition, numerous studies were conducted under laboratory conditions (Huhta, 2007). However, these experiments showed limitations as they can present lower diversity than in field conditions (Kardol et al., 2016). Consequently, the simulated biodiversity and modification of soil properties are not representative of the reality, which could be addressed by studies *in situ*.

Litterbags and teabags constitute one common way to assess decomposition *in situ* and over a long period of time (Didion et al., 2016). The litterbag technique was developed in the middle of the 19<sup>th</sup> century (Crossley and Hoglund, 1962; Edwards and Heath, 1963), while the teabag method was introduced recently (Keuskamp et al., 2013). The advantage of litterbags is that they contain organic matter that can be adapted to the context of the experiment. In addition, litterbags can be used to study the contribution of different groups of soil organisms to litter decomposition by selectively

79 excluding some fauna size classes. This selection can occur either by using different mesh sizes in  
80 order to physically exclude some organisms, or by a chemical treatment inhibiting part or all of the  
81 organisms. The exclusion of organisms by mesh size has sometimes been criticized due to a potential  
82 effect of different mesh sizes on decomposition (Anderson, 1973; Bradford et al., 2002), but this  
83 technique was approved as a reliable method to quantify the role of soil organisms in litter mass loss  
84 (Seastedt, 1984; Bokhorst and Wardle, 2013). However, contrary to the teabag method, no  
85 standardized method is currently available for one who wants to start a litterbag experiment. Yet  
86 litterbags mimic surface or buried crop residues in cropping systems and therefore allow looking at  
87 the main factors affecting their decomposition. Forecasting future studies on soil biodiversity in  
88 agricultural context, a standard protocol adapted to the specificities of annually cropped soils could  
89 be beneficial.

90 The litterbag method has been subjected to several reviews and meta-analyses, focusing on the  
91 history of the method (Huhta, 2007) and on the impacts of several factors on litter decomposition,  
92 namely soil fauna (Seastedt, 1984; Kampichler and Bruckner, 2009; Frouz et al., 2015; Zhang et al.,  
93 2015; González and Lodge, 2017; Frouz, 2018), climate and litter quality (García-Palacios et al., 2013;  
94 Zhang et al., 2015; Krishna and Mohan, 2017), nitrogen addition (Knorr et al., 2005) and pesticides  
95 (Knacker et al., 2003). In a first review, Seastedt (1984) estimated microarthropods to increase litter  
96 decomposition by 23%. The meta-analysis of Kampichler and Bruckner (2009) confirmed the positive  
97 effect of microarthropods over several land-use types, notably in agricultural soils. In a later meta-  
98 analysis, Frouz et al. (2015) found macrofauna to significantly increase litter removal but not litter  
99 mineralization. García-Palacios et al. (2013) and Zhang et al. (2015) estimated that exclusion of all  
100 fauna from litterbags, by physical or chemical methods, decreased litter decomposition by 27% and  
101 35% respectively. Recently, Huang et al. (2020) found that high diversity of earthworm functional  
102 groups increased litter and soil organic carbon decay in tree plantation and forests. In forest  
103 ecosystems, soil detritivores were reported to strongly increase leaf litter and wood debris  
104 decomposition, with macrofauna or mesofauna having a larger impact depending on the forest type

and climatic conditions (González and Lodge, 2017; Krishna and Mohan, 2017). Previous reviews were mostly focused on forest ecosystems or grasslands (González and Lodge, 2017; Krishna and Mohan, 2017), while studies on cropped soils remain scarce (Knacker et al., 2003; Kampichler and Bruckner, 2009). Furthermore, none of these reviews or meta-analyses investigated the comparative effect of different size classes of organisms. In this context, we provide here the first study to assess litter decomposition for several size classes of soil organisms in annually cropped soils.

The objectives of this study were (1) to assess the effects of different size classes of soil organisms on litter decomposition in annually cropped soils through the litterbag method and (2) to provide an overview of the existing protocols of the litterbag method in cropping systems. To this end, we conducted a meta-analysis of litterbag experiments in annually cropped soils. In particular, we aimed to quantify the effects of soil organisms of different size classes (meso- and macrofauna) in studies using bags of different mesh sizes.

## 2. Materials and methods

### 2.1 Data collection

Literature was surveyed in November 2019 for studies assessing the effect of soil organisms on litter decomposition with the litterbag method. We searched in all the Web of Science databases, with no restriction on the publication year, for papers containing the keywords “litter”, “decomposition, degradation, decay or breakdown”, “\*fauna, \*organism\*, animal\*, \*flora, \*arthropod\*, \*invertebrate\*, collembola\*, springtail\*, earthworm\*, lombric\*, fungi, bacter\* or nematod\*”, “\*bag\*” and “soil\*”. In addition, we deliberately excluded papers containing the words “bagasse”, “stream\*”, “marine” and “aquatic”. The search returned 1040 results. Further studies were added by checking articles included in previous reviews (Knacker et al., 2003; Kampichler and Bruckner, 2009; García-Palacios et al., 2013; Frouz et al., 2015; Zhang et al., 2015). In total, 1106 articles were

collected through the different search paths. Studies had to meet the following criteria to be selected:

- they should discuss the decomposition of organic matter or nutrient loss using the litterbag method and provide sufficient information about the protocol (e.g. mesh size) ;
- they should focus on agricultural soils, more precisely on annually cropped soils, but not on agroforestry, orchards, vineyards, grasslands, fallows, bare soils and field margins ;
- they should present data on organic matter decomposition (e.g. mass loss, decomposition rate), and not focus on a specific component only (e.g. lignin) ;
- they should use only physical methods based on different mesh sizes to exclude soil organisms from litterbags, and not chemicals (e.g. naphthalene) ;
- at least two different mesh sizes had to be used for the litterbag experiment ;
- the experiments should be conducted *in situ* ;
- the experiments should not assess the effect of plant protection products on litter decomposition ;
- the publication had to be written in English, French or German (which excluded mostly Chinese and Portuguese papers).

Finally, 22 studies fit all of the previous criteria. These 22 studies were used to conduct our review and the data extracted from 17 of the 22 studies were used for the meta-analysis (see the list in supplementary material). In the case of studies that took interest in several land-uses, the following analyses on study parameters and results focus only on the data related to cropland.

## 2.2 Data extraction

For the 22 selected studies, we recorded the parameters related to geographical location (country), climate, soil type, standing crop and cropping system. In order to facilitate comparisons, climate types were denominated according to the five groups defined by the Köppen climate classification:

tropical, dry, temperate, continental and polar; and soil types according to the FAO soil classes (World Reference Base for Soil Resources, 2014). When not given in the study, climate was deduced from the location of the study and by using regional climate maps, and soil types were collected in papers reporting experiments on the same sites.

We investigated the general design of the experiments: duration, number of sites and plots, number of replicate bags, number of sampling dates, sampling days, placement and depth of litterbags in soil. Information was also collected about the litterbags characteristics (bag size, mesh size, material, closure technique) and on the type of litter used in bags (litter quality, amount per bag, size of litter residues).

We collected data on litter decomposition. Twenty studies reported the decomposition as the percentage of remaining mass or initial mass loss in litterbags. Nine studies provided the decomposition rate, corresponding most of the time to the coefficient of the exponential model fitted on the data of remaining mass through time. If available, original data were extracted, otherwise they were obtained from graphics using the WebPlotDigitizer tool (Ankit Rohatgi, <https://automeris.io/WebPlotDigitizer>). If standard deviation, standard error or confidence interval were missing, we attributed the mean value of all standard deviations reported in studies selected for the same analysis. In addition, we summarized data on collected groups of soil organisms, sampling techniques and available measurements.

In order to gather decomposition data related to soil organisms of different size classes, we grouped mesh sizes used in the studies into three categories corresponding to the size classes of organisms that could enter bags. The fine mesh bags corresponded to bags with a mesh size lower than 0.1 mm, allowing access to litter to both microfauna (e.g. nematodes) and microflora (e.g. bacteria, fungi). The medium mesh bags had a mesh size comprised between 0.1 and 2 mm, allowing additional access to mesofauna which main representatives are microarthropods such as Collembola and Acari. Finally, the coarse mesh bags had a mesh size larger than 2 mm, thus enabling access to macrofauna organisms such as earthworms, carabids and other insects.



The role of the meso- and macrofauna in the decomposition of organic matter was determined from "paired observations" corresponding to the comparison between values of litterbag mass lost from two different mesh sizes (fine and medium, medium and coarse, or fine and coarse), studied under the same conditions. Each time point and each modality given in the studies provided individual paired observations with duration corresponding to the sampling time. Thus, several paired observations were reported for each study. Hereafter, we will distinguish "study" (i.e. publication) and "paired observation" (i.e. result obtained in a study) with  $n$  the number of paired observations included in the analysis.

## 2.3 Data analyses

The mass loss rate (MLR) was calculated for each mesh group as  $MLR = \%ML/t$ , where  $\%ML$  is the percent mass loss in litterbags at the time  $t$  (days). In addition, the fauna effect (FE) on litter mass loss was assessed by comparing the mass loss obtained in different bags such as  $FE = \%ML_1 - \%ML_2$  where  $\%ML_1$  and  $\%ML_2$  are the percentage of mass loss in larger (coarse or medium) and smaller (medium or fine) mesh bags respectively (adapted from Seastedt, 1984). Statistical tests were conducted to assess if differences in mass loss rate were significantly affected by factors such as climate, soil, crop, agricultural systems and litter types, and to assess if overall macrofauna and mesofauna effects on decomposition were significantly different. For this purpose, the normality and homogeneity of variances were tested with a Shapiro-Wilk test ( $\alpha > 0.05$ ) and a Bartlett test ( $\alpha > 0.05$ ) using R package *stats*. As normality was not met, a Generalized Linear Mixed-effect Model (GLMM) with a gamma distribution, a fixed effect for the studied factor and a random effect for study level was applied, followed by Tukey HSD post-hoc tests. In order to obtain only positive values, data on the fauna effect were transformed by adding to each value the maximum fauna effect. Then, ANOVA with a  $\chi^2$ -test was performed to compare models with and without the studied factor. The mass loss or fauna effect were influenced by the studied factor if the Akaike Information

Criterion (AIC) was significantly lower for the model with the factor than for the model without ( $P < 0.1$ ).

The role of the meso- and macrofauna in litter decomposition was assessed by comparing mass loss in bags of different mesh size groups. The first set of analyses investigated the effect of macrofauna comparing coarse and medium mesh bags (based on the results of 12 studies containing 201 paired observations), the second set was on the effect of mesofauna comparing medium and fine mesh bags (10 studies, 126 paired observations), and the last set on the effect of combined macro- and mesofauna comparing coarse and fine mesh bags (7 studies, 75 paired observations). Paired observations with a duration lower than 14 days were not included in the statistical analyses, but were represented on the graphical representation of litter mass loss and fauna effect. Litterbags including several groups of organisms were considered as “treatment” and bags excluding one or several groups included in the treatment as “control” (coarse vs medium, medium vs fine and coarse vs fine respectively). The effect size (ES), or standardized mean difference between treatment and control, and the 95% confidence interval (CI) were computed for individual paired observations and overall results using the Hedges’g calculation (Hedges and Olkin, 1985). A three-level random-effect model was applied, with random effects at the paired observation and at the study level. This allows to account for the lack of independence of paired observations from the same study, considering similar modalities but differing in sampling time. In addition, the heterogeneity between studies was calculated with the  $I^2$  (Higgins and Thompson, 2002). Heterogeneity above zero reveals that the variability in the observed effect size is larger than one expected based on sampling variability. This may be explained by differences between studies according to one or several factors (e.g. climate, soil type), meaning that different subgroups of studies will have a significantly different mean effect size.

The role of different soil organisms in the decomposition of litter was compared for different categorical factors (climate, soil, crop, agricultural systems and litter type) and for continuous factors (depth of bags, duration of the experiment and size of litter pieces) considered as moderators to

conduct a subgroup analysis. The effect of moderators was assessed with the Cochran Q-statistic and a  $\chi^2$ -test was performed to test its significance. A significant Q-statistic indicated that the moderator influenced the effect size ( $P < 0.05$ ) and thus that part of the total heterogeneity could be explained by this difference. Except for climate, we only presented results for subgroups with more than 10 paired observations.

These analyses were performed using R software version 3.6.3 (R Development Core Team 2020) and the *lme4* (Bates et al., 2015), *multcomp* (Hothorn et al., 2008), *metafor* (Viechtbauer, 2010) and *dmetar* packages (Harrer et al., 2019).

### 3. Results

#### 3.1 General characteristics of studies

The 22 selected studies were located all around the world, mostly across Europe (36%) but also in Asia (18%), South America (18%), North America (14%) and Africa (14%) (Table 1). In relation with their location, 54% of the studies occurred under temperate climate, and few studies under tropical (18%), continental (18%) and dry (9%) climates (Table 1). The soil type was highly variable. Several studies were conducted on Luvisol (24%) and Cambisol (14%) which are favorable to agriculture. The others occurred in nine different soil types (e.g. Ferralsol, Fluvisol, Lixisol, Kastanozem) (Table 1).

Most of the studies were conducted on annual crops under conventional agriculture (54%) and no-tillage agriculture (32%) (Fig. 1a; Table 1). Only two studies were conducted under organic agriculture, one on minimum tillage and one on relay cropping. The most common crops at the time of the study were maize (32%), barley (23%), soybean (18%) and wheat (14%) (Fig. 1b).

Collected studies had different objectives with some investigating the effect of climate (e.g. Peña-Peña and Irmler, 2018; Yin et al., 2019), land-use (e.g. Tian et al., 1998; Yang et al., 2018) or litter type (e.g. Ouédraogo et al., 2004; Carlesso et al., 2019). They also focused on various agricultural

practices, mainly tillage (e.g. House and Stinner, 1987; Domínguez et al., 2010), but also fertilization (Weil and Kroontje, 1979), conventional vs organic management (Domínguez et al., 2014; Yin et al., 2019) and genetically modified vs non-modified crops (Zwahlen et al., 2007). Finally, some authors also used litterbags to assess the decomposition activity of specific organisms (e.g. Jensen, 1985; Vreeken-Buijs and Brussaard, 1996).

### 3.2 General design of litterbags experiments

Studies were mostly conducted on a single agricultural site (82%), while studies on several sites were scarce (18%, e.g. Domínguez et al., 2010; Peña-Peña and Irmeler, 2016) (Table 1). The number of plots ranged from one to 60 and plot size varied from 0.5 x 0.5 m to 120 x 30 m. However, studies with only one plot comprised 3 to 100 subplots within this plot, which we considered as plots.

Duration of experiments ranged from 27 to 392 days, being on average 206 days (Table 1). The number of sampling dates ranged from one to 10 (Fig. 1c). Litterbags were mainly sampled between 5 and 120 days after their addition into soil, but in some studies they were sampled after more than 300 days (Fig. 1d). Seven studies considered annual or seasonal dynamics and in this case, experiments lasted up to three years. Three types of sampling schemes were identified namely short studies with few sampling dates, long studies with few or many sampling dates.

The number of replicate bags per modality ranged from 3 to 30 (Table 1). Bags were placed on the surface (32%) or buried in soil between 3 and 35 cm (50%); however information on depth was missing in several studies (18%) (Table 1). Sometimes bags were placed on the surface and then buried at the time of tillage (e.g. Jensen, 1985; Domínguez et al., 2014).

### 3.3 Litterbag characteristics

Within the selected studies, 59% used litterbags with two mesh sizes (27% medium + coarse; 18% fine + medium; 14% fine + coarse), 32% with three (23% fine + medium + coarse; 4% fine + two medium; 4% coarse + two medium) and 9% with four mesh sizes (fine + coarse + two medium). The mesh size ranged from 0.003 to 10 mm (Fig. 1e). Fine mesh ranged from 0.003 to 0.08 mm, medium mesh from 0.1 to 2 mm, and coarse mesh from 4 to 10 mm. The most common mesh sizes for each category were 0.02 mm (54%), 1 mm (29%) and 5 mm (31%).

The main material to construct litterbags was nylon or another synthetic polyester. Some studies however used stainless steel or brass in order to prevent bags from being decomposed by termites (tropical climate). The size of bags was mainly comprised between 10 x 10 cm and 20 x 20 cm (Table 1). The closure of bags was only discussed in seven publications. Bags were either sewn or glued.

Some authors improved the technique to limit litter losses in soil, after sampling and during transportation. For instance, Yang et al. (2018) sewed 0.01 mm mesh patches to the bottom of their litterbags. Jensen (1985) and Carlesso et al. (2019) used transportation bags or boxes, but they showed that mass loss during transportation was not significant in regards of the total mass loss. Tian et al. (1992, 1998) placed pieces of wood inside litterbags to avoid litter compression. Finally, to rewet the litter and standardize the initial microflora in all litterbags, Vreeken-Buijs and Brussaard (1996) soaked all the litterbags in diluted filtered soil extract one night prior their incorporation into soil.

### 3.4 Litter characteristics

Litter consisted of straw or leaves (Table 1). The most commonly used materials were C3 cereals (50%) such as wheat (27%) or rice (9%), C4 cereals (32%) such as maize (23%) or sorghum (9%), Fabaceae (27%) including crops (clover, vetch, alfalfa) and shrubs leaves (*Gliricidia sepium* (Jacq.) Steud., *Leucaena leucocephala* (Lam.) de Wit, *Senna siamea* (Lam.) H.S.Irwin & Barneby), and grasses

(14%) (Fig. 1f). Tree leaves (poplar, *Acioa barteri* (Hook.fil. ex Oliv.) Engl.), Brassicaceae (rapeseed, mustard) and manure were also used.

The C:N ratio of the litter, specified in six studies, ranged between 8 and 153. The C:N ratio was often the highest for C4 cereals (13 to 72), and remains high for C3 cereals (12 to 77). Comparatively, Fabaceae presented lower C:N ratios (9 to 57). However, C:N ratio was highly variable between studies for the same litter.

The amount of litter per bag ranged from 0.5 to 100 g, depending on the size of bags and the litter used. In some works, the size of residues was standardized by cutting litter into pieces of 1 to 10 cm length. Prior to introduction in bags, litter was often air or oven-dried between 20 °C (Valckx et al., 2011) and 105 °C (Carlesso et al., 2019).

### 3.5 Litter decomposition

The litterbags, once collected, were either brushed or rinsed under water to remove soil particles, roots, plant debris other than litter, and sometimes fauna. The bags were then dried between 20 and 105 °C, and weighed. In some cases, the litter was removed from the bags before being weighed. In nine studies, litter was burnt between 500 and 800 °C in order to measure ash free dry weight. Some studies performed additional analyses (e.g. C, N contents) to characterize the litter after biodegradation.

Enclosed litter lost more than half of its initial mass after a year or less in 57%, 46% and 23% of the coarse (n = 199 observations), medium (n = 254) and fine (n = 128) litterbags respectively (Fig. 2).

Mass loss rate was significantly influenced by climate in medium mesh bags only, and by soil and crop type in all mesh bags (Table S1). Agricultural systems did not significantly influence mass loss rate (Table S1). In medium mesh bags, mass loss was faster under tropical and continental climates than under dry ( $P < 0.001$ ) and temperate climates ( $P < 0.05$ ), and faster under temperate than under dry climate ( $P < 0.001$ ) (Fig. 2a-c). In addition, mass loss was faster in Acrisol than in Luvisol for all

mesh bags (coarse and fine :  $P < 0.001$ , medium:  $P < 0.05$ ), in Cambisol than in Luvisol in coarse ( $P < 0.001$ ) and fine mesh bags ( $P < 0.001$ ), and in Lixisol than in Luvisol in coarse ( $P < 0.001$ ) and medium mesh bags ( $P < 0.01$ ). Furthermore, mass loss was faster in fields cropped with C4 cereals than with C3 cereals for medium ( $P < 0.05$ ) and fine mesh bags ( $P < 0.001$ ).

Litter decomposition was also significantly dependent on litter type, regardless of the mesh size (Fig. 2d-e, Table S1). The degradation of C3 cereals was significantly slower than that of C4 cereals in medium ( $P < 0.05$ ) and fine bags ( $P < 0.001$ ), and slower than that of Fabaceae in all bags (coarse and medium:  $P < 0.05$ , fine:  $P < 0.001$ ). More precisely, wheat decomposed significantly faster than maize in all bags (coarse, medium, fine:  $P < 0.01$ ). It is noteworthy that C3 cereals, except for rice, were always used under temperate climate. In contrast, C4 cereals, especially maize, were used under all climate types.

### 3.6 Effect of soil organisms on litter decomposition

In parallel to monitoring litter decomposition, the fauna was in some cases extracted from the soil (27%), from the litterbags (27%) or both (9%), using MacFayden or Berlese extractors for mesofauna (50%) and hand sorting for macrofauna (45%). Microorganisms were studied in 18% of the studies. Seven studies considered two size classes and only one the three size classes (Miura et al., 2008). The most studied groups of soil fauna were Collembola (45%), Acari (36%), Lumbricidae (32%), Araneae (18%), Enchytraeidae (14%) and Nematoda (14%) (Table 1). However, some studies also took interest at Coleoptera, Isoptera, Myriapoda (Chilopoda, Diplopoda, Symphyla, Pauropoda), Crustacea (Isopoda), Annelida, Dermaptera, Diptera, Hymenoptera (Fomicidae), Lepidoptera, Orthoptera, Psocoptera, and larvae (Coleoptera, Diptera) (Table 1). Most of the studies assessed organism's density or biomass, while diversity (richness, diversity indexes) was only discussed in five studies. Microbial activity was characterized *via* respiration ( $\text{CO}_2$  measurements) or substrate-induced respiration. Only one study focused on functional groups, gathering together microarthropods

according to their feeding habits (Vreeken-Buijs and Brussaard, 1996). Finally, six studies assessed the “fauna effect” as the difference of mass loss between bags including and excluding soil fauna.

### 3.6.1 Role of macrofauna

Soil macrofauna had a significant positive effect on the decomposition of organic matter in litterbags with a mean fauna effect of 9.4% (Fig. 3a, d; range = [-20.3%, 83.5%]) and a mean effect size of 0.66 (Figs. 4a and S1; 95% CI = [0.37; 0.96],  $P < 0.0001$ ,  $n = 201$  paired observations). A moderate heterogeneity between studies was observed ( $I^2 = 72.3\%$ ). A negative mean fauna effect occurred in 22.2% of the paired observations (Fig. 3a, d).

The effect of macrofauna on litter decomposition was dependent on the climate ( $P < 0.0001$ ), soil ( $P < 0.0001$ ), crop types ( $P < 0.0001$ ) and agricultural systems ( $P = 0.0003$ ) (Fig. 4b, Table S2). Macrofauna effect on decomposition was the highest under dry ( $ES = 3.12$ ;  $n = 36$  paired observations) and continental ( $1.40$ ;  $n = 8$  from only 1 study) climates (Fig. 4b), being especially high during the early stages of decomposition and then decreasing with time (Fig. 3a). In comparison, it was lower under tropical ( $0.28$ ;  $n = 40$  from 1 study) and temperate ( $0.23$ ;  $n = 117$ ) climates (Fig. 4b), for which it remained quite steady over time (Fig. 3a). Furthermore, the macrofauna played a particularly important role for the litter degradation in Cambisols ( $4.19$ ;  $n = 26$ ), and had a lower but significant effect in Nitisols ( $0.70$ ;  $n = 12$  from 1 study), Luvisols ( $0.53$ ;  $n = 25$ ), Gleysols ( $0.43$ ;  $n = 30$  from 1 study) and Lixisols ( $0.31$ ;  $n = 58$ ) (Fig. 4b). It was not significant in Kastanozem soils ( $0.14$ ;  $n = 22$ ) (Fig. 4b). Conversely, the effect size was significantly negative in Acrisols ( $-0.26$ ;  $n = 24$  from 1 study). The effect of macrofauna also varied with the standing crop, with a maximum effect size observed in sorghum fields ( $3.12$ ;  $n = 36$ ) followed by wheat ( $0.82$ ;  $n = 10$ ), barley ( $0.49$ ;  $n = 53$ ) and soybean fields ( $0.49$ ;  $n = 10$ ). It was not significant under maize fields ( $0.10$ ;  $n = 82$ ) (Fig. 4b). However, it was difficult to discriminate the effects of dry climate and sorghum cultivation as they co-occurred in the same studies (Reddy et al., 1994; Ouédraogo et al., 2004). Finally, macrofauna



demonstrated a significant positive effect on decomposition under conventional systems (0.53;  $n = 77$ ), and a rather low and not significant effect under no-tillage systems (-0.04;  $n = 50$ ) (Fig. 4b). The type of litter did not significantly influenced the effect of macrofauna ( $P = 0.11$ ) (Fig. 4b, Table S2). However, the macrofauna was observed to play a very important role in the degradation of C4 residues such as maize (1.41;  $n = 34$ ) (Fig. 4b), especially in the early stages of degradation (Fig. 3d). Its role was less important and later for C3 residues such as wheat (0.42;  $n = 50$ ) and rice (0.42;  $n = 26$ ) (Fig. 4b). It was not significant for local residues (0.16;  $n = 11$ ) (Fig. 4b). The macrofauna effect fluctuated for Fabaceae and tree leaves for which it was sometimes positive or negative (Fig. 3d). The depth of bags had a positive significant influence on the effect of macrofauna ( $P < 0.0001$ ), but not the duration of the incubation in field ( $P = 0.14$ ) or the size of the litter pieces ( $P = 0.87$ ) (Table S3).

### 3.6.2 Role of mesofauna

Soil mesofauna had a significant positive influence on the decomposition of organic matter in litterbags with a mean fauna effect of 6.6% (Fig. 3b, e; range = [-7.5%, 48.7%]) and a mean effect size of 0.31 (Figs. 5a and S2; 95% CI = [0.11; 0.52],  $P = 0.003$ ,  $n = 126$  paired observations). The heterogeneity was low ( $I^2 = 40.2\%$ ). A negative mean fauna effect appeared in 29.5% of the paired observations (Fig. 3b, e).

The effect of mesofauna was significantly influenced by the crop types ( $P = 0.003$ ), while no significant difference was found between different climates ( $P = 0.99$ ), soils ( $P = 0.57$ ) or agricultural systems ( $P = 0.06$ ) (Fig. 5b, Table S2). Mesofauna effect on decomposition was significantly positive under temperate climate only (ES = 0.31,  $n = 110$  paired observations), but not under continental (0.49,  $n = 8$ ) and tropical climates (0.36,  $n = 8$ ) for which it increased with time (Fig. 3b). The lack of difference for the climate subgroup could be explained as almost all the experiments were conducted under temperate climate ( $n = 110$ ). Moreover, mesofauna had a non-significant positive

effect on decomposition in Fluvisol (0.67; n = 14), Cambisols (0.59; n = 20), Luvisols (0.42; n = 48) and Nitisol (0.33, n = 12), and a non-significant negative effect in Acrisols (-0.01; n = 24 from 1 study) (Fig. 5b). In addition, mesofauna played an important role on the litter decomposition in wheat fields (1.35; n = 14) compared to rapeseed (0.64; n = 12 from 1 study), maize (0.18; n = 45), barley (0.09; n = 33) and sugar beet fields (0.05; n = 10 from 1 study) for which its effect was not significant (Fig. 5b). Finally, a significantly positive mesofauna effect on decomposition was observed under conventional systems only (0.14; n = 49).

Mesofauna effect on decomposition was also dependent on the litter type ( $P = 0.03$ ) (Fig. 5b, Table S2). The mesofauna particularly increased the decomposition of C4 cereals such as maize (0.65; n = 34) (Fig. 5b), especially at the early stage of decomposition (Fig. 3e). Comparatively, it did not significantly increase the degradation of C3 cereals such as wheat (0.09; n = 43) (Fig. 5b), with low or negative effect, only increasing at the later stage of the decomposition (Fig. 3e). Finally, the mesofauna effect tended to increase with time for tree leaves (Fig. 3e) and fluctuated for Fabaceae (Fig. 5b).

The duration of the experiment significantly influenced the effect of mesofauna on decomposition ( $P = 0.002$ ), but not the depth of litterbags ( $P = 0.98$ ) or the size of litter pieces ( $P = 0.71$ ) (Table S3).

### 3.6.3 Combined impact of both macro- and mesofauna

Soil macro- and mesofauna together contributed positively and significantly to the decomposition of litter compared to microorganisms only. The mean fauna effect of combined macro- and mesofauna on litter decomposition was 10.8% (Fig. 3c, f; range = [-15.3%, 85.9%]) and the mean effect size was 0.75 (Figs. 6a and S3; 95% CI = [0.34; 1.17],  $P = 0.0004$ , n = 75 paired observations) (Fig. 6a). A moderate heterogeneity was observed ( $I^2 = 73.75\%$ ). The mean fauna effect was negative in 18.5% of the paired observations (Fig. 3c, f). The cumulative effect was lower than the effect obtained by the addition of the macrofauna and the mesofauna effects that reached 16.0%. In addition, it should be

noted that the individual fauna effects of macrofauna and mesofauna were not significantly different ( $P = 0.36$ ).

The fauna effect on decomposition was dependent on soil ( $P = 0.002$ ) and crop type ( $P = 0.01$ ), but not on climate ( $P = 0.10$ ) or agricultural system ( $P = 0.32$ ) (Fig. 6b, Table S2). Soil fauna played a very important role in the litter decomposition under continental climate ( $ES = 2.04$ ;  $n = 8$  paired observations from 1 study) (Fig. 6b), especially at the early stages of decomposition (Fig. 3c). It was also important under tropical climate ( $1.19$ ;  $n = 20$  from 1 study) (Fig. 6b), for which it increased with time (Fig. 3c). It tends to be lower under temperate climate ( $0.47$ ;  $n = 47$ ) (Fig. 6b) and seemed quite steady over time (Fig. 3c). In addition, soil fauna provided a significant increase in litter decomposition in Ferralsols ( $1.19$ ;  $n = 20$  from 1 study), and to a lesser extent in Luvisols ( $0.45$ ;  $n = 25$ ) (Fig. 6b). Contrastingly, soil fauna effect was negative but non-significant in Acrisols ( $-0.29$ ;  $n = 12$  from 1 study) (Fig. 6b). Nevertheless, it was not possible to discriminate the effects of tropical climate and Ferralsol soil type as they co-occurred in one study (Peña-Peña and Irmeler, 2016). Furthermore, soil fauna positively influenced litter decomposition in soybean ( $1.88$ ;  $n = 10$  from 1 study) and barley fields ( $0.63$ ;  $n = 33$ ), and not significantly in maize fields ( $0.33$ ;  $n = 28$ ) (Fig. 6b).

The implication of soil fauna also depended on the litter type ( $P = 0.03$ ) (Fig. 6b, Table S2). Following the trends separately observed for macro- and mesofauna, soil fauna was highly involved in the degradation of C4 cereals such as maize ( $1.35$ ;  $n = 24$ ) (Fig. 6b), particularly at the early stage of decomposition (Fig. 3f). In addition, it demonstrated a positive but non-significant effect on Fabaceae such as soybean ( $1.21$ ;  $n = 10$ ) (Fig. 6b), slightly increasing with time (Fig. 3f). This effect was lower and not significant for C3 crops such as wheat ( $0.26$ ;  $n = 20$  from 1 study) (Fig. 6b), for which it was negative at the early stage of decomposition and increased at the later stages (Fig. 3f). Finally, fauna effect tended to increase with time for the decomposition of tree leaves (Fig. 3f).

The duration of the experiment ( $P = 0.01$ ) had a significant positive influence on fauna effect, such as the size of litter pieces ( $P < 0.0001$ ) which indicates that large litter pieces required more

intervention of fauna than small litter pieces. No significant relations were found with the depth of litterbags ( $P = 0.64$ ) (Table S3).

## 4. Discussion

### 4.1 Role of soil organisms in litter decomposition

The role of the soil organisms on litter decomposition in cropping systems was confirmed as our meta-analysis demonstrated a significant positive effect of macrofauna ( $FE = 9.4\%$ ,  $ES = 0.66$ ), mesofauna ( $FE = 6.6\%$ ,  $ES = 0.31$ ) and combined macro- and mesofauna ( $FE = 10.8\%$ ,  $ES = 0.75$ ). These results are consistent with previous reviews and meta-analyses assessing the role of macrofauna (Frouz et al., 2015), mesofauna (Seastedt, 1984; Kampichler and Bruckner, 2009) and total soil fauna (Zhang et al., 2015) on litter decomposition.

Macrofauna and mesofauna effects on decomposition were not significantly different in annually cropped soils. Similarly, in forest soils the overall effects of macrofauna and mesofauna were not reported to differ, but to vary in different ecosystems (González and Lodge, 2017; Krishna and Mohan, 2017). The balance between macrofauna and mesofauna effects could be due to the distinct roles of the main macrofauna and mesofauna organisms and to the variation in taxa within both groups.

Macrofauna organisms are highly efficient for the burrowing, breakdown and digestion of litter, thus displacing a large amount of litter. After digestion most of the organic matter is returned to the soil in the form of feces (Frouz, 2018). However, the assimilation efficiency varies greatly among the different groups of macrofauna (Frouz, 2018). In the collected studies, earthworms were very often the main macro-decomposers. Earthworms are essential for the mixing of litter in soils and for its transformation through comminution (Barrios, 2007). They consume organic matter together with soil, which results in the creation of microaggregates in their feces with a reduced decomposition

rate of organic matter compared to the initial litter (Frouz, 2018). Under dry climates, the high macrofauna effect was mostly attributed to termites that are reported to be one of the main actors of litter decomposition in arid areas (Martínez-Yrízar et al., 2007). Termites have a high assimilation efficiency allowing them to convert a great proportion of the ingested litter into their own biomass (Culliney, 2013), thus releasing less organic matter directly after the digestion of litter.

Mesofauna effect on decomposition is, on the other hand, mostly indirect and attributable to microarthropods such as Collembola and Acari (Culliney, 2013). They take part in decomposition through the digestion and the breakdown of litter, but their effect on decomposition is mostly due to the regulation of microbial populations by grazing (Seastedt, 1984). Indeed, oribatid mites and Collembola have mouthparts adapted to the fragmentation of organic matter that enable them to feed on the adhering microorganisms (Seastedt, 1984). In addition, predatory microarthropods feeding on bacterial and fungal nematodes can also protect microbial populations and stimulate litter decomposition (Santos et al., 1981). The regulation of microorganisms by microarthropods was suggested to ensure continuous and regulated supply of nutrients to plants and thus to prevent nutrient loss (Culliney, 2013).

Despite the observed mean positive effect, several studies related a negative effect of soil fauna on decomposition (e.g. House and Stinner, 1987; Heisler, 1994). Those studies were not sharing specific characteristics. A main driver of the effects of macrofauna and mesofauna could be the balance between predatory and prey organisms within these two groups. Previous studies demonstrated that in sites dominated by predaceous macrofauna the litter mass loss in coarse bags decreased due to the predation on mesofauna prey (Frouz et al., 2015). In addition, the positive effect of mesofauna on decomposition may be counterbalanced by an over-grazing of microorganisms by microarthropods (Beare et al., 1992; Heisler, 1994), which could conduct to null or negative total observed effect on decomposition.

Furthermore, the effects of macrofauna and mesofauna were not additive as the mean fauna effect, combining macrofauna and mesofauna, was lower than the addition of the two effects. The non-

additive aspect of the fauna effects on decomposition could be explained by the existence of facilitative (e.g. resource use complementarity) and inhibitory (e.g. competition, predation) interactions between soil organisms (Heemsbergen et al., 2004). These interactions are assumed to explain why the combined effect of soil organisms on litter decomposition cannot be predicted with the simple sum of their individual effects (Coulis et al., 2015).

Litter decomposition was increasingly faster in fine, medium and coarse mesh litterbags respectively, which may indicate an effect of the inclusion of a larger group of soil fauna. However, the protocols set up in the selected studies allow studying the role of macrofauna and mesofauna in litter decomposition, but they do not allow discussing the role of microorganisms, which are present in all litterbags. Still, more than aiming to estimate the individual effect of soil microorganisms, the focus of future studies should be set on the interactions between soil organisms and the parameters that promote microbial activity.

## 4.2 Factors explaining the variability of the effect of soil fauna

Litter mass loss and fauna effect on decomposition were both influenced by several factors. This implies that the studied factors had direct effects on litter decomposition, but also indirect effects through their impact on soil organisms. In the following section, we will discuss the influence of climate, soil, agricultural systems, crop and litter type on the fauna effect on litter decomposition. We aim here to illustrate the trends that are observed within the meta-analysis, even though more studies would be required to conclude on the impact of these factors. In addition, one should keep in mind the existence of various interactions among studied factors (e.g. climate and soil).

### 4.2.1 Climate

Overall, macrofauna, mesofauna and total fauna effects tend to be higher under dry and continental climates, and were lower under temperate climate (Figs. 4-6). Climate and more specifically temperatures and precipitations are important drivers of litter decomposition (Knacker et al., 2003; Zhang et al., 2015) by influencing the decomposition activity of soil organisms (García-Palacios et al., 2013; Zhang et al., 2015). Litter decomposition tends to be optimum for intermediate humidity and temperature; however, the optimum will depend on the local decomposer communities and on the presence of adapted species (Knacker et al., 2003; Peña-Peña and Irmeler, 2016). For instance, Peña-Peña and Irmeler (2016) found a much higher fauna effect on decomposition in cropland during the wet season than during the dry season. Contrastingly, Kampichler and Bruckner (2009) concluded to a significantly higher effect of microarthropods in arid areas than in wet tropics, all land-uses together. In a two-year experiment, Yin et al. (2019) also demonstrated that a slight increase in temperature and change in precipitation patterns was enough to decrease the effect of soil fauna on litter decomposition.

#### 4.2.2 Soil type

The soil type influenced litter mass loss in bags of all sizes as well as the macrofauna (Fig. 4) and the combined macro- and mesofauna effects on decomposition (Fig. 6), but not the mesofauna effect (Fig. 5). The observed positive fauna effect on decomposition in Luvisols and Cambisols may be explained as they are fertile soils highly adapted to agricultural use and that do not present any particular constraints for soil organisms. Furthermore, the high variability in the fauna effect in Cambisols is explained as Cambisols are considered as “non-differentiated” soils that have heterogeneous properties. As the most represented soil types, Luvisols and Cambisols occurred in combination with different climates but it was not possible to compare their effect under similar climate. Moreover, the literature on the influence of different soil types on soil organism’s communities in agricultural soils is scarce (Girvan et al., 2003; Ivask et al., 2008; Khan and

Joergensen, 2012; Zaller et al., 2014). In addition, the few existing studies did not look at the effect of soil types on the functions performed by soil organisms. Thus, further studies are required to unveil the mechanisms causing soil types to influence the role of different soil organisms in decomposition. Furthermore, soil characteristics, rather than soil types only, are required to understand the impact of different soils on organism's decomposition activity.

#### 4.2.3 Crop

Crops cultivated on the field at the time of litterbag experiments influenced the litter mass loss in all bags and the fauna effects on decomposition (Figs. 4-6), with a significant positive effect in fields with wheat and barley (except for barley with mesofauna), and lower and non-significant effect in fields with maize. Few studies have been conducted on the effect of crop type on soil biodiversity. They demonstrated crop type influence on soil organism community composition, abundance and diversity (Eyre et al., 2012; Lüscher et al., 2014; Crotty et al., 2015; Detheridge et al., 2016; Villenave et al., 2018). Crop types were notably found to influence the abundance of predators in fields such as spiders (Lüscher et al., 2014) and of bacterivorous nematodes (Villenave et al., 2018). Crops present specificities such as plant structure, roots distribution or root exudates that may favor different organisms. In addition, crops are associated with different practices such as the frequent use of irrigation for maize crops or the relatively low need for chemicals input in wheat fields. Besides, crops previously cultivated on the field were found to influence soil organisms even after the establishment of a new crop (Crotty et al., 2016). The numerous differences between crop type and their management make difficult to explain the specific effect of crops on the activity of decomposers. Further studies are required on this point.

#### 4.2.4 Litter



The nature of organic matter added in litterbags greatly influenced the mass loss rate in litterbags of all mesh sizes. It also influenced the effects of mesofauna (Fig. 5) and combined macro- and mesofauna (Fig. 6). Macro- and mesofauna considerably increased the degradation of C4 cereals litter, especially at the early stage of decomposition. Contrastingly, they demonstrated low or no influence on the decomposition of C3 crops such as wheat. Their influence on Fabaceae and other crops was more variable and fluctuated with time. The limited availability of data on litter composition in the selected papers prevented us to observe if there was a relation between litter characteristics (e.g. percentages of soluble and more recalcitrant C, phosphorus, C:N ratio) and decomposition. In our study, we observed a higher fauna effect for maize than for wheat litter regardless of the climate and the soil type. It was surprising as maize tends to have a high C:N ratio in the collected studies, probably because of its high fiber and lignin content (Sereda et al., 2015). Whereas in previous studies, litters with low C:N ratio in cropping systems were sometimes found to decompose faster (Vazquez et al., 2003) and to present higher fauna effect than litter with high C:N ratio (Zhang et al., 2015; Peña-Peña and Irmeler, 2018).

Another potential explanation for the higher fauna effect on maize litter could be the home-field advantage effect. Indeed, there is increasing evidence that litter decomposes faster beneath the plant from which it was derived, the so-called home-field advantage effect, because of the adaptation of the local soil community to the standing plant species, either by adapted activities, changes in community composition or a combination of several processes (Milcu and Manning, 2011). The home-field advantage effect increased litter decomposition rate by 7.5% for local litter according to Veen et al. (2015). In addition, the home-field advantage effect was demonstrated to be higher for more recalcitrant litter as specialized decomposer communities are required to degrade it rapidly (Milcu and Manning, 2011). In our meta-analysis, half of the experiments using maize litter were conducted under maize fields, thus with a potential home-field advantage effect, while only few experiments using wheat litter were conducted under wheat fields.

#### 4.2.5 Agricultural practices

Agricultural systems significantly influenced macrofauna effect on decomposition (Fig. 4) but not litter mass loss in bags or other fauna effects in our study (Figs. 5 and 6). Macrofauna effect on decomposition was significantly higher in conventional than in no-tillage systems. Conversely, previous litterbag studies found no-tillage systems to exhibit higher decomposition than conventionally tilled systems (Reddy et al., 1994; Miura et al., 2008; Faust et al., 2019), but the contrary was also found (Domínguez et al., 2014). Agrosystems under conventional tillage and no-tillage management experience vastly different disturbance regimes, which affect the soil food web (Wardle, 1995). Even if the difference between systems was not significant in our study, the effect of macrofauna, mesofauna and their combined effect tend to increase litter decomposition in conventional systems but not in no-tillage systems. This is surprising as macrofauna and mesofauna densities are reported to be higher under low tillage systems, notably for macroarthropods (Reddy et al., 1994), earthworms (Parmelee et al., 1990; Miura et al., 2008) and microarthropods (Brennan et al., 2006; Dubie et al., 2011). In addition, larger organisms tend to be more sensitive to tillage than smaller organisms (Wardle, 1995; Kladvko, 2001). Tillage can indeed affect soil organisms by direct impacts, such as physical damages or trapping in soil pores (Kladvko, 2001), or indirect impacts such as changes in soil moisture and in organic matter distribution, and destruction of microhabitats for decomposers (Reddy et al., 1994; Tsiafouli et al., 2015). The lack of available information prevented us from conducting further statistical analyses on the effects of agricultural practices. Yet, different practices unevenly affect soil organisms, conducting to changes in community composition, which can in turn strongly affect litter decomposition (Bradford et al., 2002).

#### 4.2.6 Temporal evolution

The effect of macrofauna, mesofauna and both together varied with time, but with patterns depending on the context. For instance, under dry and continental climates and for C4 cereals litter fauna effects were higher at the early stage of decomposition and decreased with time. In contrast, they increased slowly with time under temperate and tropical climates and for C3 cereals litter.

The variation in the effect of soil fauna may be related to the succession of organisms in litterbags. Soil organisms successively colonize litter according to their growth and migration rate (Georgieva et al., 2005). Several studies found bacteria or fungi to successively dominate the process of crop residues decomposition, with the presence of associated predators controlling their activity (Vreeken-Buijs and Brussaard, 1996; Georgieva et al., 2005; Jacobs et al., 2011). In addition, the highest mesofauna effect on crop residues was demonstrated at the early (Beare et al., 1992) and intermediate (Lekha et al., 1989) stages of litter decomposition. The intervention of macrofauna organisms could occur depending on their feeding habitats, respectively in early stage of decomposition if they feed directly on litter and later for predators that feed on decomposer organisms.

#### 4.3 General remarks on the meta-analysis

Our analysis sometimes faced confounding effects between the different factors. For instance, studied crops were different under different climates, with a predominance of C3 crops under temperate climate and C4 crops under dry climate. In addition, some factors occurred only once and in the same study, such as climate and soil type for instance, due to the lack of studies under various climates (e.g. continental, tropical, dry) and soil types (e.g. Ferralsol). Furthermore, the effect of agricultural systems on decomposition was not clear in our analysis. However, previous case studies found alternatively management practices and soil types to have more influence on soil microarthropods and microorganisms (Fromm et al., 1993; Reeleder et al., 2006). Resolving the share of the influence of agricultural practices and soil type in decomposition could lead to reinterpret the

effect of soils on decomposition and to attribute the observed effects to agricultural management rather than to soil type only. Future litterbags studies may help to characterize soil fauna and litter decomposition for several agricultural practices under a diversity of pedoclimatic contexts.

In addition, the results of our meta-analysis show a high publication bias (Fig. S4; Rothstein et al., 2006), with a prevalence of broad studies with significant results over small and non-significant ones. This bias could lead to over- or underestimate the comparative effect of organism's size classes. Thus, we would like to encourage authors and reviewers to allow for the publications of litterbag studies in agricultural areas, even if the results do not show significant differences.

#### 4.4 Advantages and limitations of the litterbag method in cropped soils

Allowing for studies *in situ*, litterbags may provide a better estimate of the decomposition rate and fauna effect than microcosm experiments. This is especially true in cropping systems as they are submitted to various disturbances that are difficult to mimic in laboratory. In a previous review, Knacker et al. (2003) concluded that the litterbag method has more advantages than other approaches (i.e. minicontainer, cotton strip, detection of isotopes, bait-lamina) as it allows to apply numerous measurement endpoints to assess litter decomposition under field conditions (e.g. number of invertebrates, enzyme activity, chemical composition, mass loss of litter). In addition, litterbags constitute a relatively easy and cost-effective method to assess litter decomposition and soil organism activity in cropped soils. They require little space and no specific equipment, and allow for short and long-term experiments, depending on the agricultural practices in place, with numerous replicates and treatments. Furthermore, enclosed litter gives a relatively good estimate of the decomposition of crop residues buried or at the surface of agricultural soils (Ouédraogo et al., 2004).

More generally, several authors suggested that the enclosure of litter create artificial conditions that can prevent accessing the real decomposition rate (Anderson, 1973; Bradford et al., 2002; Prescott,

2005). They notably suggested that a “mesh size effect” could explain part of the differences observed between bags of different mesh sizes. Fine mesh litterbags were reported i) to be subjected to microclimate with an increased moisture responsible for an over-estimation of the results (Irmiler, 2000; Bradford et al., 2002), ii) to constitute a physical barrier that slow colonization of litter by microorganisms (Wise and Schaefer, 1994), and iii) to be colonized by a small fraction of soil mesofauna through egg deposition (House and Stinner, 1987; Vreeken-Buijs and Brussaard, 1996). In contrast, bags with a larger mesh size were found i) to allow organisms to pull litter from the bags even without degrading it (Bradford et al., 2002), ii) to increase the risk of litter loss during the handling of bags or leaching due to rainfall (Kampichler and Bruckner, 2009), and iii) to ease the admission of soil particles and roots within bags that washing or brushing after recollection do not completely removed, which increase the final weight of litter but also increase the surface of soil-litter contact and therefore the microbial decomposition activity within bags (Broadbent and Tomlin, 1978; House and Stinner, 1987; Heisler, 1994). Nevertheless, the scientific community considers litterbags as a useful method for comparative studies and it is admitted that potential bias will be similar between experiments, allowing the comparison of data collected with bags of similar mesh size (Domínguez et al., 2014; Peña-Peña and Irmiler, 2016).

#### 4.5 Adapting the litterbag protocol to agricultural areas

Existing studies on litterbag experiments occurring in cropping systems enabled to provide interesting results on the role of soil organisms in litter decomposition. However, forecasting future studies on soil biodiversity in an agricultural context, we recommend the development of a standard protocol adapted to the specificities of cropped soils. The standardization of the litterbag protocol is necessary in order to increase the number of studies in cropped soils and to ease the comparison of results. Previous authors already advocated for the need of a standard protocol for litterbag experiments and a methodology to assess litter decomposition (Knacker et al., 2003; Kampichler and

Bruckner, 2009; Krishna and Mohan, 2017). However, to this day, we are not aware of the publication of a standardized protocol to assess the effect of soil organisms on litter decomposition in agricultural soils. There is no standard design and the litterbags used for current experiments are handmade with the available means. Guidelines do exist for the use of litterbags for the risk assessment of plant protection products in soils (Knacker et al., 2003; OECD, 2006), but the suggested protocol is designed specifically to test the safety of chemicals and not to consider soil organisms of different sizes. In the recent years, the growing interest for the use of the teabag method (Keuskamp et al., 2013) was also driven by the need for a standard method to measure decomposition. However, teabags do not address the standardization needs concerning soil organism's effect on decomposition as they prevent to take into account the effect of large organisms. Here, we provide some clues to develop a standard protocol for the use of multi-mesh litterbags in cropping systems, including bag design and litter characteristics (Table 2).

In an agricultural context, the standardized litterbag protocol should take into account the various agricultural practices and the duration of the experiments. Attention should be paid to the cropping calendar and the management practices that can influence the rate of litter decomposition (e.g. phytosanitary treatments, mineral and organic matter inputs) or affect the positioning of litterbags (e.g. tillage, seedling, harvest, mechanical weeding).

Depending on the management practices in place, litterbags may be buried or placed at the surface in order to limit physical disturbances and to model the placement of crop residues. Nonetheless, regardless of the management system in place, the positioning of litterbags in the field could significantly affect litter decomposition. Decomposition in bags could notably be influenced by the distribution and community composition of soil organisms at different depths (Beare et al., 1992; Ouédraogo et al., 2004). Placed on the surface, litterbags allow to better estimate the role of mesofauna, which is for a large part located in the first centimeters of the soil, but could lead to exclude some macrofauna organisms such as endogeic and anecic earthworms. On the other hand,

buried litterbags will be better connected with soil microorganisms and less impacted by weather events (e.g. rain, UV), but they could be impacted by soil compaction.

A vast majority of the litterbag studies occurred in experimental sites, where it is easier to set an undisturbed area or to remove and replace bags at the time of main disturbances. Under real conditions, it is more difficult to request from farmers to set an undisturbed area, during tillage or mechanical weeding for instance, and to have a precise insight of the planning of their interventions ahead of time (e.g. date of harvest, tillage). Yet, the acquisition of more realistic estimates of the effect of soil biota on litter decomposition required conducting experiments under real conditions.

Finally, in order to assess more precisely the role of soil organism's in litter decomposition and to survey soil fauna in a wide range of agricultural contexts, the counting and identification of soil organisms should be achieved and reported with the decomposition results whenever possible.

## 5. Conclusion

This meta-analysis is the first, to the best of our knowledge, to deal with multi-mesh litterbag experiments in annually cropped soils and to assess the influence of different size classes of soil organisms on the litter decomposition rate. It highlights the essential role of macrofauna and mesofauna during organic matter decomposition in cropped soils, whereas these soils may be submitted to high physical and chemical disturbances. This fauna effect on decomposition may be influenced by various factors such as climate, soil, standing crop, type of enclosed litter, and in a lesser extent by agricultural systems. However, more litterbag studies would be required to conclude on the quantitative impact of these factors.

Given the variability of litterbag design and litter decomposition measurements, we pledge for a standardization of the litterbag method for the forthcoming studies, in particular in cropped soils. Here, we provide some guidelines to help to implement litterbag experiments while taking into account the specificities of cropped soils (i.e. high soil perturbations).

775 Future multi-mesh litterbag experiments should especially focus on alternative (e.g. organic or  
776 conservation agriculture) or transitioning systems and on different combinations of practices for  
777 which farmers and consumers are showing a growing interest. In addition, studies are also required  
778 on the effects of individual agricultural practices on soil organisms and litter decomposition, notably  
779 for crop rotation, mineral or organic inputs, tillage, shallow tillage, mechanical weeding and soil  
780 cover. This is necessary to determine their specific effect on the activity of different groups of soil  
781 organisms and the related impacts on the soil functioning. Finally, our understanding of the biological  
782 functioning of agroecosystems requires the identification of soil organisms and especially of their  
783 functional traits in relation to different agricultural practices and systems.

784

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786

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Table 1. Characteristics of studies selected for the meta-analysis. Time: duration of the study (days); n bag: number of replicates per modality; Size: size of bags (cm x cm); Depth: depth of bags placement in soil (cm); Fauna: studied groups of organisms.

N°	Publication	Location	Climate	Soil type	Cropping system	Time (days)	n bag	Size (cm)	Depth (cm)	Fauna	Litter
1	Broadbent & Tomlin, 1978	Canada	cont	Luvisol	C	112	8	11 x 11	5	-	maize
2	Carlesso et al., 2019	UK	temp	Cambisol	MT	180	6	5 x 6	5	-	ryegrass, wheat
3	Curry & Byrne, 1997	Ireland	temp	Gleysol	C	300	6	-	10	Lum	wheat
4	Domínguez et al., 2010	Argentina	temp	Kastanozem	NT	133	4	20 x 20	-	Ann, Ara, Colb, Ins, Lar, Lum, Myr	local, alfalfa
5	Domínguez et al., 2014	Argentina	temp	Kastanozem	C, O, NT	365	10	-	-	Colb, Lum, other	local, sorghum
6	Heisler, 1994	Germany	temp	Luvisol	C	270	4	10 x 10	10	Aca, Colb	wheat
7	House & Stinner, 1987	USA	temp	Acrisol	NT	120	9	10 x 10	0	Aca, Colb, other	clover, vetch, rye
8	Jensen, 1985	Denmark	temp	Luvisol	C	290	30	10 x 15	15	Lum, mesofauna	barley
9	Miura et al., 2008	Japan	cont	Andosol	C, NT	27	4-6	3.5 x 7	8; 18	Aca, Colb, Enc, mic, Nem, Pro	clover, wheat, sorghum
10	Ouédraogo et al., 2004	Burkina Faso	dry	Cambisol	-	90	4	15 x 18	3; 35	Ara, Colp, Der, Hym, Isop, Lum, Myr, Ort	maize, grass, dung
11	Peña-Peña & Irmeler, 2016	Brazil	trop	Ferralsol	NT	140	12	15 x 20; 20 x 20	0	-	soybean, maize
12	Peña-Peña & Irmeler, 2018	Brazil	trop	Ferralsol	C	140	12	15 x 20; 20 x 20	0	-	soybean, maize
13	Reddy et al., 1994	India	dry	Lixisol	C, NT	330	3	6 x 10	0	Aca, Colb, Isop, other	rice
14	Singh & Shekhar, 1989	India	temp	Luvisol	-	270	3	10 x 15	5; 12	-	wheat, maize
15	Tian et al., 1992	Nigeria	trop	Lixisol	-	98	4	30 x 30	0	-	tree leaves, maize, rice
16	Tian et al., 1998	Nigeria	trop	-	C, R	98	4	20 x 20	0	-	tree leaves
17	Valckx et al., 2011	Belgium	temp	Luvisol	NT	392	-	7 x 7	0	Lum	ryegrass, phacelia, mustard, rapeseed
18	Vreeken-Buijs & Brussaard, 1996	Netherlands	temp	Fluvisol	C	365	10	15 x 15	15; 25	Aca, Colb, Enc	wheat
19	Weil & Kroontje, 1979	USA	temp	Nitisol	C	70	4	10 x 15	8	Aca, Colb, mic	maize
20	Yang et al., 2018	China	temp	Fluvisol	-	365	4	15 x 20	-	Aca, Ara, Colb, Colp, Dip, Hym, Isod, Lep, Myr, Nem	tree leaves
21	Yin et al., 2019	Germany	cont	Chernozem	C, O	137	20	15 x 20	-	-	oat
22	Zwahlen et al., 2007	Switzerland	cont	Cambisol	C	240	7	10 x 10; 20 x 20	20; 10	Aca, Ara, Colb, Enc, Isod, Lar, Lum, Myr, Nem, Pso	maize

cont: continental; temp: temperate; trop: tropical; C: conventional; MT: minimum tillage; NT: no-tillage; O: organic; R: relay cropping; Aca: Acari; Ann: Annelida; Ara: Araneae; Colp: Coleoptera; Colb: Collembola; Der: Dermaptera; Dip: Diptera; Enc: Enchytraeidae; Hym: Hymenoptera; Ins: Insecta; Isod: Isopoda; Isop: Isoptera; Lar: Larvae; Lep: Lepidoptera; Lum: Lumbricidae; mic: microflora; Myr: Myriapoda; Nem: Nematoda; Ort: Orthoptera; Pro: Protozoa; Pso: Psocoptera.

Table 2. Recommendations for the standardization of litterbag experiments measuring the effect of soil organisms on litter decomposition in cropping systems.

Parameter	Goal	Standardization	Recommendations
Mesh size	Select several size groups of soil organisms	Average number and size of mesh	Select 3 mesh sizes with an opening around 0.02 mm to include microorganisms only, 1 mm to additionally include mesofauna and 5 mm to include all soil organisms.
Bag size	Enclose the quantity of litter required to conduct measurements	Single size of bag	10 x 10 cm bags limit displacement or tearing during field management and provide space for the quantity of litter required to assess decomposition over the full length of the experiment. 20 x 20 cm bags can be selected under tropical climate as litter degrade faster.
Bag material	Construct solid, biologically inert and cheap mesh bags	Single material, closure techniques	Use nylon fabric and close the edges of bags by sewing with a nylon/polyester thread or by using stainless steel staples (or monel rustproof staples for long studies). Sewing allows solid closure with limited apertures on the edges of the bags and is cheap, but may be time consuming. Staples allow gaining time, but are more expensive and may create larger apertures on the edges of the bags.
Enclosed litter	Represent crop residues typically found in the cropping systems; select standard litter to compare different sites	Standard litter management, size of litter pieces, standard litter type	Homogenize litter within all bags, select a specific part of the plant (e.g. leaves, stems) or if mixed ensure the same ratio in each bag. Cut litter in homogeneous pieces (1 to 5 cm) as the role of soil fauna differ with the size of litter. Use wheat or maize as standard litter to ease comparison between studies.
Litter amount	Fill bags with the adapted quantity of litter	Minimum litter quantity	Fill bags with a litter quantity ensuring good contact between litter and soil, and adapted to the decomposition speed. It will depend on bag size, type of litter, duration of the experiment and context (e.g. 4 - 5 g litter in 10 x 10 cm bags under temperate climate).
Positioning of bags	Allow access to organisms living in the soil; prevent field management to displace or to tear bags	Standard field placement	Place bags in the row between crop plants (Broadbent and Tomlin, 1978) and bury them vertically to limit the impact of tillage and to observe soil organisms at a greater depth (Zwahlen et al., 2007; Miura et al., 2008). In no-till systems, bags can be set at the surface.
Duration of experiment	Observe mass loss patterns during litter decomposition	Minimum duration	At least 3 months. Set length and start of the experiment according to the cropping cycle to further avoid disturbances (Ouédraogo et al., 2004; Carlesso et al., 2019).
Sampling of bags	Collect enough data on litter mass loss	Minimum number of sampling dates	Number of samplings depends on required effort (e.g. distance to field, number of bags) and on cropping calendar. More than 3 dates if the aim is to assess degradation kinetics.

Sampling of soil biota	Survey soil fauna and assess its effect on decomposition	Collection of soil organisms	Count and identify soil organisms in bags whenever possible. Extract organisms from soil sampled near area of bag deposition to get an overview of the field density and diversity.
Supplementary advice	In order to track down litterbags, use stakes and centimetric precision GPS. Stakes tend to attract megafauna (e.g. wild boars) so the ideal is to place them further away of bags, on the same crop row.		

Figure 1. Characteristics of litterbag experiments within selected studies (n = 22). (a) Cropping system. (b) Crop type. (c) Number of sampling dates. (d) Range of days within which sampling were conducted. (e) Size of mesh of litterbags. (f) Type of organic matter enclosed in litterbags.

Figure 2. Mass loss of organic matter in litterbags for different climates (a, b, c) and different litter qualities (d, e, f) in (a, d) coarse mesh (n = 204), (b, e) medium mesh (n = 266), and (c, f) fine mesh (n = 130) litterbags. Linear regressions were drawn for all data (continuous lines) and for different climates or litters (dotted lines). Coefficient of determination ( $r^2$ ) are specified in brackets in the legend. Significant correlation are indicated by \* ( $P < 0.05$ ).

Figure 3. Temporal evolution of the role of soil fauna on litter decomposition in relation to climate (a, b, c) and litter types (d, e, f) with the effect of (a, d) macrofauna (n = 217), (b, e) mesofauna (n = 134), and (c, f) both macrofauna and mesofauna (n = 81). Lines indicate the limit between positive and negative fauna effect.

Figure 4. Effect size of macrofauna on litter decomposition. Represents the effect size of all the paired observations comparing coarse versus medium mesh litterbags (incl. /excl. macrofauna) with (a) the effect sizes of all paired observations (n = 201) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. “Mean effect size” represents the overall effect size of all paired observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.

Figure 5. Effect size of mesofauna on litter decomposition. Represents the effect size of all the paired observations comparing medium versus fine mesh litterbags (incl. /excl. mesofauna) with (a) the effect sizes of all paired observations (n = 126) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. “Mean effect size” represents the overall effect size of all paired

observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.

Figure 6. Effect size of total fauna on litter decomposition. Represents the effect size of all the paired observations comparing coarse versus fine mesh litterbags (incl. /excl. macro- and mesofauna) with (a) the effect sizes of all paired observations ( $n = 75$ ) and (b) the effect sizes for each climate, soil, crop, agricultural systems and litter subgroups. “Mean effect size” represents the overall effect size of all paired observations. Effect size of each paired observation and the associated 95% confidence interval are represented by (a) black squares and bars or (b) center and length of diamonds.













