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Review

Assessing the effects of plant protection products on organic matter breakdown in arable fields—litter decomposition test systems

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Abstract

There is a need for plant protection products (PPPs) to be assessed for their effects on the breakdown of organic matter (OM), which is an important functional process in terrestrial ecosystems. Little information is available on to how to assess effects of PPPs on this complex system and formal guidelines for a standardised test method are lacking. We critically reviewed the literature to determine appropriate methods to investigate OM breakdown for the risk assessment of PPPs. Five methods appeared to be potentially suitable: namely the use of mini-containers or litter-bags to enclose OM, cotton-strip and bait-lamina assays which provide an artificial OM substrate, and stable isotopes to track the chemical decomposition of OM. These methods were compared on the basis of 10 suitability criteria, which included ecological relevance, ease of use and relevance to risk assessors. Each test method has limitations but the use of litter-bags, which is the most frequently used method, has distinct advantages over the other approaches. Accordingly, literature describing OM breakdown in litter-bags when applying PPPs are reviewed, gaps in the methodology are highlighted and recommendations for the development of a standardised and validated test method are proposed.

Keywords: Plant protection products; Environmental risk assessment; Litter-bag; Organic matter breakdown; Litter decomposition

1. Introduction

Organic matter (OM) breakdown is an ecological process that integrates numerous physical, chemical and biological activities within soil (Dickinson and Pugh, 1974). It is a vital function because, if it did not occur, all the nutrients would soon be tied up in dead organisms, and no new life could be produced (Odum, 1971). We use the term OM breakdown to refer to the breakdown of dead plant OM. Since the 1960s OM breakdown has been intensively investigated in soil ecology and agronomy (e.g. Dickinson and Pugh, 1974; Cadisch and Giller, 1997) and several methods of studying OM breakdown are described in the scientific literature (Höfer et al., 1996; Aerts, 1997; Dunger and Fiedler, 1997). It is generally accepted that disturbance of this complex process might influence nutrient transformations and cycling and, in the long run, soil fertility (Eijsackers and Zehnder, 1990).

Probably, due to the complexity of soil ecological processes, an internationally accepted and standardised test method for evaluating effects of plant protection products (PPPs) on OM breakdown, which can be applied to support the registration of PPPs by national or international competent authorities, is lacking. OM breakdown depends not only on the activity of soil macroorganisms but is a complex process in which most soil organisms are involved (Dickinson and Pugh, 1974; Swift et al., 1979; Verhoef and Brussard, 1990). Further it is rather unlikely that all toxic effects on macrofauna or microorganisms caused by PPPs can be correlated with effects on OM breakdown. We also would like to emphasise that the tendency of a number of persistent compounds to adsorb to soil particles does not necessarily exclude the bioavailability of these compounds (Calderbank, 1989) because some soil organisms, e.g. earthworms, can remobilise bound residues during gut passage (Gevao et al., 2001).

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Our objective is to review and evaluate all potential methods for assessing effects of PPPs on OM breakdown and to assess their relevance for the environmental risk assessment (ERA).

2. Scientific background

According to the concept initiated by Minderman (1968) and further developed by Swift et al. (1979) and Heal et al. (1997), OM breakdown can be understood as the rate of change of any non-living organic resource over time. The entire process of OM breakdown can be interpreted as a sequence of modules of which each represents any organic resource which is changed from one state to another over time (Heal et al., 1997). The basic module is repeated in a recurrent stepwise process (cascade) in which the products of decomposition of one resource become the initial resources of the subsequent module in the cascade. The rate of change from one module to the next is regulated by a combination of three interacting factors: the physico-chemical environment (location, soil properties, climate), the quality of the resource (nutrient quality, dimensional structure) and the decomposer community (microorganisms, i.e. archaea, bacteria and fungi; microfauna; mesofauna; macrofauna; megafauna). Further, decomposition of any resource is the result of three processes: catabolism (mineralisation by chemical processes and synthesis of decomposer tissue and humus), comminution (physical reduction in particle size and selective redistribution of chemically unchanged litter) and leaching (transport down the soil profile and removal of labile resources from the system).

The negative exponential model is a useful descriptor to define the rate of decomposition (Mellillo et al., 1989). It has long been recognised that the OM loss rate declines with time reflecting the decline in the quality of the remaining substrate. Reasons for this decline include the initial loss of more readily decomposed substrates, leaving the more resistant fractions, and possibly the formation of resistant polymeric compounds (Minderman, 1968). The recognition that the factors which control decomposition change with time is one of the features justifying the cascade model.

3. Methods to investigate OM breakdown

ERAs aim to evaluate the uncertainty associated with predictions about the effects of man-made stresses (e.g. PPPs) on populations, communities or ecosystems (e.g. the functioning of soil biomass). The following terms are employed by ecotoxicologists when assessing possible environmental hazards. An assessment endpoint (AEP) is an ecological quality that is valued by humans and needs to be protected, e.g. OM decomposition in the soil environment. A measurement endpoint (MEP) is a biological, chemical or physical property that can be measured and quantified, e.g. mass loss of litter (Suter, 1989; Kapustka and Reporter, 1993). Consequently, the first step of the literature review was to identify and evaluate appropriate methods for the determination of OM breakdown; in a second step the relevant MEPs were evaluated. The conceptual approach was based on the assumption that OM breakdown belongs to the heterotrophic activities of terrestrial ecosystems (Odum, 1971). One important indicator of heterotrophic activities, the CO_2 release or respiration, is not the subject of this paper. We have not reviewed test methods based entirely in the laboratory, those restricted to single species, and methods which do not distinguish between autotrophic and heterotrophic activities of terrestrial ecosystems or compartments. Also excluded from this review are methods which were designed to investigate only part of the OM decomposition cascade, e.g. leaching (Christensen, 1985a) or the effects of fungi on fresh leaf litter (Robinson et al., 1993).

Most of the 500 or more scientific papers on OM breakdown published since the 1960s are related to litter decomposition in forest ecosystems. However, the task of this review is to focus on OM breakdown in arable sites in which PPPs are usually applied. Since the literature describing decomposition in arable sites is scarce, important methodological aspects developed for forests or grasslands are also considered.

3.1. Identification of appropriate test methods

We found five methods which can be related to OM breakdown measured under field conditions: litter-bag (Bocock and Gilbert, 1957; Crossley and Hoglund, 1962), mini-container (Eisenbeis, 1993, 1994), cotton-strip (Harrison et al., 1988), isotope methods (N^{15}, ^{14}C, ^{13}C) (Sharkov and Lodko, 1977; Rochette et al., 1999; Thomsen et al., 2001), and bait-lamina (Von Törne, 1990a,b). The main properties of each method are compared below and are summarised in Table 1.

3.1.1. Litter-bag method

The litter-bag method was developed by Bocock and Gilbert (1957). The principle of the method is to enclose defined amounts of organic material (e.g. leaf litter, straw, or cellulose paper) in bags of non-degradable, flexible material with a size of 15–600 cm^2 and a mesh size of 2 μm to 10 mm (e.g. Beck et al., 1988; Hendrix and Parmelee, 1985; Paulus et al., 1999). The bags are either left exposed on the soil surface or buried in the soil at depths of 5–10 cm for periods that may exceed 2 y (e.g. Malkomes, 1980; Schönborn and Dumpert, 1990).

3.1.2. Mini-container method

The mini-container method (Eisenbeis, 1993) can be thought of as miniaturisation of the litter-bag technique. Mini-containers (MCs) consist of small polyethylene boxes

Table 1
Comparison of integrative functional methods used under field conditions to measure the process of organic matter breakdown

Method	Litter bag	Minicontainer	Cotton strip	^{15}N, ^{14}C, ^{13}C isotopes	Bait-lamina
Principle of the method	Decomposition of OM enclosed in gauze bags or containers	Decomposition of OM enclosed in small containers	Measurement of cellulose decomposition	Detection of isotopes from ^{15}N or ^{14}C or ^{13}C labelled organic matter	Feeding of soil organisms on bait material
Organic material used	Straw, hay, leaf litter, cellulose or equivalent	Chopped leaves, straw, cellulose or equivalent	Standardised cotton cloth material	^{15}N or ^{14}C or ^{13}C -labelled plant material	Bait material, e.g. dried, pulverized leaves mixed with agar and charcoal
Exposure of OM	Bags or containers on the soil surface or buried	Minicontainer on the soil surface or buried	Strips buried horizontally or vertically in soil	Material directly mixed into the soil	Perforated PVC strips inserted into the soil
Duration	1–12 months	2–6 months	2–26 months	Variable	1–4 weeks
Endpoints measured	Mass loss; chemical, faunal and microbial characteristics	Mass loss, chemical and microbial characteristics	Loss of tensile strength of cotton fabric	Amount of isotopes in various soil fractions	Number of empty holes indicates bait material eaten
Data assessment	Comparison of mass loss with control and reference substance	Comparison of mass loss with control	Comparison of loss of tensile strength between treatments	Not defined since the method has not been used in ecotoxicology	Empty holes in comparison to the total number of holes; vertical profile of empty holes
Remarks	See Table 8	Method rarely used, so experience is limited	Fungal growth may increase tensile strength	Mainly applied in laboratory studies	Method rarely used, so experience is limited
References	Bocock and Gilbert (1957), Crossley and Hoglund (1962), Herlitzius (1985), and Paulus et al. (1999)	Eisenbeis (1993, 1994)	Kuzniar (1950) and Maltby (1988)	Nagel et al. (1995), Sharkov and Lodko (1997) (^{14}C); Thomsen et al. (2001) (^{15}N); Rochette et al. (1999) (^{13}C)	Von Törne (1990a,b), Kratz (1998) and Irmler (1998)

(volume approximately 1.5 cm^3, dia. 16 mm) filled with an organic substrate (e.g. litter, straw, or cellulose). MCs are closed at either end with plastic gauze of variable mesh sizes (e.g. 20 μm–2.0 mm). A variable number of MCs (6–36) are inserted into PVC bars (length 38–89 cm) which can be inserted into the soil either vertically or horizontally or can be laid on the soil surface horizontally (Eisenbeis et al., 1999). MCs have been used to assess effects of various factors on OM breakdown, such as tillage and soil compaction (Lenz and Eisenbeis, 1998; Dittmer and Schrader, 2000) and PPPs (Paulus et al., 1999).

3.1.3. Cotton-strip method

The cotton-strip method was first introduced by Kuzniar (1950) and described in detail by Latter and Howson (1977). The method is used to assess the cellulolytic activity in soil profiles. Strips of sterilized cotton cloth (size often 30 × 10 cm) are inserted vertically into the field soil and, on retrieval, cut into sub-strips corresponding to selected depths of the soil profile. The tensile strength of the sub-strips is determined as a measure of decomposition. The cotton-strip method has been used to compare cellulose decomposition in various soils (Hill et al., 1985; Howson, 1991). The tensile strength may increase due to the growth of certain fungi and can be a source of error (Latter et al., 1988). In the cotton-strip method, standardised, artificial, homogenous OM (pure cellulose impregnated with a dye to highlight strips) is exposed without using any container that might interfere with the surrounding soil or soil fauna. Howard (1988) has doubted whether the rate of breakdown of pure cellulose added to soil can provide an index of litter decomposition rate, release of litter nutrients, or general biological activity.

3.1.4. Isotope methods

The chemical and spatial fate of any component and metabolite of the OM decomposition process can be traced in the soil and soil fauna via isotopes. The solid-state ^{13}C NMR technique, which is used to determine chemical changes of litter during the degradation processes under natural conditions, was reviewed by Skjemstad et al. (1997). Major limitations of the method are low natural abundance of ^{13}C and the low C content in many mineral soils (Skjemstad et al., 1997). OM labelled with ^{15}N (Nagel et al., 1995; Cortez et al., 2000) or ^{14}C (Stott et al., 1986; Fließbach et al., 2000) is used to detect OM breakdown intermediates or degradation products such as CO_2, NO_3, and NH_4. The ^{15}N and ^{14}C techniques have been used mainly under laboratory conditions to avoid environmental problems with radioactive isotopes. With isotope methods, natural OM can be exposed to soil organisms without any restrictions caused by the use of containers. All isotope techniques require rather sophisticated equipment and specifically trained experts. The isotope method has not been used to study effects of chemical stressors on OM breakdown.

3.1.5. Bait-lamina method

The bait-lamina method was developed by Von Törne (1990a,b) to measure the feeding activity of soil organisms

as an integrative indicator of the overall activity of the soil fauna under field conditions. A mixture of cellulose, bran and activated charcoal serves as bait material and is contained in small holes (dia. 1 mm) drilled into small PVC strips (length 15–20 cm, width 0.5 cm). In some studies different bait materials, e.g. dried, pulverized plant leaves mixed with agar-agar, have been used (Helling et al., 1998; Kratz, 1998). The strips are inserted vertically into the soil for a few days or weeks, usually in blocks of 16 strips. On retrieval, the percentage of emptied holes in comparison to the total number of holes is assessed. The bait-lamina method has been used to investigate the spatial heterogeneity of biotic activity in temperate soils (Irmler, 1998). Further, the method was applied to determine effects of some chemicals on the feeding activity of the soil fauna (Federschmidt and Römbke, 1994). So far, only Paulus et al. (1999) have investigated whether the feeding activity measured by the bait-lamina method correlates with OM breakdown determined by using litter-bag or mini-container methods. Each method revealed a specific pattern of responses which could not be correlated to each other and which is attributed to the different quality and way of exposure of the OM used by each of the three methods.

3.2. Evaluation of test methods used under field conditions

For PPPs it has become an accepted procedure to assess ectoxicological effects at three different tiers which exhibit increasing ecological relevance (Van Leeuwen and Hermens, 1996). At the first and second tier effects on single species are studied under laboratory conditions, whereas at the third tier structural features, e.g. abundance of taxonomic groups or species diversity, of ecosystem components are studied under field conditions. OM breakdown is a complex functional process relevant to the organisational level of ecosystems, i.e. to studies conducted under field conditions (Odum, 1971). Therefore, the methods applied to investigate OM breakdown should reflect the criteria associated with higher tier studies.

Below are listed 10 criteria which have been developed to evaluate the suitability of terrestrial ecotoxicological test systems for ERA, both in general (Edwards et al., 1996; Römbke et al., 1996), and in relation to OM breakdown (Kula and Römbke, 1998). Based on these criteria the selection of the most appropriate method to measure OM breakdown is considered and summarised in Table 2.

3.2.1. Relevance for ERA schemes

All methods summarised in Table 1 can be conducted under field conditions (Dunger and Fiedler, 1997) and are designed in such a way that the results can be integrated into existing ERA procedures for PPPs. However, whether the feeding activity of soil animals assessed by the bait-lamina method is correlated with OM breakdown has not been fully investigated.

3.2.2. Ecological relevance

The highly integrative character of OM breakdown should be reflected in the proposed testing method. The substrate used should mimic the real field situation and the measured endpoint should be closely related to key aspects of OM breakdown. The results of an OM breakdown study should represent the functioning of the entire soil ecosystem, i.e. all soil organisms should have free access to the OM. For practical reasons the criterion 'ecological relevance' was divided into two aspects (Table 2): (a) the quality of the resource used in the test, (b) the ability of relevant soil organisms to decompose the resource presented by the method.

Except for the cotton-strip and the bait-lamina methods, the resource can be natural OM. All soil organisms have unrestricted access to the resource in the cotton-strip, isotope and bait-lamina methods. If litter-bags with mesh sizes of 5 mm are used, the access to the resource is

Table 2
Evaluation of integrative functional methods used under field conditions to measure the process of organic matter breakdown (for full explanation of criteria see Section 3.1). Methods are classified as compliant with the criterion (+) or not compliant (−). Question marks denote that sufficient information to determine compliance is lacking. ERA: environmental risk assessment

Criteria for the selection of test methods	Litter-bag	Minicontainer	Cotton-strip	Isotopes	Bait-lamina
1. Relevance for existing ERA schemes	+	+	+	+	−
2. Ecological relevance					
2.1. Quality of resource (OM)	+	+	−	+	−
2.2. Access of soil organisms to OM	+	−	+	+	+
3. Experience	+	−	−	?	+
4. Flexibility					
4.1. Use in various terrestrial ecosystems	+	+	+	+	+
4.2. Use of different resources	+	+	−	+	−
5. Robustness	+	?	?	+	?
6. Practicability	+	+	?	−	+
7. Sensitivity	+	?	?	?	+
8. Data assessment	+	+	+	+	?
9. Reproducibility and repeatability	+	?	?	?	?
10. Standardisation and validation	+	−	−	−	−

sufficient to allow most soil fauna to participate in OM breakdown (Swift et al., 1979). The small size of MCs prevents some relevant soil organisms from participating in the decomposition process of the presented OM (Eisenbeis, 1994). Further, the OM enclosed in MCs is usually cut into small pieces, which unnaturally enlarges the surface of the OM for microbial activities (Eisenbeis et al., 1999).

3.2.3. Experience

Most scientific experience has been gained with the litter-bag method. Very few studies have used the MC or cotton-strip methods (Kratz, 1998; Eisenbeis et al., 1999), whereas we found no study in which the isotope methods were applied to measure ecotoxicological effects on OM breakdown.

3.2.4. Flexibility

The test method should be adaptable for use with chemicals that differ in their use patterns and physico-chemical properties. Ideally, the test should allow determination of several MEPs and should be adaptable to various test conditions as they might occur under field conditions in different climatic regions. The criterion 'flexibility' was also divided into two aspects (Table 2): (a) Can the method be used in various terrestrial ecosystems, e.g. agricultural sites, grassland and forests? (b) Can different natural resources of OM be used by the same method?

For the litter-bag, MC and isotope methods both questions can be answered with yes. In the bait-lamina method, however, different homogenised bait mixtures were used as OM resources (Helling et al., 1998) and in the cotton-strip method only one was used.

3.2.5. Robustness

Soil heterogeneity or seasonal changes of moisture and temperature may influence, but should not bias, the results of a field study. This criterion has not been studied systematically for any of the methods described in Table 1. However, since the litter-bag method has been used frequently in many different projects (Swift et al., 1979; Cadisch and Giller, 1997), one might draw the conclusion that its robustness has been taken for granted by scientists.

3.2.6. Practicability

Sophisticated and expensive equipment and intensive operator training should not be necessary to conduct the test. In the case of a field study, the demands to identify appropriate field sites should not lead to an extraordinary endeavour. In comparison to many standardised ecotoxicological studies conducted under laboratory conditions OM breakdown is a long-term process.

The isotope methods require by far the most sophisticated equipment and technical expertise compared to all other methods. The limited available literature on the cotton-strip method does not allow an assessment of the practicability of the method, although it seems that a rather complex technique is necessary to determine the tensile strength of the cotton strips (Harrison et al., 1988). The other methods are straightforward and can be easily established to conduct semi-field or field studies (Paulus et al., 1999).

3.2.7. Sensitivity

The test method should discriminate between effects caused by the test compound and other sources of variability in the data. A reasonable sensitivity towards a broad spectrum of chemicals is more desirable than a high sensitivity to a specific stressor. The set of data derived from litter-bag studies under field conditions (Tables 5–7) demonstrate the capability of the method to differentiate between background variation and effects caused by anthropogenic stressors (Malkomes, 1980). For the bait-lamina method, the same conclusion can be drawn, although the number of studies is small and the correlation of the feeding activity with OM breakdown remains to be evaluated (Larink, 1994). The sensitivity of the remaining three methods cannot be evaluated as the amount of data available is rather limited.

3.2.8. Data assessment

All methods described in Table 1 can be subjected to statistical evaluation. Results are gained such that they can be used to model the breakdown of OM over time, e.g. the half-life period of plant litter decomposition (PLD_{50}) (Wieder and Lang, 1982; Andrén and Paustian, 1987; Cadisch and Giller, 1997). For the mini-container method, however, the statistical independence of the individual containers within a plastic stick is unclear. This is particularly a problem with sticks in vertical orientation, which cannot readily be replicated due to the limitations of soil depth and vertical changes in soil structure.

3.2.9. Reproducibility and repeatability

The variability of results between different testing facilities (reproducibility) and within the same testing facility at different times (repeatability) should be determined. As a general rule, one can expect that the more complex, i.e. the closer a test method to natural conditions, the higher the variability of the results. Efforts should be made (e.g. standardise experimental approaches, optimise test design) to keep the variability of the results low.

A systematic approach, e.g. a ring-test, to study the reproducibility of the results obtained when applying the litter-bag method has not yet been undertaken, although C. Kula and S. Guske (pers. comm.) confirm that the data derived by the litter-bag method and submitted by registrants of PPPs to the German competent authority are repeatable. For the four other methods, repeatability and reproducibility have not been determined. In the case of the bait-lamina test the repeatability is low when following the original design, i.e. 16 replicate bait-lamina strips per sampling point (Von Törne, 1990a,b). However, when

increasing the number of sampling points while simultaneously decreasing the number of replicates the repeatability can be improved (Irmler, 1998).

3.2.10. Standardisation and validation

Test methods should be developed to a standard that they can be submitted with confidence to an international organisation responsible for standardisation of methods. Within such an organisation, e.g. the Organisation for Economic Co-operation and Development (OECD), the method can be fine-tuned and ring-tested to obtain an internationally accepted standardised and validated test method.

A draft guidance developed by a working group, which was established by the German competent authority for the registration of PPPs, is available for the litter-bag method (pers. comm. C. Kula and S. Guske). However, no inter-laboratory comparison or ring-test has been carried out. All other methods are far from being standardised, although the potential for standardisation is quite high for the technically simple MC and bait-lamina methods (Kampmann, 1994; Paulus et al., 1999).

3.3. Evaluation of measurement endpoints

To assess OM breakdown (the AEP) the MEPs were classified and summarised into four categories: faunal, microbiological, chemical and physical MEPs (Table 3). All the MEPs and the corresponding methods have been used intensively in soil ecological research. The criteria to rank the usefulness of the MEPs are, firstly, the closeness of the scientific relevance between the AEP and the MEP and, secondly, the practicability of the methods when applied in ecotoxicological studies. Hence, the most appropriate MEP should integrate across all aspects of the OM breakdown rather then taking into account one or few specific details of the decomposition cascade. The MEP should be technically simple without using expensive or sophisticated equipment.

Table 3
Measurement endpoints for the determination of OM breakdown in the litter-bag method

Classification	Measurement endpoint	Reference (examples)
Biological	Number or biomass of invertebrates Species composition	Dunger and Fiedler (1997)
Microbiological	Microbial biomass/ respiration	West and Sparling (1986), Beare et al. (1990), ISO 14240-1 (1997), and Alef and Nannipieri (1995)
	Enzyme activity (e.g. carboxymethylcellulase, dehydrogenase)	Burns (1983) and DIN, 19733-1 (1998)
Chemical	Composition of OM or its transformations (lignin, soluble carbon, soluble phenolics, α-cellulose)	Rowland and Roberts (1994), Constantinides and Fownes (1994), Skjemstad et al. (1997), and Joffre et al. (2001)
	Composition of nutrients	Anderson and Ingram (1993) and Thomsen et al. (2001)
Physical	Mass loss of litter	Bocock and Gilbert (1957) and Malkomes (1980)

3.3.1. Faunal MEPs

The faunal MEPs (e.g. number and/or biomass of soil fauna) are indirectly linked with OM breakdown. It is obvious that the soil fauna plays an important role in OM breakdown but the functions of microorganisms and the process of leaching should not be neglected (Swift et al., 1979). Correlations between abundance, biomass and species diversities of the soil fauna and the decomposition processes have not been established for many terrestrial sites. One of the few exceptions is a study published by Höfer et al. (2000) in which the effect of the macrofauna on OM degradation in a tropical forest was investigated. Edwards and Bohlen (1996) summarised the literature on OM breakdown for holarctic pastures and forests and demonstrated a correlation between the number and biomass of earthworms and decomposition. In most cases this correlation was established by using litter-bags of different mesh sizes to selectively exclude the earthworms from litter. Especially the large vertical burrower *Lumbricus terrestris* seems to be paramount for this correlation and has been termed as a key species or ecosystem engineer for the soil ecosystem (Jones et al., 1994). This correlation was confirmed by the observation that a decline of these worms caused by the fungicide benomyl led to a significant accumulation of leaf litter in an apple orchard (Kennel, 1990). A similar effect, i.e. an increase of the litter layer on the soil surface, was also observed after the application of high concentrations of the biocide pentachlorophenol (PCP) in a beech forest (Beck et al., 1988). In their study, the accumulation of litter was not caused by effects on the rarely present earthworms in the acid soil, but was due to effects on various taxonomic groups of the mesofauna and microflora (Schönborn and Dumpert, 1990). Although sophisticated equipment is not required for the determination of faunal measurement categories, the demand on taxonomic expertise may be high.

3.3.2. Microbiological MEPs

As with the faunal MEPs, the microbiological endpoints represent an important aspect of the biological activities involved in OM breakdown (Chapman, 1999). However, an explicit connection between established soil microbial tests, e.g. nitrogen and carbon transformation test (OECD, 2000a, b) and the AEP is lacking (Efroymson and Suter, 1999). This is because a large proportion of the microorganisms cannot be cultured, the degree of functional redundancy among populations is unknown, and only a portion of the microbial biomass is active at a given time. Microbial tests focus upon physiological processes rather than taxonomic diversity (Lawton and Brown, 1993). Thus, compared to

the zoological methods, a smaller demand for taxonomic expertise is offset by a greater requirement for laboratory instrumentation. A major disadvantage is the high variability and short reaction time of microbiological activities to soil moisture changes under field conditions (Wardle and Parkinson, 1990; Rochette et al., 1991).

3.3.3. Chemical MEPs

Some chemical MEPs concentrate on the determination of gross mineralisation, i.e. on the transformations of nutrients such as C or N, while others determine the degree of decomposition by measuring amounts of carbohydrates such as celluloses and lignins (Ziegler and Zech, 1991; Henriksen and Breland, 1999). Near infrared reflectance spectroscopy (NIRS) has also been used to characterise the biochemical composition of OM in litter decompositions studies (McLellan et al., 1991; Joffre et al., 1992) and to predict mass loss of litter (Gillon et al., 1993, 1999; Joffre et al., 2001). In particular, ^{13}C NMR spectroscopy has been used to study spatial and temporal changes in the degradation of specific litter components (Skjemstad et al., 1997). The chemical MEPs usually refer to specific fractions of the OM and the methods require a high degree of chemical expertise and, in some cases, very sophisticated laboratory instrumentation.

3.3.4. Physical MEPs

The mass loss of litter, often referred to as weight loss in the literature, is the only physical MEP. Mass loss integrates all biological and chemical processes that contribute to OM breakdown and is directly linked to the decomposition process. It is the measure that has been most often used with the litter-bag method (e.g. Bocock, 1964; Wieder and Lang, 1982; Paulus et al., 1999). Specific biological or chemical expertise is not required and standard laboratory equipment is sufficient to perform the measurement (Dunger and Fiedler, 1997). Consequently, compared to all other MEPs mass loss of OM is preferred although qualitative changes during the degradation and the mineralisation of OM cannot be related directly to mass loss (Schomberg and Steiner, 1999).

3.3.5. Recommendation of the best available method

When summarising the evaluation of the MEPs (Table 3), we conclude that the cotton-strip and bait-lamina methods are limited to just one MEP. When applying the isotope, MC and litter-bag methods, in each case several MEPs can be determined simultaneously. In particular, for the litter-bag method, analysis of chemical components of litter (e.g. Summerell and Burgess, 1989), microbial biomass (e.g. Schönborn and Dumpert, 1990), microbial respiration (e.g. Förster et al., 1996) and the abundance of soil organisms (e.g. Hendrix and Parmelee, 1985) have been measured. Also, it should be possible to integrate isotope methods into the litter-bag and MC methods. As a result, for the litter-bag method all relevant MEPs could be applied. The same is true for the MC method except that the effects of the macro- and mega-fauna cannot be determined because the containers are too small (Eisenbeis et al., 1999).

From Section 3 our first choice to develop as a standardised test method that can be applied to assess the effects of PPPs on OM breakdown is the use of litter-bags to determine the mass loss of litter.

4. OM breakdown in litter-bags

We found 119 research papers in which either OM breakdown in arable sites or the effects of chemicals on OM breakdown were studied. In 56 of these papers the litter-bag method was applied, enabling an assessment of the advantages and disadvantages of the method in ecotoxicological studies. The most important data are outlined in the following sections and summarised in Table 4 (comparison of exposure conditions of various types of straw) and in Tables 5–7 (effects of PPPs on OM breakdown).

4.1. Factors and conditions influencing OM breakdown

The litter-bag method has been used widely to assess the effects of various environmental factors and experimental conditions on OM breakdown, i.e. quality and quantity of OM, climate and soil properties, exposure, mesh size of bags and chemicals.

4.1.1. Organic matter, selection and preparation

Pure cellulose, e.g. filter paper, has been proposed as a standard organic material to determine OM breakdown in soils (Unger, 1960) and to evaluate the soil as a functional habitat for soil organisms (Schick, 1999). Cellulose substrates were used to investigate effects of different soil types (Schröder and Gewehr, 1977; Beyer et al., 1992; Kurka et al., 2000, 2001), fertilisers (Schnetter, 1971) and tree stand characteristics (Kurka and Starr, 1997) on OM breakdown. Cellulose has also been used as a substrate to assess the effects of depleted uranium (Meyer et al., 1998), carbendazim (Förster, 2001) and emissions from industrial plants (Bienkowski, 1990) on soil functions. In general, however, indigenous OM, for example leaves of trees for forest studies (e.g. Weary and Merriam, 1978; Paulus et al., 1999) and crop material for studies on arable sites (e.g. Malkomes, 1980; House et al., 1987), have been used predominantly in research studies.

The exposure period usually exceeded 1 y when wheat or barley straw was employed as decomposing material (Table 4). Summerell and Burgess (1989) and Frederiksen et al. (2001) found that approximately 50–60% of buried wheat straw was decomposed during an exposure of 0.5 y. Henriksen and Breland (1999) buried five different plant materials in litter-bags for 1 y and found the remaining C residues were largest for barley straw (49%), followed by

Table 4
OM breakdown in litter-bags exposed to arable field soils

OM	AL (mg cm^{-2})	PTL	MSL (mm)	ETL (weeks)	C-to-N ratio	LL (cm)	MEP	Reference
Barley straw	n.s.	n.s.	n.s.	104	n.s.	5.0	Mass loss	Andrén and Paustian (1987)
Barley straw	n.a.	n.s.	1.0	156	n.s.	5.0	Mass loss	Andrén et al. (1992)
Barley straw	n.s.	Water-rinsed; dried at 60 °C	0.8; 16	n.s.	n.s.	n.s.	Mass loss	Bohác et al. (1990)
Barley straw	40	Field-fresh	1.0	104	80.2	3.0	Loss of C, N, cellulose	Henriksen and Breland (1999)
Spring barley straw	33	Dried at 60 °C	1.0	62	96	0.5–5.0	Mass loss	Christensen (1986)
Spring barley straw	33	Dried at 60 °C	1.0	62 61	94	0.5–5.0	Mass loss; N-, P-content	Christensen (1985b)
Spring barley straw	33	Dried at 70 °C	0.05; 0.5; 10.0	45	n.s.	5.0	Mass loss	Jensen (1985)
Barley stems	11	Air-dried	1.0	104	n.s.	2.0	Mass loss; chemical analysis	Wessén and Berg (1986)
Spring barley stems	11	Air-dried	1.0	104	n.s.	2.0–4.0	Faunal colonization	Lagerlöf and Andrén (1985)
Rye straw	40	Air-dried	0.05; 0.2; 1.0; 5.0	13	n.s.	n.s.	Mass loss; N-content	House and Stinner (1987)
Winter rye straw	25	Dried at 50 °C	1.8	45	98	0.18	Mass loss; N_t, fauna	Beare et al. (1992)
Wheat straw	n.a.	n.s.	1.0; 7.0	156	n.s.	n.s.	Mass loss	Curry and Byrne (1997)
Wheat straw	56	n.s.	1.0	104	n.s.	n.s.	Mass loss; chemical analysis	Summerell and Burgess (1989)
Wheat straw, (internodes and leaves)	130–270	Air-dried	1.0	32	n.s.	n.s.	Fungal colonization; mass loss	Robinson et al. (1994)
Spring wheat straw	22	Water-rinsed; dried at 80 °C	0.02; 1.5	50	n.s.	0.3–3.0	Mass loss; mesofauna	Vreeken-Buijs and Brussaard (1996)
Winter wheat straw	33	Dried at 60 °C	1.0	62	94, 61	0.5–5.0	Mass loss; N-, P-content	Christensen (1985b)
Winter wheat straw	80	n.s.	1.0	52	n.s.	n.s.	Mass loss	Nieder and Richter (1989)
Winter and spring wheat straw	60	n.s.	n.s.	52	n.s.	n.s.	Mass loss	Smith and Douglas (1971)
Maize stalks; maize leaves	70	Dried at 60 °C	10.0	27.3	57.7	5.0–10.0	C_t, N_t, SIR, mass loss	Bohlen et al. (1997)

Abbreviations. OM: organic material (the term straw is used if no specification concerning the plant tissue exposed is given in the literature); AL: amount of litter; PTL: previous treatment of litter; MSL: mesh size of litter-bags; ETL: exposure time; LL: length of litter; MEP: measurement endpoint (C_t total C, N_t total N, SIR substrate induced respiration); n.s.: not stated.

Table 5
List of fungicides applied in arable, grassland or forest sites to determine effects on OM breakdown by using the litter-bag method

Active substance	C of a.s. (kg ha^{-1})	OM	SL (cm)	MSL (mm)	EL	AC	ETL (w)	MEP	E	Reference
Benomyl	0.125; 0.5; 2.0	Wheat straw	n.s.	1.0	In	i	16	CH	N^{ns}	Torstensson and Wessén (1984)
								ML	N^{ns}	
Captan	1.5	Chinese cabbage	20 × 20	0.0025	In	i	1	ML	Y^{ss}	De Jong (1998)
Carbendazim	0.36; 3.6	Hay	10 × 20	0.002; 5.0	On	d	104	ML	Y^{nsa}	Eder et al. (1992)
Carbendazim	0.36–87.5	Cellulose paper	10 × 10	n.s.	In/on	d	16	ML	Y^{ss}	Knacker et al. (2003)
Fenpropimorph	*	Wheat roots	20 × 4	1.0	In	i	16	FCF	Y^{ss}	Bjørnlund et al. (2000)
Maneb	3.0	Chinese cabbage	20 × 20	0.0025	In	i	1	ML	Y^{ss}	De Jong (1998)

Abbreviations. C: concentration (a.s. active substance, * denotes 1 ×, 10 × and 100 × recommended field application rate used); OM: organic material; SL: size of litter-bags in cm; MSL: mesh size; EL: exposure (on: litter-bags lying on the soil surface, in: litter-bags inserted in the soil, in/on: both); AC: application of chemical (d: direct treatment of litter-bags, i: indirect treatment of litter-bags, d/i: both); ETL: exposure time, w: weeks; MEP: measurement endpoints in litter-bags (ML: mass loss, CH: carbohydrate content, FCF: fungal colony forming units); E: effects on organic matter breakdown in litter-bags (Y: yes, N: no, ss: statistically significant, ns: statistically not significant, nsa: no statistical method applied); n.s.: not stated.

potato haulm (25%), ryegrass (18%), white clover (8%) and white cabbage leaves (5%).

Tables 5–7 show that in 10 out of 34 tests with PPPs wheat straw was used as OM, including one test with wheat roots. Ryegrass (*Lolium perenne*) and Johnson grass (*Sorghum halepense*) were exposed in seven, maize leaves (*Zea mays*) in six and beech leaves (*Fagus* spp.) in five tests. Leaves from four tree species (birch—*Betula* spp., oak—*Quercus* spp, hornbeam—*Carpinus* spp., red maple—*Acer* spp.) and a mixture of meadow grass (hay) were investigated once. Chinese cabbage (*Brassica chinensis*) and artificial litter (cellulose paper), were both exposed twice.

The high C-to-N ratios of cereal straw caused low decomposition rates (Christensen, 1986) and reduced the attractiveness or palatability of straw for soil invertebrates (Lee, 1985). This is especially true for the nodes and internodes of cereal stems as compared to their leaves (Harper and Lynch, 1981). While the latter tissue decomposed relatively quickly, the stems were long lasting. In a 32-week decay study (Robinson et al., 1994), only 8% of the initial weight of wheat straw leaves were left whereas for the stems 66% of the initial weight still remained. The degradation of stems and leaves were also studied separately (Malkomes, 1980; Robinson et al., 1994; Cortet and Poinsot-Balaguer, 2000).

In many studies, prior to exposure in litter-bags, the OM was either air-dried (Wessén and Berg, 1986), dried at elevated temperature of up to 80 °C (Vreeken-Buijs and Brussaard, 1996), ground (Stott et al., 1986), or subjected to forced leaching by applying water (Reinertsen et al., 1984). When ground cereal straw decomposition is accelerated (e.g. Stott et al., 1986; Roper and Smith, 1991; Fließbach

Table 6
List of insecticides applied in arable, grassland or forest sites to determine effects on OM breakdown by using the litter-bag method

Active substance	C of a.s. (kg ha^{-1})	OM	SL (cm)	MSL (mm)	EL	AC	ETL (w)	MEP	E	Reference
Carbofuran	0.286	Red maple litter	No bags	No bags	On	d	20	ML	Y^{ss}	Weary and Merriam (1978)
Carbofuran	0.6^{nuc}	Maize leaves	12 × 12	4	On	i	22	AF	Y^{nsa}	Cortet and Poinsot-Balaguer (2000)
								ML	Y^{nsa}	
Carbofuran	1.10; 11.2	Maize leaves	11 × 11	0.005; 0.7; 9.0	In	i	20	ML	Y^{nsa} (effect vanished at the end)	Broadbent and Tomlin (1982)
Chlorpyrifos	1.92	Rye straw, cellulose paper, birch and beech leaves	10 × 10	0.051; 1.3	In	d/i	20	ML	Y^{ns}	Siedentop (1995)
								AF	Y^{ns}	
Diflubenzuron	0.025; 0.25	Beech/hornbeam leaves	20 × 20	0.002; 1.0	On	d	98	ML	N^{nsa}	Paulus et al. (1999)
Fibronil	0.2^{nuc}	Maize leaves	12 × 12	4.0	On	d	22	AF	Y^{nsa}	Cortet and Poinsot-Balaguer (2000)
								ML	N^{nsa}	

Abbreviations. C: concentration (a.s.: active substance, nuc: no untreated control); OM: organic material; SL: size of litter-bags in cm (^{s} cm^{2}); MSL: mesh size; EL: exposure (on: litter-bags lying on the soil surface, in: litter-bags inserted in the soil); AC: application of chemical (d: direct treatment of litter-bags, i: indirect treatment of litter-bags, d/i: both); ETL: exposure time, w: weeks; MEP: measurement endpoints in litter-bags (ML: mass loss, AF: abundance of fauna); E: effects on organic matter breakdown in litter-bags (Y: yes, N: no, ss: statistically significant, ns: statistically not significant, nsa: no statistical method applied).

Table 7
List of herbicides applied in arable, grassland or forest sites to determine effects on OM breakdown by using the litter-bag method

Active substance	C of a.s. (kg ha^{-1})	OM	SL (cm)	MSL (mm)	EL	AC	ETL (w)	MEP	E	Reference
Alachlore	2.4^{nuc}	Maize leaves	12 × 12	4.0	On	d	22	AF	Y^{nsa}	Cortet and Poinsot-Balaguer (2000)
								ML	N^{nsa}	
Atrazine	1.0^{nuc}	Maize leaves	12 × 12	4.0	On	d	22	AF	N^{nsa}	Cortet and Poinsot-Balaguer (2000)
								ML	N^{nsa}	
Atrazine	1.5^{nuc}	Ryegrass	n.s.	1.0	In/on	d/i	72	ML	N^{ns}	Wardle et al. (1993)
								BAS	N^{ns}	
								SIR	N^{ns}	
Atrazine	*	Johnson grass	15^{s}	1.0	On	d	16	ML	Y^{ss}	Hendrix and Parmelee (1985)
Bromacil	1.6^{nuc}	Ryegrass	n.s.	1.0	In/on	i	72	ML	N^{ns}	Wardle et al. (1993)
								BAS	N^{ns}	
								SIR	N^{ns}	
Chloridazon	2.6	Wheat stems	10 × 10	1.3	In	d/i	40	ML	N^{nsa}	Malkomes (1980)
Chlortoluron	3.2	Wheat stems	10 × 10	1.3	In	i	40	ML	Y^{nsa}	Malkomes (1980)
Cycloat	2.88	Wheat stems	10 × 10	1.3	In	d/i	40	ML	N^{nsa}	Malkomes (1980)
Dichlorprop-salt	2.8	Wheat stems	10 × 10	1.3	In	d/i	40	ML	Y^{nsa}	Malkomes (1980)
Glyphosate	1.57	Wheat straw	10 × 10	0.05; 0.20; 1.0; 5.0	On	d	12	ML	Y^{ss}	House et al. (1987)
(+Alachlor)	2.24							AM	N^{ns}	
(+Linuron)	0.56							AC	Y^{ss}	
Glyphosate	**	Johnson grass	15^{s}	1.0	On	d	16	ML	Y^{ss}	Hendrix and Parmelee (1985)
								NL	Y^{ss}	
								AF	Y^{ss}	
Lenacil	1.6	Wheat stems	10 × 10	1.3	In	d	8	ML	Y^{nsa}	Malkomes (1980)
Methabenz-thiazuron	2.8	Wheat stems	10 × 10	1.3	In	i	40	ML	Y^{nsa}	Malkomes (1980)
Paraquat	**	Johnson grass	15^{s}	1.0	On	d	16	ML	Y^{ss}	Hendrix and Parmelee (1985)
								NL	Y^{ss}	
								AF	Y^{ss}	
Penoxalin	1.485	Wheat stems	10 × 10	1.3	In	i	40	ML	Y^{nsa}	Malkomes (1980)
Pyridate	0.9^{nuc}	Maize leaves	12 × 12	4.0	On	d	22	AM	n.s.	Cortet and Poinsot-Balaguer (2000)
Rimsulfuron	0.04^{nuc}	Ryegrass	n.s.	1.0	In/on	i	72	ML	N^{ns}	Wardle et al. (1993)
Terbumeton–terbuthylazine	5.0^{nuc}	Ryegrass	n.s.	1.0	In/on	i	72	ML	N^{ns}	Wardle et al. (1993)
2,4,5 T	2.25; 22.5	White oak leaf litter	15 × 15	4.0	On	d	104	ML	N^{ns}	Gottschalk and Shure (1979)
								AF	N^{ns}	
2,4,5 T	10.0; 50.0	Beech leaves	20 × 30	0.002; 0.25; 10.0	On	d	234	ML	Y^{nsa}	Beck et al. (1988) and Schönborn and Dumpert (1990)
								MB	Y^{nsa}	

Abbreviations. C: concentration (a.s.: active substance, nuc: no untreated control, * litter-bags dipped for 30 s into solution containing 0.29 or 2.9% of a.s; ** denotes 1 × and 10 × recommended field application rate used); OM: organic material; SL: size of litter-bags in cm (^{s} denotes cm^{2}); MSL: mesh size; EL: exposure (on: litter-bags lying on the soil surface, in: litter-bags inserted in the soil, in/on: both); AC: application of chemical (d: direct treatment of litter-bags, i: indirect treatment of litter-bags, d/i: both); ETL: exposure time, w: weeks; MEP: measurement endpoints in litter-bags (ML: mass loss, AF: abundance of fauna, BAS: basal CO_2 respiration, SIR: substrate induced respiration, AM: abundance of micro-arthropods, AC: abundance of carabids, NL: nutrient loss, MB: microbial biomass); E: effects on organic matter breakdown in litter-bags (Y: yes, N: no, ss: statistically significant, ns: statistically not significant, nsa: no statistical method applied); n.s.: not stated.

et al., 2000). Paulus et al. (1999) assumed that fragmentation of OM enlarges the surface areas accessible for microbial attack and enhances OM breakdown in comparison to intact leaves. Angers and Recous (1997) suggested that the improved availability and accessibility of N in small-sized particles are responsible for the increased decomposition rates.

When standardising the litter-bag method for testing the effect of PPPs, the selected OM should represent a material typical for arable sites, should be readily available in sufficient amounts of uniform quality, should easily be stored and handled, and remnants of the material should be distinguishable from other OM (e.g. roots) which might enter the litter-bags during exposure in the field. To differentiate between short-term and long-term effects the selected OM should decompose within 1 y. The ratio of different plant tissues should be considered when planning decomposition studies (Blenis et al., 1999). For instance, either the stem-to-leaf ratio of the selected OM should be the same in all litter-bags within a study, or the test should be performed with either or both of the two tissues. Cereal straw seems to comply with most of the described requirements and may therefore be an appropriate OM. This is supported by the literature, with wheat straw being the most frequently used substrate in arable sites (11 studies), followed by barley straw (nine studies) (Tables 4–7). Air-drying and cutting into pieces of roughly 5–10 cm is proposed for the development of a standard test design since all other influences of previous treatment on OM decomposition may be considered unacceptable (Taylor, 1998).

4.1.2. Climate and soil properties

Soil moisture seems to be the most important climatic factor (Stott et al., 1986; Summerell and Burgess, 1989; Parshotam et al., 2000). Dry conditions usually reduce decomposition rates while, depending on the temperature preference or tolerance of the decomposer community, temperature changes may accelerate or slow down the decomposition. Temperatures above 20 °C in combination with high moisture have been found to increase microbial activity (Willcock and Magan, 2001) whereas the activity of some earthworm species is highest at lower temperatures (Lee, 1985). Under laboratory conditions, the rate of straw decomposition increased with increasing temperature over a range of 5–30 °C (Summerell and Burgess, 1989). In field conditions, Christensen (1985b) observed a reduced decomposition rate for straw when the soil surface was covered by vegetation. One explanation for this phenomenon might be that the transpiration of plants lowers the water content of the soil which, in turn, results in retarded OM decomposition. Another explanation might be that the presence of some other organic material which is more attractive to soil organisms increases the retention period of cereal straw (Boström, 1987; Kuzyakov and Domanski, 2000).

The influence of soil type on OM breakdown is less obvious. For example, Thomsen et al. (2001) found mineralization of ^{15}N labelled ryegrass residues was not influenced by the textural composition of the soil. Similarly, Sharkov and Lodko (1997) obtained no differences in mineralization rates of wheat straw in different soils. Jensen (1985), however, reported that effects of soil type on OM breakdown were dependent on the mesh size of the litter-bags used: effects were detected with mesh sizes of 0.5 mm and, especially, 10 mm but not with a mesh size of 50 μm. Soil pH is an important factor, as soil microorganisms and enzymes as well as the soil fauna prefer defined pH ranges (Römbke, 1991; Roper and Smith, 1991) which indirectly might influence the decomposition rate of litter. It is difficult to differentiate between effects caused by soil moisture and soil type since both factors as well as other soil properties (e.g. soil pH) are closely related to each other.

4.1.3. Exposure

Litter-bags are either placed on the soil surface or buried in the soil, usually at a depth of 5–10 cm. In accordance with agricultural practice, the amount of straw exposed per unit area was usually below 100 mg cm^{-2} (Nieder and Richter, 1989; Ziegler and Zech, 1991; Recous et al., 1999). The method of exposure can influence the OM breakdown both quantitatively and qualitatively, because surface-placed OM tends to be dominated by fungal decomposers (Holland and Coleman, 1987). Decomposition of straw is retarded if exposed on the soil surface compared to buried straw (Cogle and Saffigna, 1989; Beare et al., 1992; Stemmer et al., 1999; Magid et al., 1999). Ziegler and Zech (1991) reported a higher proportion of microbially synthesised components such as non-cellulosic polysaccharides and proteins, in straw decomposing on the soil surface. Moreover, if soil animals are abundant, especially the earthworm *Lumbricus terrestris*, the C-to-N ratio of the litter exposed on the soil surface may increase over time, since the animals feed selectively on the N-rich parts of the litter, leaving the N-poor material on the soil surface (Förster et al., 1996; Bohlen et al., 1997). Finally, the intensity of patrolling of the soil surface by macro-arthropods influences the structure of biota and C transfer (Kajak, 1997).

In 14 out of 32 tests in which the effects of PPPs on OM breakdown were studied all of the litter-bags were exposed on the soil surface whereas in 13 tests they were buried (Table 5). In the remaining five tests, some litter-bags were placed on the soil surface while others were inserted into the soil. In 17 tests the litter-bags were treated directly with the PPP and in 12 tests they were first buried in the soil and then the chemical was applied onto the soil. In five tests some litter-bags were lying on the soil surface while others were inserted in the soil when the PPP was applied. However, from the available data we cannot conclude which exposure procedure is most appropriate to detect chemical effects.

Concerning the exposure of litter-bags, scientists at the German competent authority for the registration of PPPs

(pers. comm. C. Kula and S. Guske) made the following assumptions: (a) it is common agricultural practice (although not exclusively) that after harvest the remaining straw is worked into the soil; (b) the variability of results on OM breakdown is minimised when litter-bags are inserted into the soil because the climatic fluctuations affecting the degradation process are less pronounced in the soil; (c) for slowly degradable PPPs which are applied repeatedly onto the soil the expected plateau concentration is likely to be evenly distributed within the ploughing layer. Hence to mimic a worst-case situation an appropriate amount of the PPP to give the expected plateau concentration for repeated applications should be applied to the soil surface and mixed with the top soil layer (approximately 10 cm depth) before the litter-bags are inserted horizontally into the soil at a depth of approximately 5 cm; (d) one week after the expected plateau concentration has been established and the litter-bags inserted into the soil the annual maximum application rate of the PPP is sprayed onto the soil surface.

4.1.4. Mesh size

Mesh sizes ranging from 2 μm to 10.0 mm have been used in litter-bag studies (Tables 4–7). In many studies, litter-bags with different mesh sizes were applied simultaneously to study the effects of different size classes of soil organisms on OM breakdown (Broadbent and Tomlin, 1982; House et al., 1987; Eder et al., 1992). The mesh size of 20 μm excludes both meso- and macro-fauna, while a mesh size of 250 μm selectively excludes macro-fauna. Mesh sizes of larger than 5 mm allow almost all soil fauna, except mice, moles and some beetles and slugs to participate in OM breakdown (Beck et al., 1988; Cadisch and Giller, 1997). Small mesh sizes, however, may lead to higher decomposition rates because of the different micro-climate inside the bags (St John, 1980; House and Stinner, 1987). For assessing the effects of PPPs on OM breakdown, ideally all relevant soil organisms should have access to the OM exposed in litter-bags. Therefore, a mesh size of at least 5 mm would be appropriate for the development of a standard test design. Larger mesh sizes can be used, but care is needed as the straw fragments may be lost when retrieving the litter-bags from the field.

4.2. Effects of PPPs on OM breakdown

In 17 papers the effects of 25 PPPs on OM breakdown have been described (Tables 5–7). Most of the tested PPPs were herbicides (16), followed by fungicides (5) and insecticides (4). The fungicide carbendazim, the insecticide carbofuran and the herbicides atrazine, glyphosate and 2,4,5-T have been tested twice or more. In most studies only one concentration was tested. In a few studies two or more concentrations were applied (e.g. Torstensson and Wessén, 1984; Bjørnlund et al., 2000). In one study (Knacker et al., 2003) the effects of the fungicide carbendazim on cellulose decomposition was studied at four European sites at six concentrations ranging between 0.36 and 87.5 kg ha^{-1}. The applied concentrations were analytically confirmed only by Beck et al. (1988) and Knacker et al. (2003).

In 32 tests, 50 MEDs were determined (Tables 5–7). In five tests three endpoints and in eight tests two endpoints were measured simultaneously. Mass loss was most often chosen as an MED (30 times) followed by abundance of fauna (eight times). Effects caused by PPPs were obtained for 29 MEDs, of which 12 effects were statistically significant. In 15 tests (20 MEDs), the data were not subjected to a statistical treatment. Nevertheless, the authors of the respective research studies considered 13 of these endpoints as showing effects of the PPP on OM breakdown. In two studies (Wardle et al., 1993; Cortet and Poinsot-Balaguer, 2000) were untreated controls not used.

For 22 tests with herbicides, 34 MEDs were analysed, of which 18 showed effects (six effects were statistically significant). For the five fungicides studied, seven MEDs were determined, of which five showed effects (three effects were statistically significant). The fungicides benomyl and carbendazim are considered to be very toxic to oligochaetes which leads to the expectation that they would affect OM breakdown. However, no effects of benomyl were found by Torstensson and Wessén (1984), probably because the small mesh size (1 mm) of the litter-bags would have excluded the macrofauna. For four insecticides studied, nine MEDs were obtained, of which seven showed effects but only one was statistically significant. The insecticide carbofuran is also very toxic to earthworms which resulted in effects on OM breakdown (Broadbent and Tomlin, 1982). In tests with the fungicides captan, fenpropimorph and maneb effects were found when using litter-bags with small mesh-sizes (2.5 μm–1 mm) demonstrating that the microflora, mesofauna or the interactions between them were affected.

Test duration does not seem to be related to the probability of observing effects caused by PPPs since the average exposure time of OM in tests showing effects was 36 weeks whereas the exposure time of tests showing no effects was on average 61 weeks (Tables 5–7).

4.3. Test performance

The test design of each of the 56 litter-bag studies reviewed was unique. Usually, the influence of one controlled factor on OM breakdown was assessed. Nevertheless, by comparing the research findings we identified areas where guidance would be required on how to conduct litter-bag tests.

4.3.1. Number of samples and duration of trials

The number of replicate litter-bags per sampling time point and treatment ranged from two (Siedentop, 1995) to 25 (Andrén and Paustian, 1987). The number of sampling times ranged from one (Malkomes, 1980) to more than 10 (e.g. Gottschalk and Shure, 1979) with a median of five sampling times per test. In most cases there was one plot per

treatment whereas in few tests up to four replicate plots per treatment were used (Cortet and Poinsot-Balaguer, 2000). At the minimum, the litter-bags were exposed to OM degradation for 1 week (De Jong, 1998) and at the maximum for 234 weeks (Beck et al., 1988; Schönborn and Dumpert, 1990).

4.3.2. Handling after retrieval of litter-bags

In most cases the remaining OM was air dried followed by drying at elevated temperatures, usually at 60–80 °C. Some investigators washed off the adhering soil particles before drying the litter (Torstensson and Wessén, 1984). The amount of mineral soil attached to the litter remnants can be a source of error when studying rates of litter loss (Potthoff and Loftfield, 1998). Therefore, the ash-free mass of the remaining material was calculated by combusting the straw remnants in a muffle at temperatures of 500 °C (e.g. Curry and Byrne, 1997) or even higher at 900 °C (Smith and Douglas, 1971) for 1–8 h (House and Stinner, 1987; Donegan et al., 1997). Assuming that the ash represents the soil fraction, the ash weight was then subtracted from the total dry weight. Additionally, Malkomes (1980) proposed a formula that also takes into account the soil OM content.

4.3.3. Reference substance

Reference substances are usually applied in ecotoxicological laboratory studies to check the sensitivity and suitability of a test system. A reference substance is usually a chemical with a well known (toxic) effect on the respective organism or function tested. For field studies the reference substance should not cause unnecessary risks to the environment. It should be a registered or notified compound of medium persistence and low human toxicity. Application should be simple and the toxicity, preferentially to a wide range of soil organisms, should be known.

Apart from the banned biocide PCP, none of the chemicals studied seems to be appropriate to serve as a reference substance since they do not fulfil the requirements mentioned. In particular, the reproducibility of effects on OM breakdown using mass loss as the MEP has not been demonstrated for any of the PPPs listed in Tables 5–7. The possibility that a mixture of relevant chemicals might be suitable as a toxic reference has not yet been addressed in research studies and requires investigation. One study has been published in which a substance of known toxicity was used to validate the test system by eliminating all biological activities (House et al., 1987).

4.3.4. Validity

Except for the study by House et al. (1987), test validity has not been addressed. The working group established by the German competent authority for the registration of PPPs proposed that a study should be considered valid if at least 50% of the initial OM is degraded during the study period (pers. comm. C. Kula and S. Guske). The possibility that a reference substance could be used for determining test validity has not been considered by the working group. An appropriate reference substance could, for instance, indicate whether the application of the chemical and the exposure of the OM is appropriate to determine effects on OM breakdown. An alternative method to evaluate the quality of the application procedure for PPPs could be to analytically determine residue concentrations of the relevant substance in the soil surrounding the litter-bags or on the OM enclosed within the bags.

4.3.5. Evaluation of data

Depending on the duration of the study and the frequency of sampling, some authors have tried to apply mathematical models to describe the decomposition rate (Andrén and Paustian, 1987; Curry and Byrne, 1997). From a scientific point of view a definite number of sampling time points to calculate, for example, the half life period of Plant Litter Decomposition (PLD_{50}) cannot be prescribed. Nevertheless, in accordance with the modelling of the degradation of chemicals in soil (ISO, 1992) there should be at least five sampling times during a 1 y exposure.

5. Conclusions and recommendations

The important influence of microbial activities, soil fauna, climatic conditions and soil properties on OM breakdown and their resultant effect on OM breakdown as well as the effect of a chemical stressor on OM breakdown cannot be predicted from tests on single species of the soil fauna. Therefore, effects on OM caused by PPPs have to be determined experimentally by measuring the OM breakdown directly. It has been demonstrated that the litter-bag method with mass loss of OM as the MEP at present is the most appropriate technique available.

Most of the litter-bag studies reviewed were designed to study ecological aspects of OM breakdown rather than ecotoxicological effects of a chemical on OM breakdown. Each study was designed on an ad-hoc basis, hence the effects of PPPs on OM breakdown from different studies are difficult to compare. The high degree of effects observed is probably due to the fact that PPPs known to cause effects on oligochaetes were selected for the studies.

A standardised guideline to test the effects of PPPs on OM breakdown cannot be extracted from the literature. Nevertheless key elements for a guideline can be identified. The recommendations are based on 24 test criteria (Table 8). However, the literature does not advise on how to proceed when non-cereal crops are used in the litter-bag method. Guidance is also lacking on the optimal timing of litter-bag burial and retrieval in relation to agricultural practice. Probably several representative standard application situations need to be defined, if realistic worst-case exposure situations and, at the same time, common agricultural practices should be covered by the litter-bag method.

Table 8
Factors affecting the performance of litter-bag assays to determine effects of PPPs on OM breakdown

Study design	Findings extracted from literature	Recommendations	Comments
Study site	Selected site should be relevant for the intended use of the PPP	Arable site	Bare soils preferred to sites with vegetation
Vegetation of study site	Vegetation should be relevant for the intended use of the PPP	Standard crop, e.g. one cereal	Standard crop improves comparability of results between test sites, repeated tests etc.
Number of treatments	In most tests 1 plus control	At least 5 plus control, to establish a dose–response relationship	Effects based on a dose–response relationship to increase the value for ERA
Number of plots per treatment	≥ 4	≥ 4 (K and G)	Value is in accordance with recommendations for earthworm field test (ISO, 1999)
Size of plots (m^2)	≥ 100	≥ 100 (K and G)	Value is in accordance with recommendations for earthworm field test (ISO, 1999)
Distance between plots (m)	≥ 2.0	≥ 2.0 (K and G)	Value is in accordance with recommendations for earthworm field test (ISO, 1999)
Litter-bag material	Non-degradable	Inert, flexible synthetic material	Material should assure retrieval of remaining OM
Litter-bag mesh size	Micro-, meso- and macrofauna should have access to litter	5–10 mm (K and G)	Mesh size should allow interaction of the entire biocenosis with OM
Litter-bag size	The size should be large enough to contain a realistic amount of litter	10×20 cm (K and G)	Size should allow inclusion of a sufficient amount of OM in the bag while simulating a natural layering of the OM
Number of bags per plot per sampling date	More than four if manageable	Eight or more (K and G)	Number of bags should allow statistical evaluation of data
Organic matter (OM)	Cereal straw	Wheat straw with similar amounts of leaves and stems in each litter bag	The two fractions of the wheat straw represent fast and slow decomposing OM
OM mass in litter-bags ($g\ m^{-2}$)	Realistic amount of OM for the field site chosen	200 g dw m^{-2} (K and G)	200 $g\ m^{-2}$ is representative for crop fields
Straw length	Different lengths are used according to mesh sizes	5–10 cm (K and G)	Length should be realistic compared to the field situation and manageable with regard to the mesh size chosen
Amount of test substance applied	Depending on the objective of each study, specific amounts are applied	In agreement with agricultural practice; for example PEC plus RAR (K and G)	For example, plateau (residue) concentration when test substance is repeatedly applied in the field plus recommended application rate
Application method	Depending on the objective of each study, a specific method is chosen	PEC on the soil followed by mechanical treatment using a rotary hoe before bags are buried RAR on litter-bags lying on the PEC treated soil	Test substance may remain in the top 1–2 cm of soil if not rotary hoed
Litter-bag exposure	On soil surface or buried	Buried into soil depth of approximately 5 cm (K and G)	Insertion into soil represents the field situation; depending on specific crops or agricultural practice modifications should be possible
Duration of litter-bag exposure	6–12 months	Until at least 50% of OM is degraded	Exposure time corresponds to the time required for straw decomposition under representative field conditions
Number of sampling times	Specific regimes are chosen depending on the objective of each study	In total 5, 3 or 4 of which are taken within first 6 months (K and G)	Recommended pattern allows detection of short-term effects on leaves
Reference substance	Carbendazim was chosen once as a model chemical	Broad-spectrum biocide or a mixture of several products	Reference substance should assure effects on some major component of decomposer community
Validity criteria			
Decomposition in control plots	Not taken into consideration as a validity criterion	Mass loss equal to or larger than 50% but smaller than 100%	To calculate decomposition rate, the mass loss should not be 100%

(*continued on next page*)

Table 8 (*continued*)

Study design	Findings extracted from literature	Recommendations	Comments
Decomposition in plots treated with the reference substance	No data found	Statistically significant reduction of OM breakdown at least at one sampling	To confirm the success of the application method of the test substance
Variability of data	High variability of data observed between and within studies	No recommendation (K and G)	The natural variability of data has not been determined under the test conditions recommended
Evaluation of data			
Effects	Any deviation from a control	Statistically significant differences in OM mass loss between controls and treated litter bags at two consecutive samplings or at the end of the study (last sampling)	Effects data should have a form to be useful to prepare decisions by risk assessors
Recovery	No data found	If effects occur, repeated exposure of litter-bags with uncontaminated OM on the same field site	To assess whether there are lasting effects on OM breakdown

Abbreviations. ERA: environmental risk assessment; RAR: recommended application rate; PEC: predicted environmental concentration corresponding to the plateau concentration of a repeatedly applied test substance; PPP: plant protection product; K and G: recommendation in agreement with conclusions drawn by a working group established by the German competent authority for PPP registration (pers. comm. C. Kula and S. Guske).

Some aspects of the proposed study design are based on expert judgement rather than on scientifically justified conclusions from research studies. Accordingly, we complete this review with a list of research areas that would extend the scientific background of the study design proposed in Table 8:

- The variability of OM breakdown depending on the quality and exposure of OM should be determined for representative regional soils and climates, e.g. corn-belt in the USA, wheat-growing area of Australia, upland rice-cropping areas in Southeast Asia, Northern and Southern Europe.
- A suitable reference substance should be identified which causes effects on OM breakdown at field conditions typical within the respective region.
- Whether changing the leaf-to-stem ratio of decomposing organic material significantly changes the degradation rate should be investigated.
- Whether a model describing the degradation rate of OM influenced by PPPs might be an appropriate tool for risk assessors to identify effects should be investigated.
- Whether recovery of altered OM breakdown caused by PPPs can be determined by repeated exposure of untreated litter-bags (determination of resilience of soil ecosystems) should be investigated.

Finally, we recommend that risk assessors consider whether the decision tree and trigger values within the ERA scheme for PPPs might allow the earthworm field test and the test on OM breakdown to be conducted simultaneously on the same field site.

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