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Recovering decomposing plant residues from the particulate soil organic matter fraction: size versus density separation

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Abstract A detailed size separation of particulate organic matter (POM) from soils amended with straw from *Hordeum vulgare* or *Vicia sativa* revealed that the loss of C during the first 56 days of incubation mainly occurred from particles >2,000 μm, without a concomitant reduction in the size of these large particles. Preliminary studies of POM from non-amended soil had shown that the stable heavy (>1.4 g cm^{-3}) POM fraction was mainly (>80%) composed of particles <400 μm, whereas the light fraction was dominated by larger particles (>80%). Therefore we decided to compare the POM <1.4 g cm^3 with POM >400 μm. There was a very close relationship between POM>400 μm and POM <1.4 g cm^{-3} with regard to amounts of C and N, as well as the appearance of these fractions under the microscope. Similarly there was a close relationship between changes in the C content of the POM fractions and the CO_2 respired, and this was also the case when comparing changes in POM-N with net N mineralization. This indicated that the biological activity during decomposition was actually localized in the POM. Due to the lighter workload and lower expenditure for reagents in connection with size separation of POM, we recommend the size separation procedure in connection with studies of residue decomposition in arable systems.

Keywords Residue decomposition · Particulate organic matter · Size fractionation · Density fractionation

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Introduction

Considerable effort has been directed at understanding the role of soil organic matter in determining soil fertility, and therefore in developing methodology that can elucidate specific fractions or pools that make significant contributions to the "active" soil organic matter (Jenkinson and Rayner 1977; Goh et al. 1984; Balesdent et al. 1988; Hsieh 1993). The quantitative determination of these pools will allow verification of the rate constants and pool sizes to be used in dynamic models describing non-steady state and short-term conditions that are desirable when making an analysis, for example, of a growing season. The addition of homogeneously labelled ^{14}C-labelled plant materials resulted in the labelling of all measurable soil fractions at the outset of an experiment, due to the redistribution and interaction between water-soluble components and the soil matrix, (Magid et al. 1996). As microbial decomposition took place the specific activity of particulate organic matter (POM) and of dissolved organic C decreased, indicating that these fractions contained stable as well as labile components, and thus were unsuitable for study using a first-order kinetic model.

Using densimetric techniques (Henin and Turc 1950; Henin et al. 1959; Greenland and Ford 1964) free particulate light-fraction material has been identified as an active soil organic matter pool, by acting as a readily decomposable substrate for soil microorganisms and a source of plant nutrients (Greenland and Ford 1964). Its use in soil organic matter studies has been reviewed by Christensen (1992) and further developed and tested by Cambardella and Elliot (1992, 1993). A problem associated with this approach is the use of density reagents. Organic solvents have commonly been used as density agents (Greenland and Ford 1964; Turcheneck and Oades 1979; Dalal and Mayer 1986), but their toxicity and the potential for C adsorption to soil materials has led to the use of inorganic density reagents (Janzen et al. 1992), which, however can also modify the organic

material (Sollins et al. 1984). Magid et al. (1996) observed that the two density agents sodium polytungstate and Ludox TM40 affected the subsequent biodegradation of isolated materials, and thus rendered further bioassays problematic.

Magid et al. (1997a) demonstrated that it was possible to recover decomposing rape straw from field sites using size-density fractionation, as an alternative to using litterbags. This implies that the recovered plant tissues have been in unrestricted contact with the surrounding soil, and this could be a methodological advance for studies of decomposition of certain litter types in arable systems. The recovery of decomposing plant material from soil is helpful for determining the actual mass loss, and thereby estimating the substrate utilization efficiency of the soil microbial biomass (Magid et al. 1997b; Mueller et al. 1998). Furthermore, the recovered materials may be used for assessing the successional stages of the decomposer communities. Changes in litter quality parameters during decomposition may indicate which factors limit decomposition. From the attempts to recover decomposing plant tissues directly from soil, it was clearly indicated that the large, light POM fraction represents both rapidly decaying plant tissues and more stable native POM (Magid et al. 1997a). Thus this approach may allow the study of biologically meaningful entities if proper controls are included, even though it isolates components with widely differing decomposition rates.

However, the use of low-viscosity density reagents is expensive and tedious in connection with the large number of measurements needed to perform process studies at the field level. Since gaining a better insight into the decomposition of plant litter in arable systems is a priority in both tropical and temperate low-input systems, it was decided to further explore the possibilities for methodological simplification. The main objective of this study was to elucidate the potential of a size-separation method for recovering decomposing plant residues in fractions, compared to the methods based on both size and density fractionation. In previous studies it was assumed that intact plant residues contain entrapped air, which is lost as the particles disintegrate during the decomposition, and thus they become more heavy (Magid et al. 1996). Since decomposing material will also decrease in size, we decided to test the size separation of POM as a means of recovering decomposing plant material, as an alternative to size-density separation.

Materials and methods

Experimental design

The experiments were conducted on a loamy sand (5.5% clay, 9.3% silt, 41.6% fine sand, 43.5% coarse sand, $pH_{(CaCl2)}$ 6.5, 1.03% C, 0.093% N.) sampled from the plough layer of an arable soil. The soil was initially air dried and sieved at 1 mm, then moistened to 60% of water-holding capacity and pre-incubated for 3 weeks in the dark at 24 °C.

The experimental design included additions of barley (*Hordeum vulgare*) and vetch (*Vicia sativa*) straw (Table 1). A control treatment with soil, but without additions of plant material, served as the reference. Air-dried straw material was chopped to ca. 2-cm lengths and mixed with 1,200 g soil (dry matter, DM) to a concentration of 2.0 g plant material (DM) kg^{-1} soil, corresponding to 4.8 t straw ha^{-1} to a depth of 15 cm. The soil samples were placed in 3-l jars containing a CO_2 trap (30 ml of 1 M NaOH), and a beaker containing water to maintain a water-saturated atmosphere. For each treatment 15 jars (3 l) were prepared, allowing triplicate harvesting at five points in time. Additionally five jars containing only the CO_2 traps and water served as blanks. The jars were then incubated in the dark at 24 °C. Three jars per treatment were removed after 0, 7, 14, 28 and 56 days for fractionation and for inorganic-N analysis, while CO_2 trapping continued in the remaining jars.

Chemical analysis

Soil respiration was monitored by changing NaOH in the incubation jars on days 1, 2, 4, 7, 14, 21, 28, 49 and 56 and measuring the trapped CO_2. The HCO_3^- and carbonate ions in the NaOH solutions were precipitated with excess $BaCl_2$. Total CO_2 was determined by titrating the residual NaOH with 0.1 N HCl to a pH of 8.5.

Inorganic N was measured on days 0, 7, 14, 28 and 56, after shaking the soil (20 g dry weight) with 100 ml of 2 M KCl for 1 h. NH_4^+-N and NO_3^--N were determined in the extracts by standard colorimetric methods (Keeney and Nelson 1982) using flow-injection analysis.

Fractionation

The soil collected at 0, 7, 14, 28 and 56 days from each jar was divided into three portions equivalent to 400 g dry weight; two portions were used for density and size fractionation, respectively, and the last one for subsequent studies. All samples used for fractionation were immediately frozen, and later used in a randomized design, in order to minimize the operator variability.

Isolation of POM

Soil (400 g) was initially dispersed in 100 ml of 5% NaCl, shaken by hand and allowed to stand for 45 min. Then the samples were poured gradually onto a sieve (< 100-μm mesh size) and washed with tap water. The aggregates were destroyed by pushing the soil through the sieve during the washing procedure until the water passing the sieve became clear. The material retained on the sieve was transferred into a white bucket. Tap water was added and the

Table 1 Characteristics of the plant material

Plant material added	C (%)	N (%)	C-to-N ratio	Lignin (%)	Water-soluble C (%)
Hordeum vulgare	43.0	0.44	97.7	11.3	4.1
Vicia sativa	37.1	3.24	11.5	9.5	24.3

bucket was swirled, and organic material was separated from the mineral material by flotation-decantation. Swirling and flotation-decantation was repeated several times, until there were no more visible organic particles in the mineral fraction, and the mineral fraction was then discarded. This POM was then separated according to density or size by the following procedures.

Density fractionation

The isolated POM was further separated by density using an aqueous sodium polytungstate [$Na_6(H_2W_{12}O_{40})$] solution at 22 °C and at ρ 1.42 ± 0.02 g cm^{-3}. After 5–10 min settling time under normal gravity, the POM separated into a light fraction $\rho \leq 1.4$, and a heavy fraction $\rho > 1.4$, and the floating light fraction was skimmed from the surface with a 100-μm sieve. The heavy fraction material was stirred once and allowed to resettle, in order to ensure complete separation of light and heavy fraction material. In a preliminary study the density fractions were further separated by size. Each density fraction was poured onto sieves with mesh sizes of 2,000; 800; 400 and 100 μm; and washed with tap water. The light fraction was then separated into fractions of 100–400 μm, 400–800 μm, 800–2,000 μm and ≥2,000 μm, while the heavy fraction was separated into fractions of 100–400 μm and ≥400 μm.

Size separation

POM was immediately separated by size and yielded four fractions: 100–400 μm, 400–800 μm, 800–2,000 μm and ≥2,000 μm.

All POM fractions were oven-dried at 70 °C for 24 h. Dry samples were weighed, the material was ground, and analysed for total C and N using an elemental analyser.

Statistical analysis

Data were analysed statistically using the General Linear Models procedures for comparison (SAS Institute 1985). The significance of differences was estimated using the Tukey test with $\alpha = 0.05$.

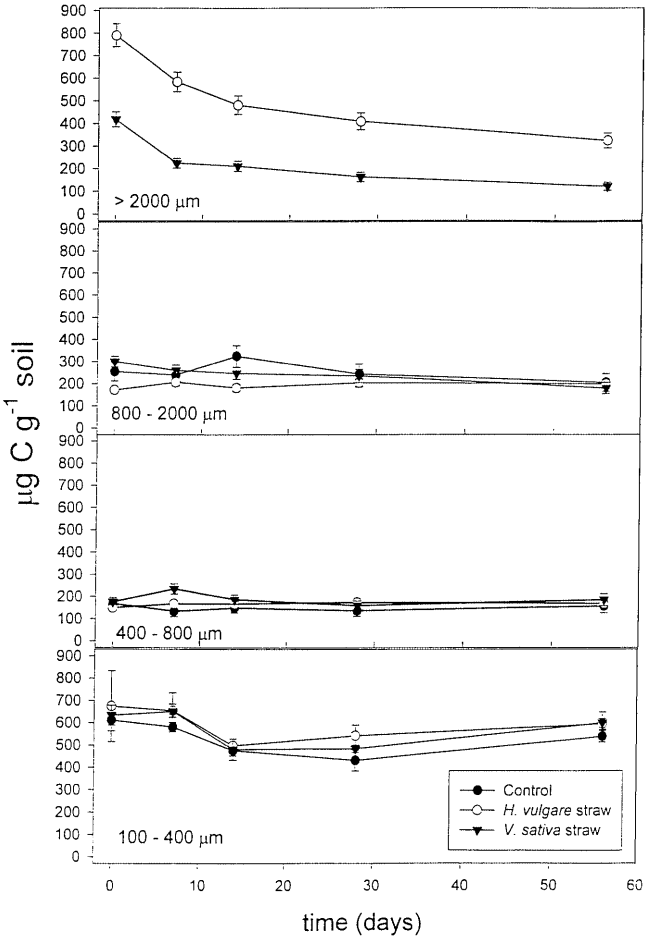

Fig. 1 C contents in size fractions from particulate organic matter (POM) from the *Hordeum vulgare, Vicia sativa* and control treatments. *Error bars* indicate the SEM

Results and discussion

As expected, the detailed size separation of POM from *H. vulgare*- and *V. sativa*-amended soils showed that the main changes in C content during decomposition occurred in POM >2,000 μm (Fig. 1). In the remaining fractions the differences in C contents between treatments were small and not significant. For the 400- to 800- and 800- to 2,000-μm fractions the C content decreased slightly with time, whereas the 100- to 400-μm fraction showed an initial decrease followed by an increase, which was largest but not statistically significant in the amended soils. This could indicate that the smallest fraction was replenished by POM from larger fractions during the course of decomposition. However, on the basis of the C content itself the transfer from larger to smaller fractions was slight compared to the overall C mineralization. This indicates that a considerable part of the C was lost as CO_2 from the large fresh particles without a concomitant reduction of the particle size, and subsequent transfer to smaller size classes. Detailed studies of C depletion from tissues of *H. vulgare* and *V. sativa* have not been found in the literature. However, it is conceivable that, for example, a straw particle could retain its macroscopic shape for a long time, while losing a large fraction of the original C content. At the same time its N content could increase (see the results of the barley treatment below) due to proliferating microorganisms embedded in the decaying tissue. Examples of plant tissues as microbe-embedded structures have been shown by Foster (1988) and Lee and Foster (1991).

The C-to-N ratios of the various size classes in POM consistently showed that in fractions <2,000 μm the *V. sativa*-amended treatment had generally the lowest ratio (Fig. 2). Since the added material was >2,000 μm at the outset, this difference between treatments indicates that a transfer of N from the larger to smaller fractions occurred during the decomposition. However, it is unclear to which extent this transfer was due to immobilization of N and/or C mineralization in "native POM" from the smaller fractions during their decomposition, or due to size reduction and subsequent transfer of some large *V. sativa* particles. The fact that the difference in C-to-N ratios was apparent in the 800- to 2,000-

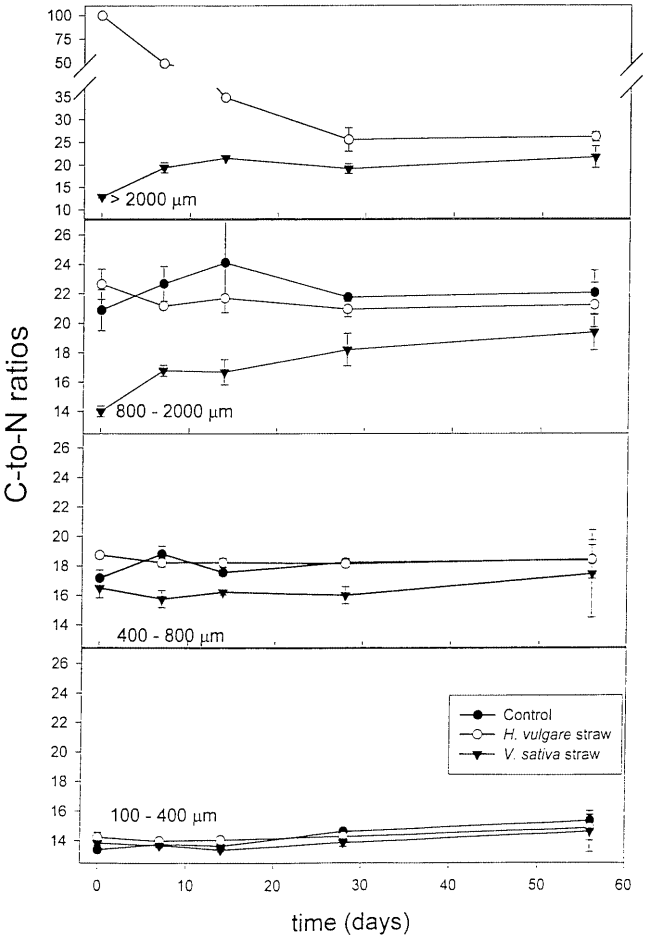

Fig. 2 C-to-N ratios in size fractions from POM from the *H. vulgare, V. sativa* and control treatments. *Error bars* indicate the SEM

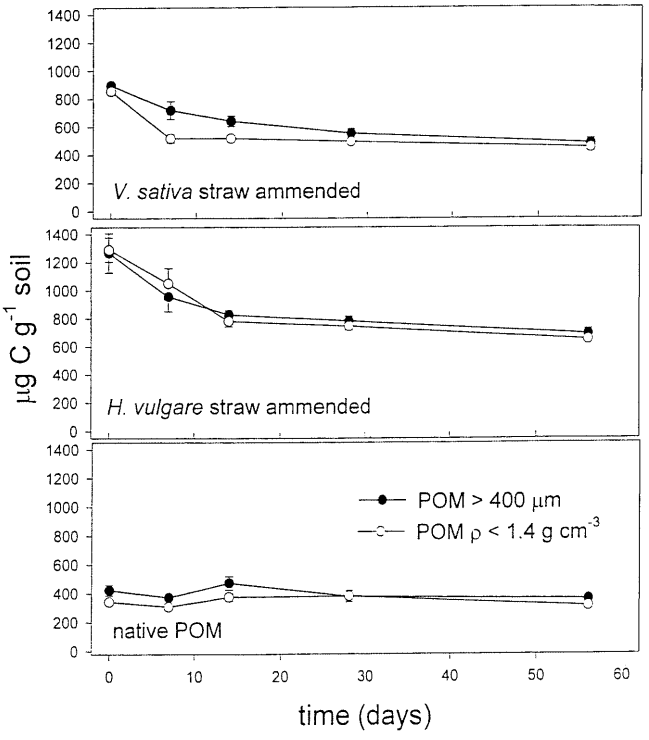

Fig. 3 Comparison of C content in POM fractionated by density ($\rho < 1.4$ g cm^{-4}) or size (>400 µm). *Error bars* indicate the SEM

µm fraction of the *V. sativa* treatment already at 0 days indicates that some fragmentation of the added material occurred during the extraction of POM from soil. The difference in the C-to-N ratios was most marked in the 800- to 2,000-µm fraction, that showed a steadily increasing trend during decomposition as did the >2,000-µm fraction, indicating that the rapidly decomposing materials in the *V. sativa* treatment were rich in N. In contrast, the C-to-N ratio in the *H. vulgare* amended treatment decreased from 100 to 25 in the >2,000-µm fraction, whereas no significant difference relative to the control could be detected in the smaller POM fractions.

A preliminary examination of POM from non-amended soil had shown that the stable heavy (>1.4 g cm^3) POM fraction was mainly (>80%) composed of particles <400 µm, whereas the light fraction was dominated by larger particles (>80%). Therefore we decided to assess the possible correlation between the POM <1.4 g cm^3 and POM >400 µm. Figure 3 clearly shows that the differences between the C content of the two fractions were very small and insignificant, with the exception that density fractionation yielded substantially less material than size fractionation in the *V. sativa*-amended treatment at 7 and 14 days.

Values for POM-C and CO_2-C from the control soil have been subtracted from the treatments, so the dynamics of added materials are described on their own in Fig. 4. The decay pattern depicted by POM >400 µm is similar to the cumulative CO_2 pattern (Fig. 4). The differences in C-to-N ratios between POM <1.4 g cm^3 and POM >400 µm were negligible (data not shown). The similarity between the materials found as POM <1.4 g cm^3 and POM >400 µm was further confirmed by light microscopy. Thus it seems likely that size separation of POM can replace density separation of POM, in studies of decomposing plant litters, leading to a lighter workload and lower expenditure for reagents.

These results confirm previous findings (Magid et al. 1997a) that by this technique decomposing plant materials can be recovered from soil for further characterization, without the use of litterbags. The restricted access imposed by the litterbag may impede the access of soil fauna (Tian et al. 1992) as well as the supply of soil particles with their associated nutrients (e.g. P) and thus limit the rate of decomposition. Stemmer et al. (1998) examined the development of enzymatic activities of soil POM fractions, and interpreted these to elucidate plant litter decomposition. Thus, this POM fractionation approach may be pertinent in studies of arable systems, in which plant materials are usually mixed

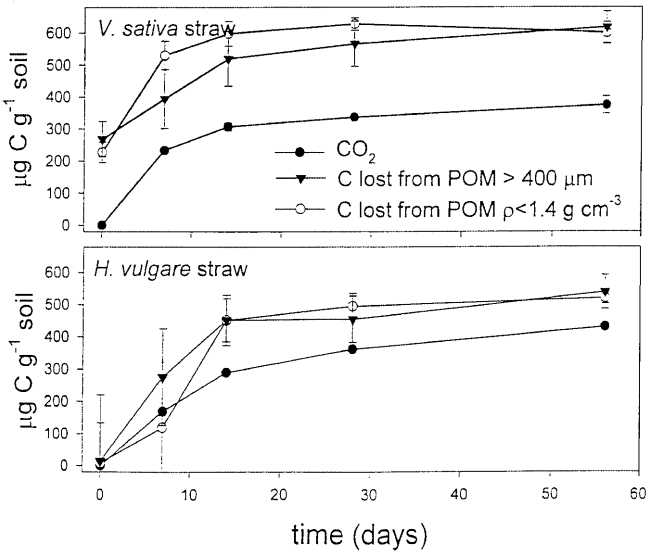

Fig. 4 Cumulated C loss from POM fractionated by density ($\rho < 1.4$ g cm^{-4}) or size (>400 µm) in the *H. vulgare,* and *V. sativa* treatments, compared with cumulative CO_2. (Values for POM-C and CO_2–C from the control soil have been subtracted from the treatments, so the dynamics of added material are described on their own.) *Error bars* indicate the SEM

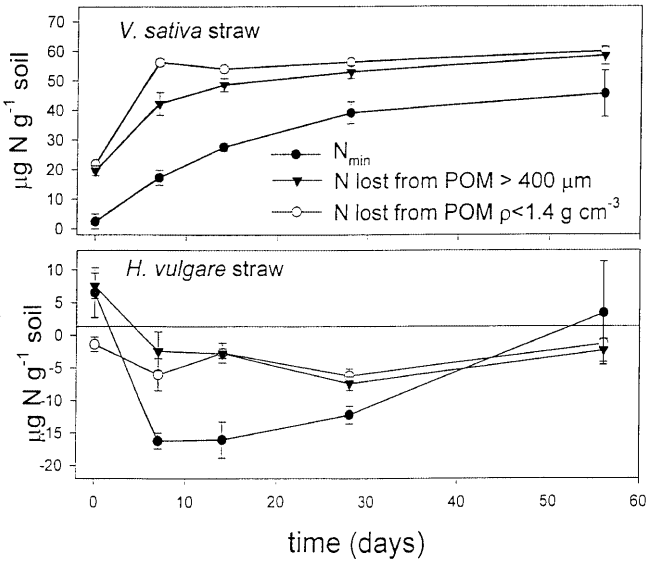

Fig. 5 Cumulated N loss from POM fractionated by density ($\rho < 1.4$ g cm^{-4}) or size (>400 µm) in the *H. vulgare,* and *V. sativa* treatments, compared with mineralized N. (Values for POM-N and mineralized N (N_{min}) from the control soil have been subtracted from the treatments, so the dynamics of added material are described on their own.) *Error bars* indicate the SEM

with the underlying soil through tillage. A drawback connected with the initial POM fractionation procedure used in this study is that water-soluble materials may be leached from the plant residues during isolation. As stated previously, this can be expected to cause substantial errors when looking at highly decomposable plant residues, such as green leguminous materials (Magid et al. 1997a). This is confirmed in Fig. 4. Only a small amount of material was lost from the *H. vulgare*-straw-amended treatments at $t=0$, due to its low content of water-soluble components. By contrast, approximately 250 µg C g^{-1} soil was lost from the *V. sativa* treatments. In future studies on the decomposition of green plant materials this inadequacy must be addressed, in order to obtain materials representative of the "litter-decomposer community" association.

The amount of C respired as CO_2 was generally lower than the C loss from POM fractions (Fig. 4), which is consistent with the idea that some of the C lost from the decomposing residues is used for microbial growth. Except for the discrepancy in the *V. sativa* treatment at 0 days due to the loss of soluble materials, there is a close relationship between changes in the C content of the POM fractions and the CO_2–C respired (Fig. 4) and between the N lost and the mineralized N (Fig. 5). In Fig. 5 the depicted POM-N and mineralized N (N_{min}) values are differences between those of the treated soils and control soil. In this way the dynamics of the added *H. vulgare* and *V. sativa* can be assessed. The close links between POM-C and evolved CO_2 or between POM-N and N_{min} indicate that the microbiological activity during decomposition is actually localized in the POM.

The amount of free POM may have been considerably underestimated in studies on SOM fractionation due to one or several methodological artifacts: (1) pretreatment prior to fractionation involved a harsh dispersal treatment (i.e. ultrasound at high energy levels, or addition of steel or marble balls), or (2) there was no size separation of soil materials prior to density fractionation. In our initial exploratory methodological assessment (data not published) we found that the use of an ultrasonic probe (350 W, 600 s, 70 g soil, 250 ml water) reduced the amount of C found as POM by more than 70%. On the other hand, Magid et al. (1996) showed that without the use of a prior size separation of the soil materials, the densiometric approach alone yielded considerably less ^{14}C-labelled plant material in the light fractions. This was attributed to the interaction between clay and silt particles with the decomposing plant material during the centrifugation step that is needed to separate small particles by density. The interaction between clay, silt and POM decreases if the suspension is allowed to settle some time before centrifugation is initiated (Greenland and Ford 1964). Since such procedures have been commonly used, the importance of the large free POM as a focus of microbiological activity may have been underestimated.

In conclusion, a detailed size separation of POM from soils amended with straw from *H. vulgare* or *V. sativa* revealed that the loss of C during the first 56 days of incubation mainly occurred from particles >2,000 µm, without a concomitant reduction in the size of these large particles. There was a very close relationship between POM >400 µm and POM <1.4 g cm^3

with regard to their contents of C and N. Examination by optical microscopy confirmed the similarity of the fractions. Due to the lower expenses and workload incurred, we recommend the size separation of POM for studies of residue decomposition in arable systems. However, the method needs to be improved so as to reduce losses of easily soluble C compounds by washing from green plant materials. The close relationship between changes in the C content of the POM fractions and the CO_2 respired, and the N content of POM and the mineralized N indicated that the microbiological activity during decomposition was actually localized in the POM. This emphasizes the importance of the large free POM as a focus of microbiological activity, and points towards the large POM as a "residue-decomposer community embedded" structure.

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