Plant Soil (2008) 303:253–264
DOI 10.1007/s11104-007-9504-1

REGULAR ARTICLE

Dynamics of C, N, P and microbial community composition in particulate soil organic matter during residue decomposition

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Received: 12 June 2007 / Accepted: 26 November 2007 / Published online: 21 December 2007

Abstract Decomposing residues can be an important source of nutrients for plants, especially of N and P, but the relationship between N and P release and microbial community dynamics have rarely been studied. Two pea (*Pisum sativum* L.) residues with contrasting chemical composition, shoots from flowering pea (Pea-Y) with 2.9 mg P and 36 mg N kg^{-1} and from mature pea (Pea-M) with 0.3 mg P and 13 mg N kg^{-1}, were added at a rate of 20 g kg $soil^{-1}$ to a sandy soil low in nutrients. Particulate organic matter (POM) was isolated on days (d) 0, 5, 15, 28, 42 and 61 after residue addition and analysed for C, N, P and microbial community structure (fatty acid methyl ester analysis). The recovery of POM from residue-amended soils decreased over time to 30–40% of added amounts for both residues. Apart from d 0, the N concentration in POM was lower in residue-amended soil than in the control. Due to a rapid decrease in P concentration during the first 5 days in Pea-Y and a slow increase over the whole experiment in Pea-M, P concentrations in POM on d 61 were similar in all treatments. In Pea-Y, the dynamics of C, N and P were coupled, with amounts of C, N and P decreasing during the first 15 days and remaining stable thereafter. In Pea-M, a steady loss of C from POM was contrasted by a slight increase in P. As a result, the C/P ratio decreased from 1,330 on d 0 to 390 on d 61. The C/N ratio of Pea-M decreased only during the second phase of decomposition. The different nutrient dynamics in Pea-Y and Pea-M led to similar amounts of N and P in POM towards the end of the incubation. Microbial community composition in the POM in Pea-Y and Pea-M remained distinct from the control, even though it changed over time. POM was shown to be an important source of potentially available nutrients after addition of plant residues. In the unamended soil, stable nutrient amounts in POM suggested very low net nutrient release from native POM compared to POM after residue addition.

Responsible Editor: Elizabeth (Liz) A. Stockdale.

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Keywords Fatty acid methyl ester · Microbial community composition · Nitrogen · Particulate organic matter · Phosphorus

Introduction

Baldock and Skjemstad (1999) distinguished between different pools of the non-living soil organic matter (SOM): dissolved organic matter (OM), particulate organic matter (POM), humus and inert OM. POM is defined as partially decomposed plant residues that are not closely associated with soil minerals and represents the 53–2,000 μm size fraction of SOM. POM is dominated by relatively fresh, undecomposed plant residues with a recognizable cellular structure (Liao et al. 2006; Yamashita et al. 2006), but may also include fungal hyphae, seeds, spores, and fauna skeletons (Gregorich et al. 1994). This macro organic matter decomposes more quickly than the total SOM because it is free of mineral particles which can protect against decomposition by micro-organisms (Gregorich and Janzen 1995) and contains a greater fraction of undecomposed C than humus or inert OM (Baldock and Smernik 2002). Hence, POM is more sensitive than total SOM to changes in management practices and may indicate changes in C turnover (Chan et al. 2002). In general, the POM fraction contains 20–45 and 13–40% of total soil C and N, respectively (Haynes 2005). Compounds within the POM have different fates, they may be respired or incorporated into the soil microbial biomass or they can move into the other SOM pools such as dissolved OM or humus. Particulate organic matter is one of several measurable SOM pools used to model SOM dynamics in soil (Skjemstad et al. 2004) and may also be useful for modelling of organic P turnover.

Chemical properties of plant residues such as C/N ratio, N concentration, lignin and polyphenol concentration can affect their decomposition rate (Vigil and Kissel 1991; Wang et al. 2004). Residues with a wide C/N ratio usually decompose more slowly than those with a narrow C/N ratio, and plant residues with a high N content have a high decomposition rate and nutrient release (Kumar and Goh 2000). However the results of Wang et al. (2004) suggest that only the initial phases of residue decomposition are affected by residue C/N ratio. Other studies have indicated that lignin and cellulose may be more important for the rate of decomposition than the C/N ratio. For example, Tian (1992) found that plant lignin concentrations were negatively correlated with decomposition rate. Nitrogen release during decomposition increased with increasing N content in residues, but decreased with increasing polyphenol and lignin content. The C/P ratio is considered a critical indicator of whether P is mineralised or immobilised during SOM decomposition (Curtin et al. 2003). Up to 40 and 60% of P in residues is water-soluble and rapidly released after incorporation into soil (Martin and Cunningham 1973). Friesen and Blair (1988) studied the fate of P from ^{32}P-labelled residues added to soil and found that 11 days after incorporation of the residues, 50% of ^{32}P was recovered in the soil inorganic P pool. In later stages of decomposition, P is released from residues more slowly by mineralisation of organic P compounds. However, net immobilisation of P is likely to occur if residues added to soil have a C/P ratio greater than 300 (Brady and Weil 1996; Iyamuremye et al. 1996). Residue properties such as total P, water-soluble P and C, but also N/P ratio determine whether there is net mineralisation or net immobilisation of P after residue addition (Nziguheba et al. 2000; Kwabiah et al. 2003a, b). Hence, in order to understand nutrient release during decomposition, not only nutrient content of the original residues, but also their carbon chemistry need to be determined.

Crop residues decompose in two distinct phases, an initial rapid phase, in which about 70% of C initially present in the residues is lost as CO_2, followed by a slower phase during which the more resistant fraction is decomposed (Wang et al. 2004). Aneja et al. (2006) found a strong decrease in sugars and starch during the first 2 weeks of decomposition of beech and spruce leaf litter whereas the percentage lignin increased. Since microbial species decompose a given compound at different rates (Killham 1994), the microbial community composition is likely to change during decomposition of residues. In compost, it has been shown that bacteria dominate the initial phases of decomposition whereas fungi dominate in the later phases (Cahyani et al. 2002), however there are only few studies that have examined microbial community composition on residues during decomposition in soil.

Salas et al. (2003) added two different types of residues, sorghum and crotalaria (with similar C/N and C/P ratios) to two weathered soils to investigate

the changes of P in POM. They showed that after an initial strong decrease to levels similar to those in non-amended controls, the amount of P in POM increased again during the later stages of decomposition of added plant residues. Thus, after incubation for 60 days they found that the amount of P in POM after addition of sorghum residues was nearly the same as at the beginning of the experiment, whereas in case of crotalaria, the amount of P in POM was lower than at the start. They explained the increase of the amount of P in POM in the later stages of decomposition by colonisation of the POM by fungi which could transfer P from the soil into the POM. This was supported by microscopic observations that showed extensive fungal growth on sorghum-POM but less on crotolaria-POM.

Since POM is a labile soil organic matter pool, it is a potential nutrient source for plants. Therefore it is important to understand short-term nutrient release from POM. The aim of the present experiment was to determine the pattern of C, N and P release from POM after addition of two residues with different C chemistry and N and P content to soil. Additionally, we studied the microbial community composition in the POM to assess how the community changes during decomposition. We used fatty acid methyl ester (FAME) analysis to assess microbial community composition. This method is based on the differential fatty acid composition in the membrane of organisms; differences in abundance of certain fatty acids indicate changes in microbial community composition.

Materials and methods

Soil

Soil samples were taken in March 2004 from a P fertilisation trial on a Calcarosol (Australian soil classification according to Isbell 1996) at the Mallee Research Station in Walpeup, Victoria, Australia. The field experiment was started in 1940, initially with a fallow–wheat–oats rotation and from 1960 onwards a fallow–wheat rotation (McClelland 1968). The treatment chosen for the experiment received no P fertiliser since the start of the field trial. The soil had the following properties: clay 12%, sand 81%, silt 7%, pH 6.4, total organic C 2.9 g kg^{-1}, total N 0.17 g kg^{-1}, total P 74 mg kg^{-1}, resin-extractable P 3.6 mg kg^{-1}.

Plant residues

Shoot residues from field-grown pea (*Pisum sativum* L.) at two different growth stages were chosen: Pea-Y (flowering) and Pea-M (maturity) (Table 1). These two stages were chosen to have plant material with strongly contrasting chemical properties and therefore degradability and nutrient release. The plant material was air-dried, cut in one-cm pieces and then ground to particle size≤5 mm. To assess the effect of C chemistry of the residues on decomposition rate and nutrient release, the initial residues were analysed by solid-state ^{13}C magic angle spinning (MAS) nuclear magnetic resonance (NMR) (Smernik and Oades 2002). Briefly, NMR spectra were obtained at a ^{13}C frequency of 50.3 MHz on a Varian Unity 200 spectrometer. Samples were packed in a 7 mm diameter cylindrical zirconia rotor with Kel-F end-caps and spun at 5,000±100 Hz in a Doty Scientific MAS probe. Cross polarization (CP) spectra were acquired using a 1-ms contact time and a 4-s recycle delay; 1,000 scans were collected for each spectrum. Free induction decays (FIDs) were acquired with a sweep width of 40 kHz; 1,216 data points were collected over an acquisition time of 15 ms. All spectra were zero-filled to 32,768 data points and

Table 1 Properties of the residues of flowering and mature pea shoot residues, Pea-Y and Pea-M, respectively, used in the study

Residue property	Pea-Y	Pea-M
Age of plants at harvest	Flowering	Mature
Total C (g kg^{-1})	428	427
Total N (g kg^{-1})	36	13
C/N ratio	12	33
Total P (mg kg^{-1})	2,920	330
C/P ratio	147	1,294
Water-extractable P (mg kg^{-1})	1,113	62
O-Alkyl (% of total C)[a]	13.8	9.5
Amide (% of total C)[a]	10.9	7.3
Lignin (% of total C)[a]	7.7	7.7
Phenolic (% of total C)[a]	2.9	3.0
O-Alkyl to lignin+phenolic ratio[a]	1.3	0.9

[a] Determined by nuclear magnetic resonance spectroscopy (Baldock and Smernik 2002). Only selected C compounds are shown.

processed with a 50-Hz Lorentzian line broadening and a 0.01-s Gaussian broadening. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm. Spin counting experiments were performed using the method of Smernik and Oades (2000).

Experimental design

The incubation experiment had three residue treatments (addition of Pea-Y or Pea-M residues, and a control treatment without residues). Residues were added at 20 g dry matter kg dry soil^{-1} which corresponds to 13 Mg ha^{-1} incorporated into a 0–5 cm soil layer with a bulk density of 1.3 g cm^{-3}. This rate is equivalent to that used by Salas et al. (2003). Reverse osmosis (RO) water was added to bring the soil moisture to 80% of water-holding capacity. For the control treatment without residues, only water was added. All treatments were thoroughly mixed by manual stirring. There was one jar (250-ml jars containing moist soil equivalent to 210 g dry soil) for each treatment per sampling date for the treatments with residues added (2×6=12 jars with residues). At each sampling time, the soil was mixed before dividing it up into four 50 g sub-samples which were then used for POM isolation. Because of the low amount of POM in the control treatment, there were 12 replicates for each sampling date, and POM from three sampling replicates per treatment replicate was combined for chemical analyses (3×6=18 control jars). The jars were placed in large plastic containers with a few small holes in the covers to allow for gas exchange. Vials with RO water were placed inside each plastic container to maintain a humid atmosphere and minimise loss of soil moisture during incubation. There were six sampling dates: 0, 5, 15, 28, 42 and 61 days after start of the incubation. The containers were incubated in the dark at 25°C for 61 d. Every 10 days the soil moisture content was re-adjusted by weight. The weight loss due to evaporation ranged from 1–2% of initial weight in each 10-day period.

Physical fractionation of particulate organic matter

At each sampling date, POM was isolated by physical fractionation, using the procedure described by Salas et al. (2003) with some modifications. Soil equivalent to 50 g dry soil was dispersed in 200 ml of a 5 M NaCl solution and shaken over night in an end-over-end shaker followed by centrifuging at 900 g for 15 min. The supernatant containing buoyant residues was decanted first onto 250 and then onto 53 μm sieves. The soil pellet was re-dispersed in 200 ml of a 1 M NaCl solution and the suspension decanted first onto 250 and then onto 53 μm sieves. The soil on the sieves was gently rubbed and rinsed with RO water until there was no further particle flow through the sieve. The material retained on the two sieves was rinsed into a beaker to separate sand from POM by flotation. The POM was frozen at −81°C for at least 2 h before freeze-drying the samples for 3 days.

The percent recovery of residues at each sampling time was calculated as: residue recovery (%)= (amount of POM (g kg^{-1}) in residue treatment− amount of average POM (g kg^{-1}) in control treatment)/amount of residues added (g kg^{-1})×100. Similar equations were used to calculate percent recovery of residue C, P and N.

Analytical methods

Soil texture and pH were estimated by mid-infrared spectroscopy (Janik et al. 1998). For total P, the soils were digested in 6:1 HNO_3:$HClO_4$. Phosphorus extracted with 0.5 M H_2SO_4 from non-ignited soils was considered to be inorganic P, while the increase in H_2SO_4-extractable P after ignition (500°C, 1 h) was assumed to originate from organic P (Saunders and Williams 1955). Resin-extractable and microbial P were determined by extraction of moist soil with anion exchange membranes (Kuono et al. 1995).

The concentration of P in POM was determined by combustion of POM in porcelain crucibles at 550°C for 4 h followed by dissolution of the ash in 20% HCl. The P concentration in all solutions was measured colorimetrically (Murphy and Riley 1962). Total C in the soil and C and N in the POM were determined by dry combustion on a LECO2000 CN Analyzer. Nutrient concentrations are based on POM dry weight.

Fatty acid methyl ester analysis

Fatty acid methyl esters were extracted from 0.1 g of freeze-dried POM as described previously (Pankhurst et al. 2001). Due to varying amounts of POM

recovered, the number of replicates ranged from one to four. No FAME data exists from day 42 due to insufficient sample or loss of sample. The concentrations of the individual FAMEs were calculated on the basis of weight percentage (wt.%) of the total FAMEs. Fatty acid nomenclature was used as described in our previous studies (Marschner et al. 2006). To minimise the error caused by fatty acids derived from residues and soil organic matter, only signature fatty acids for bacteria and fungi were used in the statistical analyses. The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0 and cy19:0 were chosen to represent bacterial fatty acids. The fatty acid 18:2ω6 was used as an indicator of fungal biomass (Frostegård et al. 1993). Indices of richness and dominance of signature fatty acids were calculated according to Zak et al. (1994) with richness $H=\Sigma((n_i/N)\times\ln(n_i/N))$ and dominance $D=\Sigma(n_i\times(100/N))^2$, where n_i is the wt.% of a given signature fatty acid (i) and N the sum of wt.% of all signature fatty acids.

Statistical analyses

Since there was only one jar per treatment and sampling data, the four analytical replicates can not be considered to be independent. Treatments were compared by one-way ANOVA taking the average of a given treatment for a given sampling time and using the sampling times as replicates (GenStat 5th edition, Rothamsted Experimental Station 2000).

Microbial community composition based on log transformed FAME patterns was analysed by principal component analysis (PCA) (CANOCO 4.5, Microcomputer Power, Ithaca, NY, USA). The log transformation was used because there were a few samples with concentrations of certain FAMEs which differed substantially from the others and therefore skewed the results. Log transformation is commonly used before statistical analysis in order to achieve normally distributed data.

Results

Residue properties

The shoot residues from flowering peas, Pea-Y, had a lower C/N and C/P ratio than the mature pea residues, Pea-M (Table 1). Compared to Pea-M, total P was approximately seven times higher and water-extractable P 18 times higher in Pea-Y. The percentage of the initial (undecomposed) plant residue dry matter that remained on the 250 and 53 μm sieves in the absence of soil was 62% and 85% for Pea-Y and Pea-M, respectively. Pea-Y had a higher percentage of *O*-alkyl-C and amide-C content and a higher *O*-alkyl/lignin+phenolic ratio than Pea-M (Table 1).

Recovery of POM and concentration and amount of C in POM during decomposition

There were significant differences in the recovery of residues in POM. On d 0, the recovery was 62% for Pea-Y and 82% for Pea-M (Fig. 1). Residue recovery remained higher for Pea-M than Pea-Y up to d 28, whereas on d 42 and d 61 the recovery was similar for both residues.

The concentration of C in POM (g C kg^{-1} POM) was higher in residue-amended soils than in the control on d 0; but decreased in the first 5 days in POM from residue amended soil (Fig. 2). After d 5 it averaged 300 g C kg^{-1} POM in all treatments.

The amount of C in POM (g C kg^{-1} soil) on d 0 ranked in the order control<Pea-Y<Pea-M (Fig. 3) and remained lower in the control than in the residue-amended soils until d 61. The amount of C in POM changed little throughout the experiment in the control, but decreased over time in residue-amended soils. Compared to d 0, the amount of C in POM at

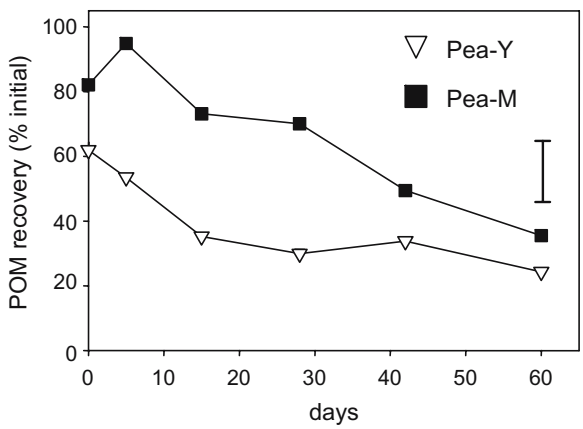

Fig. 1 Particulate organic matter recovery (% of initial) with addition of residues of flowering and mature pea shoot residues, *Pea-Y* and *Pea-M*, respectively, over time. *Bar* indicates least significant difference

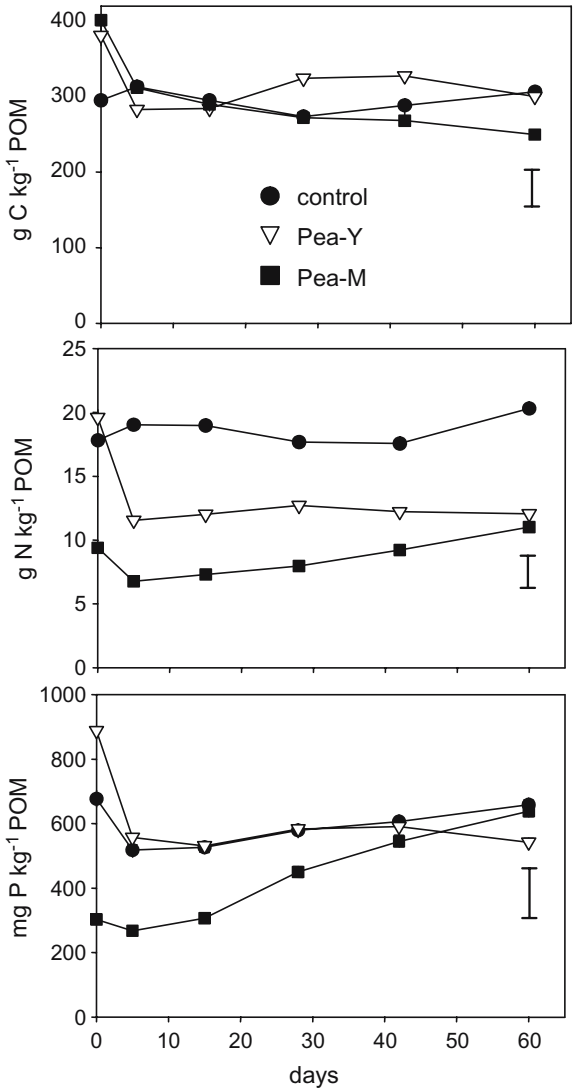

Fig. 2 Concentration of C (g), N (g) and P (mg) in particulate organic matter (kg^{-1} POM) in soil without addition of residues (*control*) and with addition of residues of flowering and mature pea shoot residues, *Pea-Y* and *Pea-M*, respectively, over time. *Bar* indicates least significant difference

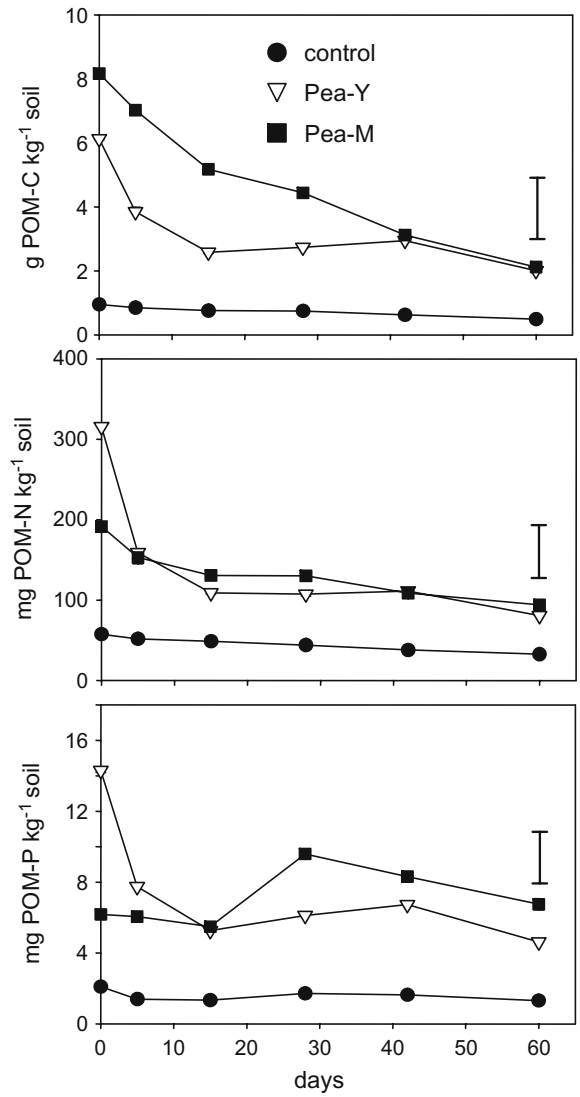

Fig. 3 Amount of C (g), N (mg) and P (mg) in particulate organic matter (kg^{-1} soil) without addition of residues (*control*) and with addition of residues of flowering and mature pea shoot residues, *Pea-Y* and *Pea-M*, respectively, over time. *Bar* indicates least significant difference

the end of the experiment was reduced by 67% and 74% in Pea-Y and Pea-M, respectively.

Concentration and amount of N in POM

From d 5 onwards, the N concentration in POM (g N kg^{-1} POM) of the control was higher than in the residue-amended soils and remained stable over time (Fig. 2). In Pea-Y, the N concentration in POM decreased strongly in the first 5 days and remained stable thereafter. The N concentration in POM from Pea-M did not change significantly throughout the incubation period.

On d 0, the amount of N in POM ranked in the order control<Pea-M<Pea-Y (Fig. 3). The amount of N in POM decreased significantly in both residue treatments during the first 5–15 days of incubation, with a greater decrease in Pea-Y than Pea-M. The amount of N in POM continued to decrease slowly in both residue treatments, while it did not change over time in the control where it remained lower than in the soils with added residues throughout the incubation.

Concentration and amount of P in POM

The P concentration in the POM ranged between 300–900 mg P kg POM^{-1} (Fig. 2). Until d 15, the P concentration in POM in Pea-Y and the control was higher than in Pea-M, but there were no differences between the three treatments thereafter. The P concentration in the native POM changed little over time. Compared to d 0, the P concentration in POM of Pea-Y on d 5 was reduced by 37%. After d 15, the P concentration in POM in Pea-Y remained stable (Fig. 2). In Pea-M, the P concentration in POM showed a different trend, remaining stable until d 15 and increasing thereafter. Compared to d 0, the P concentration in POM in Pea-M on d 61 had increased by 111%.

Recovery of P in POM on d 0 was 27% and 100% of P added with residues for Pea-Y and Pea-M, respectively (data not shown). On d 0, the amount of P in POM (mg POM-P kg soil^{-1}) ranked in the order control<Pea-M<Pea-Y (Fig. 3), and throughout the incubation it was lower in the control than in the residue-amended soils. In Pea-Y, the amount of P in POM decreased strongly in the first 15 days and remained stable thereafter. In Pea-M, the amount of P in POM increased from d 15 to d 28, followed by a decrease which led to similar amounts on d 61 as at the beginning of the incubation. At the end of the experiment, 32% and 109% of the amount of P in POM on d 0 were recovered in Pea-Y and Pea-M, respectively.

C/N and C/P ratios of POM

Initially, the C/N and the C/P ratios of POM in the control and Pea-Y were lower than in Pea-M (Table 2). The ratios remained stable over time in the control and Pea-Y. In Pea-M, the C/N ratio of POM decreased gradually over time, while there was a strong decrease in the C/P ratio in the first 28 days. On d 61, the C/P ratio was similar in all treatments, but the C/N ratio of POM was higher in the two residue-amended soils than in the control.

Relationships between C, P and N dynamics in POM

In Pea-Y, the temporal changes in concentration and amount of C, P and N were similar, decreasing in the first 15 d and then remaining more or less stable until the end of the incubation (Figs. 2 and 3). Thus, C, P and N dynamics were coupled and all three nutrients were lost and/or mineralised at the same rate as indicated by the absence of changes in C/N and C/P ratio.

During the decomposition of Pea-M, P and N dynamics followed a different pattern. Concentrations of P and N in POM did not change in the first 15–28 d (Fig. 2). This was followed by a strong increase in P concentration to levels of the control, whereas the N concentration increased more slowly and remained lower than in the control until the end of the incubation. The C/N ratio did not change in the first 15 d but decreased from d 28 to d 61. In contrast, the C/P ratio decreased significantly throughout the first 28 d and did not change in the later stages of decomposition (Table 2).

Microbial community composition in POM

The two dimensional plot of the FAME data (expressed as log weight %) explained 71.8% of the total variance. The microbial community composition

Table 2 C/N and C/P ratios of particulate organic matter isolated from soil without addition of residues (control) and with addition of residues of flowering and mature pea shoot residues, Pea-Y and Pea-M, respectively, over time

	Treatment	Day						LSD
		0	5	15	28	42	61	
C/N	Control	16.6	16.5	15.6	15.5	16.5	15.1	
	Pea-Y	19.4	24.5	23.7	25.5	26.8	25.0	6.6
	Pea-M	42.7	46.1	39.7	34.2	28.9	22.6	
C/P	Control	434	621	587	472	476	480	
	Pea-Y	429	510	518	541	557	554	28
	Pea-M	1,330	1,168	945	605	491	390	

LSD Least significant difference

in POM of the control differed from that in POM of residue-amended soils (Fig. 4). In the control, the microbial community composition in POM changed little over time, except for the samples from d 5 which differed quite strongly from the other sampling times (Fig. 4). The microbial community composition in POM from Pea-Y and Pea-M was similar on d 0, 5 and 15, but differed at the later sampling dates. For both residue-amended soils, the microbial community composition in POM changed strongly from d 0 to d 5. In POM from Pea-Y, the microbial community composition changed again from d 5 to d 15 but then remained stable until d 61. In POM from Pea-M however, the microbial community composition changed throughout the experiment.

The FAME richness (number of fatty acids; see "Materials and methods" for definition) on d 0 was greater in POM of the control than in residue-amended soils (Table 3). Richness remained constant over time in the control whereas it increased strongly from d 0 to d 5 in POM in the residue-amended soils. The dominance index showed the reverse trend to the richness index being nearly twice as high in POM of soils with residues than the control on d 0 (Table 3). A high dominance index indicates that there are some fatty acids with very high abundance whereas the concentration of most other fatty acids is low. The dominance index decreased strongly from d 0 to d 5 in POM of residue-amended soils. From d 15 onwards, there were no differences in richness and dominance between treatments.

The percentage bacterial fatty acids in POM in the control and in POM from Pea-M was similar and remained unchanged in the first 28 d; but then decreased to d 61, the decrease being greater in POM from Pea-M than in the control (Table 3). The percentage bacterial fatty acids in POM from Pea-Y showed a different temporal pattern in the first 5 days. On d 0 it was lower than in the other two treatments but increased to d 5 when the percentage bacterial fatty acids was similar in all treatments.

The differences between the treatments with respect to the percentage of the fungal fatty acid in POM were greatest on d 0, being more than twice as high in POM from Pea-Y than in the control and Pea-M (Table 3). In Pea-Y, the percentage fungal fatty acid in POM decreased strongly from d 0 to d 5, leading to similar values in all treatments from d 5 to d 28. The percentage fungal fatty acid increased from d 28 to d 61 in all treatments; the increase being strongest in Pea-M.

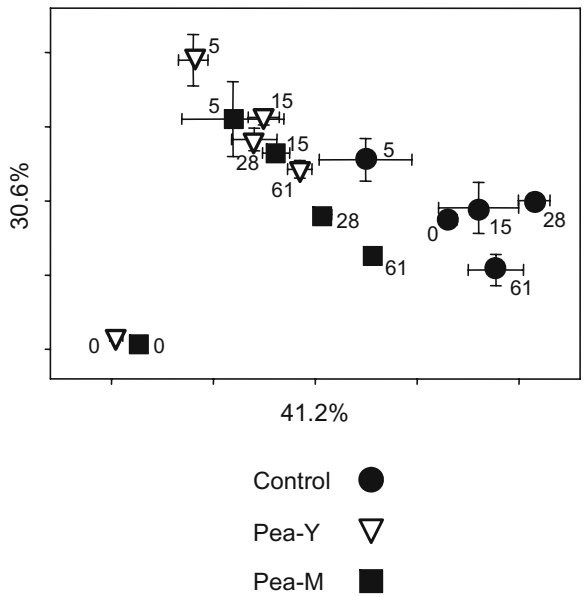

Fig. 4 Microbial community composition in particulate organic matter (based on FAME patterns) without addition of residues (*control*) and with addition of residues of flowering and mature pea shoot residues, *Pea-Y* and *Pea-M*, respectively, over time. *Vertical* and *horizontal lines* indicate standard error (n=2–4). *Numbers* indicate days after residue addition

Discussion

Recovery of POM

On d 0, POM recovery was lower for Pea-Y than for Pea-M of the amount added (Fig. 1), which can be explained by loss of POM during the isolation of POM from soil, especially for the younger Pea-Y material which was softer, more easily fragmented into pieces small enough to pass through the sieves, and had a higher proportion of soluble P (Table 1). The similar recovery of POM in the presence (Fig. 1) and absence of soil (see above) indicates that the physical separation of soil and residues by floating-off was very efficient.

Amount of C in POM

The decrease in recovery and amount of C in POM throughout the experiment indicated substantial decomposition of the added residues (Figs. 1 and 3). We

Table 3 Richness and dominance of FAME patterns and bacterial and fungal fatty acids (in percentage of signature fatty acids, see "Materials and methods" for details) of particulate organic matter isolated from soil without addition of residues (control) and with addition of residues of flowering and mature pea shoot residues, Pea-Y and Pea-M, respectively, over time

	Treatment	Day					LSD
		0	5	15	28	61	
Richness	Control	1.64	1.63	1.72	1.73	1.78	
	Pea-Y	1.10	1.70	1.65	1.66	1.76	0.28
	Pea-M	1.17	1.66	1.57	1.57	1.68	
Dominance	Control	3,338	3,246	3,003	2,961	2,608	
	Pea-Y	4,872	3,025	3,275	3,189	2,695	977
	Pea-M	5,115	3,170	3,643	3,615	2,959	
Bacteria (%)	Control	91.7	89.9	90.9	91.9	86.2	
	Pea-Y	79.2	88.8	89.1	88.2	84.7	4.7
	Pea-M	90.0	90.0	90.1	89.1	82.2	
Fungi (%)	Control	8.3	10.2	9.1	8.1	13.8	
	Pea-Y	20.8	11.2	10.9	11.8	15.3	4.7
	Pea-M	10.0	10.0	9.9	10.9	17.8	

LSD Least significant difference

had expected a slower decomposition of Pea-M compared to Pea-Y throughout the incubation because of its lower P and N content and consequently, higher C/P and C/N ratios, however by d 61, a similar percentage of either residue remained in POM. The microbial community composition in POM differed between Pea-Y and Pea-M (Fig. 4) suggesting that residues are colonised by a specific microbial community that is capable of decomposing the given compounds. A study by Bünemann et al. (2004) supports this hypothesis of a build-up of a decomposer community that is capable of decomposing recalcitrant compounds efficiently. In their study with four different soils, the rate of crystalline cellulose decomposition was initially greater in soils with a history of plant residue inputs than in soils from which crop residues had been continuously removed. However, in the latter group of soils, cellulose decomposition rates subsequently increased and were maintained longer, resulting in similar cumulative respiration in all soils after 120 days.

Concentration and amount of N in POM

The decrease in the amount of N in POM in residue-amended soil during decomposition suggests net release of N from the pea residues. According to Stevenson and Cole (1986), a residue C/N ratio less than 25 leads to net mineralisation, explaining the decrease in the amount of N in POM in Pea-Y (initial C/N 12) in the present study. Although the C/N ratio of Pea-M residues and of POM from Pea-M was higher (initial C/N 33) than the critical value suggested by Stevenson and Cole (1986), no net N immobilisation into POM was found. These results are in agreement with Salas et al. (2003) who also found a decrease in the amount of N in POM from crotalaria residues. On the other hand, the amount of N in POM from sorghum did not change over time in their study.

Concentration of P in POM during decomposition

In residue-amended soils, POM is a mixture of native and added POM. On d 0 in residue-amended soils, the proportion of POM derived from native POM was only 10–20% (data not shown). Therefore, the concentration of P in POM would be expected to be more similar to that of the original residues than to that of native POM. However for Pea-Y, leaching of P during isolation of POM must have occurred since the concentration of P in POM on d 0 was only 29% of the initial P concentration in the residues (Table 1; Fig. 2). A substantial loss of water-soluble materials was also reported by >Magid and Kjaergaard (2001). Similarly, Salas et al. (2003) found that 75% of total P added with residues was lost on d 0. For Pea-M on the other hand, significant leaching did not occur since the concentration of P in POM on d 0 was similar to the P concentration in the original plant residue. The

difference in P dynamics between Pea-Y and Pea-M is in accordance with residue characteristics such as tissue softness and soluble P concentration in the residues (Table 1), the latter being 18 times higher in Pea-Y than in Pea-M.

The increase in P concentration in POM after d 15 in Pea-M suggests P immobilisation, which is in agreement with the initial P content of Pea-M residues of 0.03%. According to Fuller et al. (1956), net immobilisation is likely at P concentrations of less than 0.2%, whereas net mineralisation occurs at >0.2%. At the start of the incubation, POM in the Pea-Y and Pea-M treatments contained a large proportion of freshly added material and therefore differed in P concentration (Fig. 2). Freshly added organic matter is likely to contain more easily decomposable compounds than the native POM, thus over time, the proportion of freshly added residue material in POM decreased, whereas the relative proportion of native POM increased. The apparent increase in P concentration in POM in Pea-M is therefore partly due to a decrease in the relative proportion of the low P residues in the POM.

Amount of P in POM

The dynamics of the amount of P in POM (POM-P kg^{-1} soil) in the present study differed in a number of features from those in the study by Salas et al. (2003). Salas et al. (2003) found that of the amount of P in POM on d 0, 75 to 95% had been released on d 5, resulting in similar amounts of P in POM as in the non-amended control. The lower P release in the first 5 days in the present study (Fig. 3) may be due to the lower proportion of water-soluble P in the residues (38% and 19% of total P in Pea-Y and Pea-M, respectively) compared to 63% to 73% in the residues used by Salas et al. (2003).

Salas et al. (2003) found an increase in the amount of P and N in POM from d 5 to d 60. They explained this by immobilisation of P and N in microorganisms on the POM, particularly fungi that could transfer P and N from the surrounding soil into the residues. In the present study, the amount of P in POM did not increase consistently in any of the treatments (Fig. 3). The increase in P concentration in POM in Pea-M suggests some immobilisation, but, as mentioned above, it can also partly be explained by the relative decrease of the low P residue in the POM during the incubation and the faster rate of C than P mineralization.

The pH of the soils used by Salas et al. (2003) was 4.8–5.8, which favours fungi since their pH optimum for growth is 4–6 (Killham 1994) and may explain the strong colonisation of the POM by fungi in their study. The soil used here had a pH of 6.4, which favours bacteria rather than fungi. Single-celled bacteria are less capable of translocating P from the soil into the POM than fungi that may do so via their hyphae. Similarly, Frey et al. (2003) showed that soil N can be translocated into residues but that the transfer is significantly decreased by application of a fungicide. In the present study, the percentage of the fungal fatty acid increased from d 28 to d 61 (Table 3), suggesting that there is an increase in colonisation of POM by fungi in the later stages of decomposition. However, in Pea-Y the percentage fungal fatty acid fluctuated during the incubation; thus there is no clear pattern in fungal colonisation of POM by over time.

Microbial community composition in POM

Using *Carex* and *Sphagnum* litter differing in total N and C content, Thormann et al. (2003) found temporal changes in community composition of micro-fungi during decomposition which were distinct for the two litter types. In a recent study by Aneja et al. (2006), both the bacterial and the fungal community composition change during the first 8 weeks of decomposition of beech and spruce leaf litter. The results of the present study show that the microbial community composition in POM changes rapidly in the first 2 months after residue addition.

In agreement with the study by Aneja et al. (2006), in which beech and spruce leaf litter were colonised by distinct bacterial and fungal communities, the present study demonstrated that this is also the case for POM from pea residues of different growth stages (Fig. 4). It also showed that the microbial community in POM from freshly added residues differs from that of the native POM in the first 60 days after residue addition. Although the percentage of residues in POM decreased over time, the microbial community composition in POM in residue-amended soils remained distinct from that of the POM in the control. This is most likely due to an amendment-induced change in the soil microbial community; addition of substrate will change the relative competitiveness of native

microbial species. Some microorganisms may also have been introduced with the residues. There are some temporal changes in the microbial community composition in POM of the control, but these are small compared to those in POM from Pea-Y and Pea-M. The microbial community composition in the native POM had a high richness (high number of fatty acids) and a low dominance index (i.e. all fatty acids had approximately the same abundance) (Table 3) which suggests a stabilised community (Marschner and Rumberger 2004).

The distinct microbial communities in the POM from the two residues can be explained by the differential original chemical composition as well as the differences in composition during the incubation. However, it should be noted that (1) these findings apply only for a the first 60 days after residue addition, and (2) the microbial community detected in POM in this study is likely to comprise only a fraction of the microorganisms colonising POM in the soil. Most of the loosely adhering microorganisms would be lost during the washing procedures used to isolate POM. Thus, only the tightly adhering microorganisms were assessed here. Nevertheless, POM isolation is the only method to recover residues after mixing into the soil.

Conclusions

This study showed that POM from added residues is rapidly decomposed, with 30–40% of added residues remaining in POM after 60 days. The potential importance of POM as a source of nutrients was confirmed since 50–75% of N and, in the case of Pea-Y, about 70% of P added with residues were lost from POM over 60 d. In contrast, P increased in Pea-M, indicating immobilisation. Not all nutrients lost from POM will be available to plants; they may remain in residue particles <53 μm, be taken up by the microbial biomass, and P can be fixed. Nevertheless, using the same residues and soil, we showed in an earlier study that P availability in the immediate vicinity of decomposing Pea-Y residues is increased up to eightfold compared to the control, whereas P availability was slightly reduced in the vicinity of Pea-M (Ha et al. 2007).

In the absence of added residues, nutrient amounts in POM were stable during the 61-day incubation, suggesting that native POM may be poorly degradable. Interestingly, despite large differences in the initial residues, nutrient amounts in POM became similar over time with a trend towards the amounts found in the native POM. This suggests that there is only a limited capacity to use POM as a longer term nutrient storage pool. The study also showed that soil microbial communities change in response to residue addition and rapidly adapt to the chemical composition of the residues leading to similar decomposition rates despite differences in initial chemical composition.

Acknowledgements This work was carried out within the project "Biological cycling of P in agricultural soils in Southern Australia" funded by the Australian Grains Research and Development Corporation (GRDC). KVH acknowledges the support by the Vietnamese government for his studies in Australia. The authors thank Shahriar Iqbal (University of Adelaide) for providing the CP/MAS ^{13}C NMR data of the residues and Rebecca Stonor and Bruce Hawke for excellent technical assistance.

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