Particulate Soil Organic-Matter Changes across a Grassland Cultivation Sequence

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ABSTRACT

Many models have been constructed in an attempt to describe the dynamics of soil organic-matter (SOM) turnover, most of which include 2 to 3 kinetically defined organic-matter pools. Physical and chemical definition of these conceptualized SOM pools has been difficult. We describe a simple method for dispersion of soil to isolate a particulate organic-matter (POM) fraction that may represent an important SOM pool in grassland soils. The POM fraction was isolated by dispersing the soil in 5 g L^{-1} hexametaphosphate and passing the dispersed soil samples through a 53-µm sieve. We compared POM and mineral-associated C among three tillage treatments (20 yr under cultivation) and an undisturbed grassland at Sidney, NE. The POM C in the native sod represented 39% of the total soil organic C. Twenty years of bare-fallow, stubble-mulch, and no-till management reduced the C content in this fraction to 18, 19, and 25%, respectively, of the total organic C. The mineral-associated organic-matter fraction showed no reduction in C content in the bare-fallow treatment compared with the grassland soil but increased in the no-till and stubble-mulch treatments. Nitrogen dynamics generally mirrored those observed for C. Analysis of the POM fraction for lignin and cellulose content indicated that this fraction was 47% lignin and had a lignocellulose index of 0.7. The stable C-isotope composition of the POM fraction suggests that wheat-derived POM turns over faster than grass-derived POM. We suggest the POM fraction closely matches the characteristics of a SOM pool variously described as slow, decomposable, or stabilized organic matter.

S OIL ORGANIC MATTER is an important source of inorganic nutrients for plant production in natural and managed ecosystems. The ability to quantitatively estimate SOM fractions is especially important for understanding SOM dynamics in intensively managed systems as we move toward adoption of more environmentally sound and sustainable practices. The use of conventional cultivation methods for crop production in North American grassland soils has resulted in a significant decline in the SOM content. Conventional tillage practices can lead to losses of 40% or more of the total soil C during a period of 60 yr (Jenny, 1941; Haas et al., 1957; Tiessen and Stewart, 1983). Our ability to enhance management of cropping systems for sustained production is partly dependent on our understanding of how different fractions are impacted by cultivation and how they are related to changes in nutrient-supply capacity of the soil.

Many conceptual and mathematical models have been constructed that attempt to describe the processes of organic-matter formation and turnover (Jansson, 1958; Parnas, 1975; Jenkinson and Raynor, 1977; Smith, 1979; Paul and van Veen, 1978; van Veen et al., 1984; Parton et al., 1987). Models that best represent the composite behavior include 2 to 3 organic-matter

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pools that are kinetically defined with different turnover rates. Generally, these pools are conceptualized as one small pool with a rapid turnover rate and one to several pools of greater size and slower turnover rate. Physical or chemical isolation of these pools, however, has been difficult.

Many studies of SOM have utilized chemical extractants or physical methods to fractionate SOM (Stevenson and Elliott, 1989). Chemical fractions are not clearly related to the dynamics of organic matter in natural and cultivated systems (Jenkins, 1971; Oades and Ladd, 1977; Duxbury et al., 1989). Physical disruption of soil organic matter by shaking or sonication reduces the chemical alteration of organic material that may accompany chemical extractions, but SOM may be redistributed among fractions during the disruption of the soil (Elliott and Cambardella, 1991). The combination of physical separation followed by chemical extraction (Anderson et al., 1981; Dormaar, 1983), as well as sequential physical separation methods (Turchenek and Oades, 1979; Tiessen and Stewart, 1983), have been used with some success to elucidate SOM dynamics.

A relatively new tool used to trace the fate of recent organic inputs into SOM is the stable-isotope analysis of ¹³C. Cerri et al. (1985) used this method to describe the turnover of organic matter in a 50-yr-old cane (*Saccharum officinarum* L.) field. Balesdent et al. (1987) measured SOM turnover for a forest soil that had been planted to continuous corn (*Zea mays* L.) for 23 yr, using ¹³C as a tracer. They concluded that the cropping of a C₄ plant on soils that only had C₃ plants growing on it previously could be used for in situ labeling of the organic matter. This tool can be applied to the study of bulk SOM and to the characterization of organic fractions (Balesdent et al., 1987; Martin et al., 1990).

We present a simple method that combines chemical dispersion of the soil with a subsequent, easily standardized, physical separation based on particle size. The method isolates a POM fraction from soil that we suggest closely matches the characteristics of the slow (Parton et al., 1987), the decomposable (van Veen and Paul, 1981), or the stabilized (Paul, 1984) organic-matter pools conceptualized in simulation models. Our objectives were to isolate and characterize the soil organic-matter fraction that is depleted as a result of long-term cultivation and to apply this information to an evaluation of tillage effects on soil organic-matter fractions.

MATERIALS AND METHODS

Field Sampling

We collected soils from an experimental site located at the High Plains Agricultural Research Laboratory near Sidney, NE. The soil type is a Duroc loam (a fine-silty, mixed,

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Abbreviations: SOM, soil organic matter; POM, particulate organic matter; LCI, lignocellulose index; LF, light fraction; LSD, least significant difference.

Table 1. Ash-free lignin and cellulose content of the particulate organic-matter (POM) fraction of a dispersed soil

50
46
48
43

mesic Pachic Haplustoll). Mean annual precipitation is 446 mm, 63% of which falls between April and May. The site had never been cultivated prior to 1969, when it was plowed and sown to winter wheat (*Triticum aestivum* L.). One wheat crop is removed every other year and the alternate year the ground is fallow. Plow depth was 20 cm at sod breaking. Three tillage treatments were initiated in addition to the control native-sod plots: bare fallow (plowing), stubble mulch, and no-till. The bare-fallow treatment was tilled to a depth of 10 to 15 cm using a moldboard plow in the spring of the fallow year followed by 2 to 3 cultivator or rotary rod-weeder operations. Stubble-mulch fallow was cultivated with 0.9- and 1.5-m sweeps 2 to 4 times a year at a depth of 10 cm, followed by a rotary rod-weeder. Weed control during fallow in the no-till treatment was accomplished with herbicides (Fenster and Peterson, 1979).

The experimental design was a randomized complete block with three field replicates. The three tillage treatments and the native-sod control were represented in each of the three field replicates, which in turn were represented in each of two blocks, west and east, which alternated the crop and fallow portions of the rotation. Treatment plots within field replicates were 8.5 by 46.0 m. We sampled each field replicate within the west block of the experiment in late July 1990. The west block had been in fallow since the summer of 1989. Soil cores were taken with an 8-cm-diam. steel coring bit to a depth of 20 cm without removing surface residues. We collected a soil core every 4.5 m along the length of each plot, for a total of 10 cores per plot. The exact horizontal location along the width of the plot for each core was randomly located. To eliminate edge effects, we established a 0.50-m buffer zone along the edges of each plot.

We gently broke apart the soil cores by hand in the laboratory and passed them through a 2-mm sieve while still moist. The large pieces of stubble and root that had passed through the sieve were removed by hand. The sieved soil was dried overnight at 50 °C, after which the soil cores taken from each plot were composited and stored at 4 °C. We estimated bulk density using the total weight and volume of the pooled samples.

Laboratory Methods

Ten-gram subsamples of soil from each plot were dispersed in 30 mL of 5 g L^{-1} sodium hexametaphosphate by shaking fo 15 h on a reciprocal shaker. The dispersed soil samples were passed through a 53- μ m sieve and, after rinsing several times with water, the material that was retained on the sieve was dried at 50 °C overnight. The soil slurry passing through the sieve contained the mineral-associated and water-soluble C and N. Water in the slurry was evaporated in a forced-air oven at 50 °C and the dried sample was ground with a mortar and pestle and analyzed for total organic C (Snyder and Trofymow, 1984) and total Kjeldahl N (Nelson and Sommers, 1980). Hexametaphosphate does not interfere with the wet oxidation methods used to quantify C and N. The difference between the C and N values for the evaporated soil slurry and those obtained from a nondispersed soil sample was considered to be equal to the C or N retained on the sieve. Since the sand was removed from the mineral-associated fraction during the dispersion, it was necessary to correct the C and N values for this fraction for sand prior to calculating this difference.

For the purposes of this study we also analyzed the POM remaining on the sieve. Large sand grains were picked out by hand with forceps and as much of the remaining sand as possible was removed by dry panning. The POM plus residual fine sand was ground in a wiggle-bug to pass a 250- μ m screen. Ash-free lignin and cellulose content were determined on the ground samples (Goering and Van Soest, 1970). The LCI was calculated as the ratio of lignin to lignin-plus-cellulose (Melillo et al., 1989).

Carbon-isotope ratios for the POM remaining on the sieve were determined by mass spectrometer analysis. The ground POM samples were found to be free of contaminating carbonates and were combusted using a Carlo Erba CHN analyzer (Carlo Erba Strumentazione Milane Italy) in a stream of pure O₂. The CO₂ gas was separated from the N gas chromatographically, purified cryogenically on a triple trap, and analyzed using a SIRA 10 isotope-ratio mass spectrometer (VG Isogas Ltd., Cheshire, England). Analytical precision for C is better than 0.2‰ (Larry Tieszen, 1991, personal communication). Isotope ratios are expressed as δ^{13} C values in parts per thousand (Winter et al., 1976).

$$\delta^{13}C \%_{00} = \left\{ \left[\frac{(^{13}C/^{12}C) \text{ sample}}{(^{13}C/^{12}C) \text{ reference}} \right] - 1 \right\} 1000.$$

The relative proportion of wheat-derived C in the POM fraction is calculated using the equation.

$$(\delta_0 - \delta_e)f + \delta_e = \delta_t$$

where $\delta_0 = \delta$ of wheat straw, $\delta_e = \delta$ of native sod POM, $\delta_t = \delta$ at time *t*, and *f* is the fraction of wheat POM C (Skjemstad et al., 1990).

Å density separation of the POM retained on the sieve was made using sodium metatungstate, as suggested by Oades (1988). We suspended the POM in a solution of sodium metatungstate adjusted gravimetrically to a density of 1.85 g cm⁻³ and gently swirled the suspension. After allowing the density separation to occur overnight at room temperature, we aspirated the POM floating on the surface of the liquid. The material that sank to the bottom of the liquid was analyzed for organic C to assess how much of the POM C was associated with material that had a density >1.85 g cm⁻³. We fractionated the POM that floated on the surface at a density of 1.85 g cm⁻³ into five size fractions by sieving through a nest of sieves that had openings of 500, 250, 125, 90, and 53 μ m in diam., and examined them using a scanning electron microscope.

Differences among tillage treatments were tested by a one-way analysis of variance. We used Fisher's LSD mean separation test with a 0.05 significance level.

RESULTS AND DISCUSSION

Method Development

Determination of POM-C and POM-N, calculated as the difference between material retained on the screen and that passing through, were consistently more precise and technically easier to obtain than direct analysis for C and N of the POM fraction retained on the sieve. We suggest this calculation by difference be used rather than direct analysis of the POM fraction for total C and N. For specific analysis of the POM fraction, however, material retained on the sieve must be used.

The POM fraction contained 47% lignin and 18%



Fig. 1. Particulate organic matter (POM) isolated from (a) native sod soil, (B) no-till soil, (c) stubble-mulch soil, and (d) barefallow soil. The POM fraction isolated from all the tillage treatments and the native-sod control passed through a 500- μ m screen but was retained on a 250- μ m screen. Magnification 32.8×.

cellulose on an ash-free basis averaged across treatments (Table 1). It is diffucult to estimate the ash content of the POM fraction since the sand fraction separates out with the POM fraction after dispersion. Therefore, estimations of ash content are more representative of the amount of residual sand rather than the ash content of the POM.

Most of the POM fraction floated in sodium metatungstate adjusted to a density of 1.85 g cm⁻³. The percentage of the POM-C associated with POM that has a density >1.85 g cm⁻³ was 7.8%, averaged across treatments. Examination of the material retained on the sieve that was more dense than 1.85 g cm⁻³ under a light microscope revealed it was composed mostly of sand grains and charcoal. Direct microscopic inspection of the floating material using a scanning electron microscope indicated that the POM fraction was composed mostly of root fragments in various stages of decomposition (Fig. 1a–d). The native-sod soil had more POM in the 500- and 250- μ m size classes than the tilled soil. Bare-fallow soil had less POM in the larger sizes than stubble-mulch or no-till soil. Most of the POM from the three tillage treatments and the native-sod control was <125 μ m. The smaller POM pieces seemed more decomposed than the larger pieces.

Table 2. Organic-C contents of nondispersed soil (total soil C), particulate organic matter (POM), and mineral-associated organic matter to 20 cm.

Tillage treatment	Total soil C	Mineral- associated C	РОМ С	POM C/ total soil C
	·····	g/m²		%
Bare fallow	3055c†	2492c	563c	18
Stubble mulch	3494b	2813a	681c	19
No-till	3729b	2803a	926b	25
Native sod	4237a	2566b	1671a	39

[†] Values in the same column followed by the same letter are not significantly different at $P \le 0.05$ according to Fisher's least significant difference mean separation test.

This was true for the three tillage treatments as well as the native sod control.

Treatment Effects

Total soil organic C in the native-sod plots was significantly greater than that for any of the three tillage treatments ($P \le 0.05$)(Table 2). No-till and stubble mulch were equivalent in their total soil organic-C contents and had significantly more total organic C

Table 3. Organic-N contents of nondispersed soil (total soil N), particulate organic matter (POM), and mineral-associated organic matter to 20 cm.

Tillage treatment	Total soil N	Mineral- associated N	POM N	POM N/ total soil N
		%		
Bare fallow	295c†	247d	48c	16
Stubble mulch	325b	272Ь	53c	16
No-till	326b	286a	40b	12
Native sod	366a	259c	107a	29

[†] Values in the same column followed by the same letter are not significantly different at $P \le 0.05$ according to Fisher's least significant difference mean separation test.

than the bare-fallow treatment ($P \le 0.05$). No-till had a consistently but not significantly higher total soil organic-C content than stubble mulch (Table 2). The mineral-associated C content in the bare-fallow treatment was significantly lower than that of the nativesod control. The no-till and stubble-mulch tillage treatments, although not different from each other, were significantly higher in mineral-associated C than the native-sod control or the bare-fallow tillage treatment $(P \le 0.05)$ (Table 2). This enrichment may have resulted from decomposition of the POM originally contained in the grassland soil when first plowed and the subsequent movement of this organic matter into the mineral-associated fraction. The POM-C in the native sod comprised 39% of the total soil organic C. Twenty years of bare-fallow, stubble-mulch, and notill management reduced the C content of this fraction to 18, 19, and 25% of the total soil C, respectively. Considering that the inputs from crop production are not different between tillage treatments (Fenster and Peterson, 1979), these patterns imply that decomposition in the bare-fallow and stubble-mulch treatments is more rapid than in the no-till treatment. This could be due to differences in temperature, moisture, or substrate availability between the three tillage treatments, all of which can affect decomposition rates. Nitrogen pools in the whole soil and the dispersed POM and mineral-associated fractions generally mirrored those observed for C with two exceptions; the amount of mineral-associated N for the no-till treatment was significantly greater than for the stubble-mulch and barefallow treatments ($P \le 0.05$) and the amount of POM-N for the no-till treatment was significantly less than for the stubble-mulch and bare-fallow treatments (P \leq 0.05)(Table 3).

The C/N for whole soil was significantly different among treatments, with the native-sod and no-till C/N being equal to one another, and higher than the stubble-mulch and bare-fallow C/N ($P \le 0.05$). The no-till POM appeared to be enriched in C relative to N when compared with the bare-fallow and stubblemulch treatments ($P \le 0.05$), indicating that no-till retains more C than N in this fraction after 20 yr of planting to wheat (Table 4). An explanation for this is that wheat straw decomposes more slowly in no-till than in the other two tillage treatments. Many studies have reported that plant residue decomposes more slowly on the soil surface than when it is incorporated into the soil, primarily as a result of microclimate differences at the soil surface (Brown and Dickey,

Table 4. Carbon/nitrogen ratios of nondispersed soil, particulate organic matter (POM), and mineral-associated organic matter.

Tillage treatment	Whole soil	РОМ	Mineral-associated organic matter
Bare fallow	10.4c†	11.7c	10.1a
Stubble mulch	10.9bc	13.6bc	10.4a
No-till	11.4ab	20.1a	10.0a
Native sod	11.6a	15.5b	10.0a

[†] Values in the same column followed by the same letter are not significantly different at $P \le 0.05$ according to Fisher's least significant difference mean-separation test.

Table 5. Stable isotope ratios (δ ¹³C) for particulate organic matter (POM) and calculated contributions to the POM from grass and wheat.

			Org	anic C to 2	0 cm
	δ¹³C	$\delta^{13}C$ Wheat C remaining	POM total	POM wheat	POM grass
		%		<u> </u>	
Native sod	-21.4a†	0	1671	_	1671
No-till	-23.1b	31	926	287	639
Stubble mulch	-23.1b	31	681	211	469
Bare fallow	- 22.1a	13	563	73	490
Wheat straw	- 26.8c	_	-	-	_

[†] Values in the same column followed by the same letter are not significantly different at $P \le 0.05$ according to Fisher's least significant difference mean separation test.

1970; Doran, 1980; Douglas et al., 1980; Holland and Coleman, 1987; Nyhan, 1975). No-till POM also had a significantly higher C/N when compared with the native-sod control ($P \le 0.05$)(Table 5). Since wheat straw (C/N = 80)(Buyanovsky and Wagner, 1987) has been shown to have a higher C/N ratio than blue grama grass [*Bouteloua gracilis* (Willd. ex Kunth) Lagasca ex Griffiths] (C/N = 40) (Clark, 1977), one of the dominant mixed-grass prairie species (Pinck et al., 1950), and assuming that wheat straw decomposes more slowly under no-till than under the other two tillage treatments, it follows that no-till POM would have more C relative to N than native-sod POM. The C/N of mineral-associated organic matter was not significantly different for any of the tillage treatments or for the native-sod control ($P \le 0.05$)(Table 4).

The δ^{13} C value for POM isolated from the barefallow treatment was not significantly different than the δ^{13} C value for the native-sod POM ($P \le 0.05$)(Table 5), although the latter had three times more POM-C (Table 2). The no-till and the stubble-mulch POM δ^{13} C values were not different from each other, but were significantly more negative than the native-sod or bare-fallow values ($P \le 0.05$)(Table 5). The POM from all of the tillage treatments and the native-sod control exhibited a stable C-isotope signature that was different from wheat straw ($P \le 0.05$)(Table 5).

Cropping to wheat switched the cultivated plots from a mixed-grass system having a δ^{13} C value somewhere between that for C₃ and C₄ plants to a monoculture of winter wheat, a C₃ plant. The C₃ and C₄ photosynthetic systems discriminate differently against ¹³C. We assume that the discrimination against ¹³C was constant for all C₃(δ^{13} C = -28) as well as C₄(δ^{13} C = -12.5) species (Tieszen et al., 1979). Assuming that the POM derived from wheat stubble or grass residue has a stable isotopic composition similar to that of the vegetation, and assuming that the stable C-isotopic composition of the native-sod POM has not changed since the beginning of the experiment, one can make some estimates of the amount of new POM contributed by wheat to this pool. The stable C-isotope composition of the POM fraction isolated from 20-yr-old stubble-mulch and no-till plots showed that 69% of the POM-C was still derived from the previous grassland in these two treatments. Only 13% of the POM-C came from wheat in the bare-fallow treatment (Table 5).

A small proportion of the POM fraction consisted of charcoal ($\approx 5-10\%$ as determined by direct microscopic observation). The presence of this charcoal in the POM fraction could potentially complicate the interpretation of these data. The charcoal was produced from the historical burning of the native grassland and would have a δ^{13} C signature similar to that of the noncharcoal part of the grass-derived POM frac-tion (Skjemstad et al. 1990). The presence of charcoal could influence calculations of residence time of this fraction but not calculations of the proportion of wheatderived C. The δ^{13} C signal of the POM in the barefallow treatment was derived mostly from the native grassland, with little material from wheat. In this treatment, the potential exists for charcoal to significantly affect interpretation of residence time of this fraction. Microscopic observation of the POM fraction in the bare-fallow treatment, however, showed that charcoal was still a minor component. Therefore, we believe that the presence of small amounts of charcoal have not significantly influenced the interpretation of the results. As decomposition of this fraction proceeds, the presence of charcoal may become an increasingly important control on the turnover rate of this pool of SOM.

The above analysis suggests a slower turnover of the POM derived from native grassland soil than from residues derived from wheat. Assuming the POM fraction decays exponentially, the estimated half-life of grass-derived POM is ≈ 13 yrs. It has a LCI that indicates it is in the latter stages of decomposition along the decay continuum (Melillo et al. 1989). The characteristics of this POM fraction suggest it may represent a large portion of the slow organic-matter pool conceptualized by Parton et al. (1987).

In this study, 39% of the total soil organic C from the top 20 cm of a native grassland soil was associated with the POM fraction. More than 50% of the organic C in the POM fraction was lost as a result of 20 yr of bare-fallow management. Using δ^{13} C as a tracer, Balesdent et al. (1988) identified an easily mineralized fraction of soil organic matter with a half-life of 10 to 15 yr that was exhausted after 30 to 40 yr of cultivation. Tiessen and Stewart (1983) reported that, under virgin prairie, $\approx 40\%$ of the total soil C was associated with floatable organic matter in the A horizon of a Typic Cryoboroll and that 60 yr of continuous cultivation reduced C in this fraction by 65%. The floatable organic-matter fraction described by Tiessen and Stewart (1983) was dispersed from the soil by ultrasonic vibration and isolated by sieving the dispersed suspension through a $50-\mu m$ sieve. It appears that their floatable organic-matter fraction is the same as the POM fraction described here.

The LF organic-matter pool, defined originally by Greenland and Ford (1964) to be material having a density of <2.0 g cm⁻³, is composed of partially decomposed plant residue having a C/N ratio of <25. In grassland soils, the LF can account for up to 48% of the total soil C and up to 32% of the total soil N (Greenland and Ford, 1964). Spycher et al. (1983) reported similar values for total C and N content for LF material isolated from the top 3 cm of a forest soil. The loss of total SOM from soils during the first years of cultivation is higher than in subsequent years and often declines exponentially (Greenland and Ford, 1964). This rapid decomposition is probably the result of LF decomposition. After microbial decomposition of the more labile components of the LF pool, what remains are the more stable forms of organic matter, such as lignin and lignin-encrusted cellulose (Jenkinson, 1971; Paul, 1984). The POM fraction described here has a similar density (≤ 1.85 g cm⁻³) and C/N ratio (20) to LF organic matter. We suggest that the POM fraction is similar to LF organic matter as it was originally defined by Greenland and Ford (1964).

Spycher et al. (1983) reported that LF organic matter isolated from the top 3 cm of a forest soil has a C/N ratio of 30. However, they disrupted their soils by gentle stirring of a soil-water suspension for 30 s, a very minimal disruption level compared with hexametaphosphate dispersion. Therefore, it is likely that the C/N ratio of the LF pool depends not only on ecosystem type, but also on the intensity of disruption.

We observed a depletion of organic matter in the POM fraction of the cultivated soils with a subsequent increase in the organic-matter content of the mineralassociated pool. The redistribution of SOM from labile fractions to the more humified fractions has been demonstrated by many other researchers (Ford and Greenland, 1968; Ladd et al., 1977; Anderson et al., 1981). Since mineral-associated organic matter has been shown to have relativley low nutrient availability (Tiessen and Stewart, 1983; Anderson et al., 1981), cultivation not only reduces the total organic-matter content of soil but reduces nutrient availability.

We have presented arguments that link a physically and chemically defined SOM fraction to an analogous conceptual SOM pool represented in simulation models. We are currently investigating what the relationship is between the POM and the transient organic C pool (Tisdall and Oades, 1982; Oades, 1984), suggested to be the pool that is depleted as a result of cultivation (Elliott, 1986).

We suggest that the POM fraction that is separated out by this simple physical fractionation method accounts for the majority of the SOM initially lost as a result of cultivation of grassland soils. For soils cultivated for long periods of time, or soils developed under other than grassland vegetation, the POM fraction could be of less importance in providing mineralized N than microbially produced decomposition products that are physically occluded within the ag-

gregate structure. This suggestion is supported by observed changes in the mineral-associated fraction (Tables 2 and 3) and information obtained from ongoing work (Cambardella, 1991 unpublished data). This caveat, however, does not minimize the usefulness of this method for enhancing our understanding of SOM dynamics at the research site at Sidney, NE. Testing the method at other sites and with other soil types will yield additional information to fill in gaps in our knowledge concerning the formation and turnover of SOM and the role the POM fraction plays in supplying nutrients to the soil.

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