DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

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Research and Development

Final Project Report

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Executive summary (maximum 2 sides A4)

Introduction

The breakdown of organic matter (OM) is a critical process in terrestrial ecosystems that regulates nutrient availability for plants and is important for the maintenance of soil fertility in sustainable agriculture. To reduce the potential environmental risk posed by the use of agrochemicals, European Directive 91/414/EEC and its subsequent amendments stipulate that plant protection products should be evaluated for their possible effects on OM breakdown.

This regulatory requirement poses a number of problems for risk assessors in EU member states, not least of which is the question of how to measure the effects of plant protection products on such a complex set of organisms, interactions and processes. Current opinion, based on recommendations from a working group led by the German Biologische Bundesanstalt (BBA), is that litter bags should be used to assess effects of plant protection products on the mass loss of plant litter. In the litter-bag approach, mass loss of

litter is considered to be an integrative functional endpoint that can provide information on the overall progress of decomposition without the need to study in detail the complex individual biological and chemical components of the system.

Aims of the work

The overall aim of this project is to determine the state of knowledge on OM breakdown in agroecosystems and the organisms that are involved in the process, and on the methods that might be used to assess effects of pesticides on OM breakdown. This information is required to optimise the risk assessment scheme for plant protection products with regard to the OM breakdown functional endpoint for compliance with the EU legislative requirements. In particular, clarification is required on whether the litter-bag test is actually the most appropriate method for assessing effects of chemicals on OM breakdown and, if so, how litter bags should be designed and exposed to agrochemicals under field conditions. There is also a need to establish whether existing lower- and middle-tier tests could be used to predict chemical risk to OM breakdown or, alternatively, whether different tests might be appropriate.

Methods

This project was carried out by performing three critical reviews of the scientific literature. These reviews determined the state of knowledge on: (1) Test methods that are available for assessing effects of plant protection products on the breakdown of OM. (2) Soil micro-organisms and microbial processes that are involved in the breakdown of OM. (3) The role of the soil fauna in OM breakdown in agroecosystems.

Principal findings

1) Five test methods that have relevance both to the assessment of OM breakdown and to the risk assessment for environmental effects of plant protection products are reported in the literature. A critical appraisal of these methods revealed that only the litter-bag method has been sufficiently well developed so far to be suitable as a higher-tier test for effects of agricultural chemicals on OM breakdown.

2) Although the finding above supports the recommendations of the BBA working group that the litter-bag test is appropriate for assessing effects of agricultural chemicals on OM breakdown, a number of disadvantages of using the litter-bag approach have been identified. These mainly concern areas of the test methodology where standard protocols are required but are currently unavailable. For example, guidance is required on how litter bags should be exposed to different types of pesticide application, and on how OM breakdown should be assessed in a range of different arable crops, soils, and climatic regions of the EU. A clear link between the mass loss endpoint and a risk classification for chemicals is also currently lacking.

3) The EU risk assessment scheme for plant protection products is relatively poorly equipped to predict chemical effects on OM breakdown. Currently, only the litter-bag test can predict the risk to OM breakdown, but apart from the current persistence triggers, no trigger pathway for this test exists. Efficiency of the risk assessment would be improved if the existing data on single species/groups or additional lower and middle-tier tests could be organised and/or developed in such a way that chemical safety to OM breakdown could be determined without triggering the litter-bag test. To achieve this, a better understanding would be required of the ecological relevance of existing single-species tests, and of the relative susceptibility of the test species to agricultural chemicals compared with the distribution of species' sensitivities in the field.

4) Suggestions are given as to how the inadequacies of the risk assessment scheme might be overcome. Microbiological multi-enzyme ('BIOLOG') and catabolic potential ('CRP') rapid screening tests appear to offer promise as 'early-warning' indicators of risk to OM breakdown that, with futher development, might be appropriate as middle-tier tests in a sequential testing scheme. Also (or instead), information on species sensitivity distributions could be used to clarify the relative ecotoxicological importance of the existing single-species tests and identify candidate soil organisms that could be used as worst-case indicators of chemical risk.

5) Recommendations arising from this work are grouped in two categories: (a) improvements to the risk assessment scheme that are required principally to address EU legislation (these focus on improvement of the litter-bag method): and (b) opportunities to further improve the efficiency of the risk assessment scheme (principally concerning optimisation of the lower- and middle-tier tests and trigger pathways).

Scientific report (maximum 20 sides A4)

Introduction

The breakdown of organic matter (OM) is a critical process in terrestrial ecosystems that regulates nutrient availability for plants and is important for the maintenance of soil fertility in sustainable agriculture (Odum 1971). OM breakdown is a complex of manifold interacting processes conducted by microorganisms and soil animals. Decomposing plant litter provides habitats for a wide range of organisms, not all of which are directly involved in the decomposition process but which may be important in regulating its temporal dynamics. To reduce the potential environmental risk posed by the use of agrochemicals, European Directive 91/414/EEC and its subsequent amendments (Directive 96/12/EC) stipulate that plant protection products should be evaluated for their possible effects on OM breakdown.

This regulatory requirement poses a number of problems for risk assessors in EU member states, not least of which is the question of how to measure the effects of plant protection products on such a complex set of organisms, interactions and processes. Current opinion, based on recommendations from a working group led by the German Biologische Bundesanstalt (BBA), is that litter bags should be used to assess effects of plant protection products on the mass loss of plant litter (Kula and Guske 2000; 2001). In the litter-bag approach, mass loss of litter is considered to be an integrative functional endpoint that can provide information on the overall progress of decomposition without the need to study in detail the complex individual biological and chemical components of the system. The need for this 'black box' approach (Dighton 1997) is endorsed by the sheer complexity of the system, our poor knowledge of which organisms actually participate in the breakdown of organic matter and of the extent to which there is functional redundancy among potential decomposer organisms.

Scientific objectives

The objectives of this work are to determine the state of knowledge on OM breakdown in agroecosystems and the organisms that are involved in the process, and on the methods that might be used to assess effects of pesticides on OM breakdown. This information is required to optimise the risk assessment scheme for plant protection products with regard to the OM breakdown functional endpoint for compliance with the EU legislative requirements. In particular, clarification is required on whether the litter-bag test is actually the most appropriate method for assessing effects of chemicals on OM breakdown and, if so, how litter bags should be designed and exposed to agrochemicals under field conditions. There is also a need to establish whether existing lower- and middle-tier tests could be used to predict chemical risk to OM breakdown or, alternatively, whether different tests might be appropriate.

Methods

This project was carried out as a desk-based study and the findings reported here were obtained by three critical reviews of the scientific literature: (1) A review of the test methods that are available for assessing effects of plant protection products on the breakdown of OM (ECT Oekotoxikologie, Germany); (2) A review of knowledge on soil micro-organisms and microbial processes that are involved in the breakdown of OM (University of Southampton, UK); (3) A review of the role of the soil fauna in OM breakdown in agroecosystems (University of Bremen, Germany). The three reviews have each been completed and, inkeeping with project milestones, were submitted together to an international journal of soil biology in November 2001. As the original review papers are lengthy, the report here provides an overview of the principal findings from the three review papers.

Principal results

(a) Relevance of the litter-bag method

Five methods that could have relevance both to the functional process of OM breakdown and to the risk assessment of pesticides are reported in the literature. These are the litter bag (e.g. Kula and Guske 2000; 2001), minicontainer (e.g. Eisenbeis et al. 1999), cotton-strip assay (e.g. Harrison et al. 1988), stable C and N isotopes (e.g. Nagel et al. 1995) and bait-lamina assay (e.g. von Törne 1990). These methods were compared on the basis of 24 suitability criteria, which included their relevance to the OM breakdown process, feasibility for inclusion in the risk assessment, and the availability of appropriate test protocols. Only the litter-bag test was found to be sufficiently well developed and relevant to be suitable as a technique for assessing pesticide effects on OM breakdown in the field. It may be concluded that the litter-bag method is currently the most appropriate ecotoxicological test method for the OM breakdown functional endpoint. Stable C and N isotope analyses might ultimately prove useful in 'tracking' the chemical transformations that occur during OM breakdown, possibly with a view to early detection or prediction of effects of agricultural chemicals. However, the

current paucity of published information on the use of such methods in ecotoxicology and decomposition studies precludes a detailed assessment of their pros and cons.

(b) Limitations of the litter-bag method

Experience with the litter-bag method is increasing and draft test protocols have been developed (e.g. Kula and Guske 2001). Nevertheless, risk assessors still face problems when using this method. In particular, guidance is required on:

- how (where within a field, at what depth and at what time) litter bags should be exposed under field conditions to agrochemical applications that differ in:
  - chemical type
  - application method
  - timing of application
  - frequency of application within a season
- how to conduct the risk assessment in different crop types (is the use of a generic substrate in the litter-bag test, e.g. cereal straw, appropriate for all types of arable crop, or should the substrate be crop specific ?)
- standardisation of the litter substrate (in terms of particle size and chemical composition, e.g. stem : node and C : N ratios)
- a methodology that can be validated under a wide range of agroecological conditions, e.g. different soil types and climatic conditions (a means of validating field tests, e.g. using toxic reference substances or chemical fate data currently does not exist)
- derivation of risk classification trigger values from the litter mass loss data

Current guidance is that the higher-tier litter-bag test should be conducted under field conditions (Kula and Guske 2001); the test is relatively time-consuming to perform, with mass-loss assessments required throughout the crop season. Whether the duration of a field litter-bag test could be reduced (e.g. by shifting the test from the field to extended laboratory, e.g. mesocosm, conditions) without loss of predictive capability for OM breakdown is not known at present.

(c) Limitations of other existing tests

Tests in the risk assessment relevant to OM breakdown are those concerning microflora and microbial processes (e.g. C and N transformations; OECD 2000a, 2000b) and those concerning earthworms (e.g. van Gestel et al. 1989) and other soil fauna, e.g. Collembola (ISO 1999), Enchytraeidae (ISO 2001) and Acari. Although the importance of soil microorganisms in OM breakdown is wholly accepted (Chapman 1999), an explicit connection between soil microbial tests and assessment endpoints is lacking (Efroymson and Suter 1999). This is because a large proportion of the microflora cannot be cultured, the degree of functional redundancy among populations is unknown (but probably high), and only a portion of the microbial biomass is active at a given time. For these reasons, microbial tests should focus upon functions rather than taxonomic diversity (Lawton and Brown 1993). But ecotoxicological tests on microorganisms and microbial processes are very difficult to relate to OM breakdown for three principal reasons: First, the degree of functional redundancy among microflora is unknown, so tests are difficult to interpret in terms of their functional relevance. Denitrification is a case in point: it is practised by many microbial populations, some of which might be expendable without any tangible impact on N cycling. Furthermore, if there is functional redundancy, elimination of sensitive populations by a stressor (e.g. pesticide) could reduce community sensitivity if more tolerant species perform the same function. Second, some tests are too general. Soil respiration tests (ISO 1997), for instance, do not detect only the activities of decomposer organisms. Third, some microbial tests are too specific. Nitrification (OECD 2000a), for example, utilises one of the decomposition products (ammonia) as a substrate and hence is less relevant to the early stages of OM breakdown. Soil enzymes are intimately involved in the decomposition of plant litter and enzyme tests (e.g. dehydrogenase or phosphatase) have been considered as predictors of pesticide risks to OM breakdown. However, Somerville and Greaves (1987) advised against the use of enzyme tests, because the role of individual enzymes in the overall decomposition process is difficult to quantify, agreed methodology is lacking, and activities of certain enzymes can be affected by the presence of soil fauna.

Of the ecotoxicological tests on soil fauna, only earthworm tests (e.g. ISO 1998) are used routinely in the risk assessment scheme; other tests on Collembola, Enchytraeidae and Acari have standard methods and are available as 'further' tests to provide additional information in specific circumstances, for example if a pesticide is applied directly to soil or thought likely to pose a risk to soil fauna. However, for many other functionally important groups of soil fauna (e.g. Diplopoda, Isopoda, Mollusca, Nematoda and Protozoa) standard test methods are lacking (van Straalen and van Gestel 1998). Data on non-target terrestrial arthropods are often available but these are mainly foliage or surface dwelling natural enemy ('beneficial') species of uncertain relevance to soil dwelling arthropods which may be involved in OM breakdown. The importance of lumbricids in OM breakdown is undisputed. Only for earthworms have attempts been made to compare chemical sensitivity among relevant species (e.g. Neuhauser et al. 1986). The Collembola reproduction test (ISO 1999) has been strongly criticised since the euedaphic, parthenogenetic test species *Folsomia candida* is not typical of

farmland Collembola (Hopkin 1997). Inevitably, the desire to use easily-cultured species like *F. candida* in ecotoxicological tests has resulted in a tendency to mainly use species with r-selected life history tactics. The available test species therefore represent a narrow focus of attention in comparison to the complex multi-species interactions that contribute to the breakdown of OM. Even for earthworms, no guidance exists on how relevant the results of the single-species risk assessment tests are to the mass loss endpoint assessed by the litter-bag method.

(d) Limitations of the overall risk assessment scheme

At present, a test for effects of a pesticide on OM breakdown (i.e. performing the litter-bag test) is required (Directive 96/12/EC, Annex II, Point 10 - specifically 10.6.2) if:

- ? the pesticide (active substance or soil metabolite) is persistent, or, where of intermediate persistence,
- ? it poses a high risk to soil microbial processes or earthworms (as determined from existing data), or
- ? there is a high risk to other soil fauna (as determined from exisitng data on non-target arthropods or 'further' single-species tests, e.g. upon Collembola, Enchytraeidae or Acari)

Persistent chemicals are defined for the purposes of this risk assessment as those for which 90% dissipation of the active substance (or soil metabolite) usually under field conditions (DT_{90}) exceeds 365 days. An assessment of effects on OM breakdown is not required if DT_{90} < 100 days (Directive 96/12/EC). But guidance is lacking on how to conduct the risk assessment for chemicals of intermediate persistence (where DT_{90} is in the range 100-365 days): expert judgement is required here, which could draw upon information from the existing and 'further' single-species tests on soil fauna. However, the relevance of these single-species tests to the OM breakdown endpoint is presently unclear. Furthermore, none of the other lower-tier tests can predict OM breakdown. The lack of clear trigger pathways for the litter-bag test means that any uncertainty as to the safety of a pesticide for OM breakdown indicated at lower-tier testing can currently only be resolved by conducting a litter-bag test in the field, which has the disadvantage of being time consuming and hence relatively costly. To optimise efficiency of the risk assessment scheme, it would be beneficial if either the ability to use existing data could be clarified or one or more 'middle-tier' tests could be included that would give early clarification of the risk to OM breakdown so that unnecessary field testing could be avoided.

(e) Development of 'middle-tier' tests that might predict OM breakdown

An efficient trigger pathway for a tiered sequence of tests in the risk assessment scheme requires knowledge of the chemical sensitivity of the test species in relation to the actual distribution of species sensitivities in agroecosystems. In theory, several carefully-chosen single-species middle-tier tests could provide sufficient ecotoxicological information to allow triggers in the risk assessment to be set such that a given plant protection product does not cause harmful effects for the majority (e.g., 95%) of soil fauna species (van Leeuwen and Hermens 1995). This 'distribution-based extrapolation' (DIBAEX) approach could have relevance to the protection of OM breakdown and might provide information on how to proceed with the risk assessment for chemicals of intermediate persistence (i.e. where DT_{90} is in the range 100-365 days). However, the DIBAEX approach requires that a number of assumptions are met and that a minimum number of single-species tests is performed (van Leeuwen and Hermens 1995); the economic and practical feasibility of using such an approach in the risk assessment scheme is presently unclear.

Dighton (1997) suggested that despite the limitations of single-enzyme tests reported by Somerville and Greaves (1987), enzymes might have predictive value for pollution risk to nutrient cycling if multiple enzyme activities could be studied simultaneously using the 'BIOLOG' microtiter plate method. The laboratory assay prepared commercially by Biolog Inc. consists of a microtiter plate containing up to 95 carbon substrates and a tetrazolium redox indicator dye; when incubated with an inoculum of a microbial community, the colorimetric changes indicate which of the substrates have been utilised, i.e. which enzymes have been active (Firestone et al. 1998). This method has been used mainly to investigate microbial community structure and its changes, such as those caused by pesticides (e.g. El Fantroussi et al. 1999). The usual substrate for the litter-bag test is straw, which is mainly degraded through the activity of lignocellulases. A test system incorporating this substrate, or analogues of it, would therefore be appropriate. At present, with the exception of cellobiose and ?-methyl-D-glucose, the Biolog substrate arrays do not detect activities associated with cellulose decomposition. However, it may be possible to use more ecologically relevant substrate arrays to mimic substrates of greater importance to OM breakdown. Key advantages of the Biolog approach are:

- ? it is not dependent on the identification of particular microbial species
- ? it can be automated as a high-throughput screening tool
- ? results can be obtained quickly (in 70 hours)
- ? the method could be modified for the OM breakdown endpoint

The Biolog method also has some drawbacks, in particular:

- ? it is suitable only for culturable microorganisms
- ? the redox dye is toxic to filamentous fungi; however, non-toxic dye is available and would permit both bacterial and fungal catabolic activities to be assessed

Even if certain functionally important microorganisms cannot grow on Biolog plates, the test might still have sufficient predictive value of the risk to OM breakdown. Further validation work would be needed to clarify this, by investigating correlations between the Biolog and litter-bag endpoints for an appropriate range of compounds and conditions.

An alternative (or additional) approach to Biolog is the catabolic response profile (CRP), also known as in-situ catabolic potential (ISCP) (Degens and Harris 1997). This approach directly assesses the catabolic diversity of microbial communities (e.g. those involved in OM breakdown) by adding a range of simple organic substrates directly to (pesticide treated and untreated) soil and measuring the short-term catabolic activity as CO_2 output. The main advantages of the CRP method are:

- ? the method is not restricted to culturable organisms
- ? the range of substrates could be tailored specifically to assess OM breakdown
- ? the test is quick (approximately four hours) and results are reproducible

The CRP approach does have some drawbacks, for instance:

- ? problems of functional redundancy might occur if substrates are utilised by a wide range of microflora (careful choice of substrates should overcome this)
- ? clarification is required on which aspects of catabolic diversity are most relevant to OM breakdown

With further work either or both of the Biolog and CRP methods might improve the efficiency of the risk assessment for pesticide effects on OM breakdown by giving an early indication of chemical risk to the functional process, thereby avoiding unnecessary triggering of the field litter-bag test.

Status of the project

(a) Have the scientific objectives of the work been met ?

As the foregoing report indicates, detailed reviews of the literature have been completed and have revealed a number of pros and cons of the risk assessment scheme for effects of agricultural chemicals on the OM breakdown process. The question of whether the litter-bag test is appropriate as the functional endpoint test has been answered, but problems with the litter-bag test, with other tests in the risk assessment scheme, and with the overall scheme itself, have been identified; these form the basis of recommendations (see below) on how future research and development might be prioritised to optimally address the requirements of the risk assessment scheme.

(b) Technology transfer

The three detailed reviews of the literature were completed on time and have been submitted to a peer-reviewed international journal for publication together as a mini-series entitled 'Assessing the effects of pesticides on organic matter breakdown in arable fields'. A summary article outlining key findings from the three reviews has been accepted for inclusion in the May 2002 issue of SETAC Globe. A summary of the project's findings will be circulated to delegates at the SETAC EPFES Workshop (Workshop on Functional Endpoints in Soil) in Lisbon in April 2002 and a platform presentation reporting the project's findings will be made in the 'Functional Endpoints' session at the SETAC-Europe meeting in Vienna in May 2002.

Discussion

(a) Reliability of the results

Some areas of the work were poorly represented in the published literature. Information on the role of soil organisms in OM breakdown was biased towards certain groups (e.g. earthworms) with very little information available on other groups (e.g. Diptera, Diplopoda,

Isopoda and Mollusca). Data on soil organisms relevant to OM breakdown in agricultural systems was also lacking. Studies conducted using litter bags were each unique in design, which in most cases precluded meaningful comparisons between studies. The conclusion that the litter-bag test is the most appropriate functional endpoint test at present in part reflects the lack of appropriate information on other test methods. Stable isotopes, for instance, might offer promise as a means of 'tracking' the chemical transformations in OM breakdown but relevant methods have not been adequately assessed in ecotoxicological risk assessments and decomposition studies so their pros and cons are difficult to weigh up at present.

(b) Implications of the findings

Overall, the current risk assessment scheme for plant protection products appears to be relatively poorly equipped to deal with the functional endpoint of OM breakdown. The litter-bag test has a number of limitations (although there has been progress towards a standardised methodology), none of the existing lower-tier tests can predict OM breakdown, and the overall risk assessment scheme lacks any trigger pathways whereby safety to OM breakdown of a chemical may be decided without invoking the full field litter-bag test. Key priorities would be (1) to clarify how the litter-bag test should be used in the field in a standardised manner whereby results can be validated, (2) to determine the degree of perturbation to OM breakdown that equates to an environmentally unacceptable effect (as the litter bag test has been shown to be highly sensitive, acceptance of any effect of a chemical on mass loss might lead to exaggeration of actual risk), (3) to clarify the relevance of lower-tier tests and whether development of alternative microbiological screening methods such as BIOLOG or CRP can be justified, (4) to clarify whether any of the existing (or additional) lower-tier tests can provide guidance on the risk to OM breakdown for chemicals of intermediate persistence (DT_{90} in the range 100 and 365 days). These priorities can be stated as a series of recommendations, as indicated below.

Future directions: recommendations

The recommendations arising from this work can be divided into those which are required to support the mandatory risk assessment for OM breakdown as prescribed under European legislation, and those which might enable the efficiency of the overall risk assessment scheme to be improved.

(a) Research requirements

- ? appropriate exposure scenarios need to be determined for different agricultural management conditions.
- ? a means of validating litter-bag tests is required (e.g. using appropriate toxic reference substances or information on chemical fate).
- ? clarification of the ecological relevance of the litter mass loss endpoint is needed (e.g. by comparison of litter mass loss with other parameters indicative of environmental stress).
- ? guidance should be developed on how litter mass loss data should translate into trigger values for risk classification, ideally with guidance on follow-up testing if risk is deemed unacceptable or uncertain.
- ? guidance should be developed on how to interpret the results of litter-bag tests carried out in different crops, for instance correlations between mass loss of cereal straw and the breakdown of residues of broadleaf crops should be investigated to determine whether a generic litter substrate (e.g. cereal straw) can be used with correction factors for different crop types or whether crop-specific test substrates would be required.
- ? performance of the litter-bag test under a representative range of climatic and agricultural scenarios within the EU should be investigated to allow harmonisation of regulatory decision making among EU member states.

(b) Research options to improve efficiency of the risk assessment scheme

- ? Information is required on how to optimally structure the series of existing and middle-tier tests in the risk assessment scheme (a) to reduce unnecessary triggering of field litter-bag tests by giving an early indication of risks and (b) to provide an appropriate test scheme for the assessment of risk to OM breakdown posed by chemicals of intermediate persistence (i.e. where DT_{90} is in the range 100 to 365 days). Opportunities exist to develop BIOLOG and/or CRP methodology to address these requirements. The relevance of the DIBAEX approach to the risk assessment scheme requires clarification. Possibly, less complex information on species sensitivity distributions than that required for DIBAEX could improve the risk assessment scheme, e.g. by using species sensitivity data to identify susceptible test species as worst-case indicators.
- ? The possibility of combining the ISO earthworm field test with litter-bag tests should be investigated as the combination of results could improve the interpretation of both field tests.
- ? One of the limitations of the litter-bag test, that it is time-consuming to perform, might be overcome if litter-bag tests could be conducted as extended-laboratory methods, e.g. by using litter bags within soil mesocosms or 'terrestrial model ecosystems'. TME methods can be highly standardised but require initial capital investment and validation through sufficiently wide-

ranging comparisons with field data. Opportunities exist to investigate the economic feasibility of such an approach relative to its potential scientific benefits and its feasibility of use in contract regulatory testing.

? Stable isotopes have been used widely to study chemical transformations and could have a future role in the risk assessment for OM breakdown but so far the methodology has not been sufficiently well developed for application in ecotoxicology. Possibly, integration of stable isotope methods with the litter-bag test might provide sufficient information on C and N transformations in the field to allow risk to be determined without requiring the full time course of the litter-bag test to be completed. Expert opinion on the relevance of stable isotope methods is required and an appropriate first step would be to convene a workshop to address the subject.

Perhaps the most difficult task for optimising the risk assessment to address the OM breakdown endpoint will be the determination of how relevant the existing and additional single-species tests (eg. on arthropods and earthworms) and lower-tier microbial function tests are to the litter mass loss endpoint. This will influence whether these tests can form part of the trigger pathway for OM breakdown, or whether they should remain as 'supporting' tests for a trigger pathway that involves some combination of middle to higher tier tests (eg. extended laboratory, soil mesocosm/TME) and Biolog or CRP tests.

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