Canola residues decomposition: the effect of particle size on microbial respiration and cycling of sulphur in a sandy soil

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Abstract

A study on canola residue decomposition was conducted under controlled conditions for 183 days to elucidate the effect of its particle size (<1 mm, 5–7 mm, 20–25 mm) on microbial respiration, microbial-S and plant-available S (SO₄²⁻) after incorporation into a nutrient-deficient sandy soil.

Variation in the particle size of canola residue did not affect net cumulative CO₂-C respiration (as % of added C), the size of microbial-S pool, and the extent of sulphate-S immobilisation, except for the slower decomposition of the <1 mm residue in the soil between days 7–28 of incubation. In total, 33–35% of the residue-C added was respired during 183 days of incubation. About 75% of S added in canola residue to the soil was extracted as sulphate-S upon extraction with 0.01 M CaCl₂ immediately after incorporation (day zero), indicating that canola residue is a good source of readily-available S. The initial sulphate-S in the amended soil on day zero was reduced to 65% on day 183 after incorporation. On average, more microbial-S was present in the amended (3.2 mg/kg soil) than in the non-amended soil (2 mg/kg soil) throughout. In conclusion, the cumulative respiration at the end of 6-month incubation was similar regardless of the particle size of canola residues. Furthermore, adding canola residue to the soil significantly increased (4-fold) the initial soil sulphate-S level, which decreased by one-third over time. This finding indicates microbial immobilisation and transformation of inorganic-S to stable organic-S in the amended soil.

Key Words

Crop residues, carbon mineralisation, sulphur mineralisation, microbial biomass, soil respiration.

Introduction

Crop residues may be a good source of nutrients in many agro-ecosystems, thus reducing dependency on fertilisers. Western Australia is the biggest canola (Brassica napus L.) growing state in Australia. Canola has relatively high demands for nitrogen (N) and sulphur (S) per yield unit because of larger content of these nutrients in seeds compared to most grain crops. The proportion of S contained in canola stems at harvest is reported to be larger than in pod walls and seeds (McGrath and Zhao, 1996). Relatively high content of these nutrients in canola residues emphasizes the need to return them to soil for nutrient recycling. At present, little is known about decomposition dynamics of canola residues upon incorporation into nutrient-poor soils of Western Australia. Crop residue decomposition and subsequent release of nutrients in soil can be altered by a number of factors, including nutrient and water availability, soil temperature, physical and chemical nature of crop residues and their amount, soil type and soil–residue contact (Swift et al. 1979). In the present study, we characterized the effect of the size of canola stems residue on decomposition and nutrient (S) release dynamics.

A varied effect of the particle size of crop residues on decomposition and nutrient release dynamics after incorporation into soil has been reported in the literature, depending on biochemical and physical properties of residues and soils (Sims and Frederick 1970; Bending and Turner 1999). It is, however, not known how canola crop residue and its particle size would affect C and S mineralisation after incorporation into S-deficient soil. Residue particle size and its distribution in the soil can be altered by tillage and harvest operations. Compared to large size residues, residues of small size would decompose at a relatively faster rate because of their greater dispersion in the soil volume and greater accessibility of the substrates and nutrients in them to microbial attack. Although small-sized canola residues would be more intimately mixed with the mineral soil, the physical protection of decomposing substrate to microbial attack would be small in a sandy soil (Sims and Frederick 1970). However, differential decomposition rates of biochemical components of canola residues of different sizes could also be expected (for example, there could be a
greater lignin degradation of ground material) and this could affect C and S mineralisation of added organic matter (see Figure 1). Breakdown of lignin could cause release of low molecular weight polyphenols related to humic and fulvic acids (Stevenson 1994). These polyphenols can form insoluble calcitrant complexes with a variety of organic-N containing compounds in senescent microbial tissues and decomposition products (Bending and Read 1996, Bending and Turner 1999). Similarly, Wu et al. (1993) have shown that as microbial-S decreased in soil amended with barley straw (C:S ratio – 206:1) during incubation, there was no increase in soil sulphate-S, but microbial-S was probably directly transformed into stable soil organic-S.

Because canola stems residue has high C:N and C:S ratios, its incorporation into a nutrient-poor soil could also lead to greater immobilisation of plant-available nutrients in soil to meet microbial demands (Wu et al., 1993; Magid et al. 1997). Figure 1 lists various other possible expectations concerning C and nutrients (N, S) mineralisation that one could make when canola residue of different sizes is incorporated into soil. This project on the role of crop residues in improving soil fertility is currently in progress. Here, we report data on microbial-S and sulphate-S mineralised and/or immobilised during incubation of a sandy soil in the presence of canola stems residue. Currently, we are also making other measurements, such as, microbial-C and -N, water-soluble C and N, and C, N, S and P associated with decomposing organic matter with the overall aim to elucidate the effect of canola residue on detailed dynamics of these nutrients in soil after incorporation.

Materials and methods

Soil and plant material and preparation for incubation

An infertile brown sandy soil collected from the top 0–10 cm layer of a bushland near Lancelin, Western Australia, was used in the experiment. The soil was air-dried, sieved (2 mm sieve), any visible undecomposed plant material removed, and the soil analysed for selected properties (pH-H2O – 5.21, clay – 4.5%, silt – 6.5%, sand – 89%, total C – 7.5 g/kg, total N – 0.21 g/kg, total S – 0.035 g/kg). Lancelin soil (750 g, air-dried) was filled in plastic containers (8 cm diameter × 12 cm height) (bulk density of approximately 1.5 Mg m–3 soil to a depth of 10 cm) and moisture content was adjusted to 10% (w/w). The soil in each container was inoculated with 1 g of wet (moisture 40% (w/w)) soil plus green waste based manure mixture (mixed at 60 m3/ha) applied as suspension in water. The inoculation was done to raise the initial soil microbial population level in the Lancelin soil. Two such containers (i.e. 1500 g soil) were then placed in a sealed 5-L bucket (representing one treatment and one replicate) and preconditioned aerobically for a week in the dark at 20 °C.

Canola residues used in this study were collected (from a recently harvested field at Meckering in western Australia), air-dried, and stored at 4 °C in a sealed plastic bag for up to 4 months before use. There were three particle size treatments: the canola stubble was either ground (<1 mm) using a grinder, or chopped to 5–7 mm and 20–25 mm length by manually cutting with a pair of scissors. The canola residues mainly consisted of stems (upper and lower), side branches, and pedicles (but no leaves or pods and pods wall). A portion of each size of canola residues was further ground in a coffee grinder and used for various basic analyses. The results are presented in Table 1 on dry weight basis.

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<table>
<thead>
<tr>
<th>Quality parameters</th>
<th>Canola residue particle size</th>
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<tbody>
<tr>
<td></td>
<td>&lt;1 mm</td>
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<tr>
<td>Total N content (g/kg)</td>
<td>3.7</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>131</td>
</tr>
<tr>
<td>Total S content (g/kg)</td>
<td>1.6</td>
</tr>
<tr>
<td>C:S ratio</td>
<td>300</td>
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<tr>
<td>Water-soluble C (g/kg C)</td>
<td>39.5</td>
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Because of wider C:N and C:S ratios, canola stubble incorporation to a nutrient-poor soil would cause immobilisation of nutrients because microbial demand for nutrients during their growth on added C substrates (especially recalcitrant cellulose and lignin) would increase.

Hypothesis: Immobilisation of mineral N and S would increase with the decrease in particle size

Is it because of: a) greater build up of microbial biomass and/or their slower turn over, thus retaining immobilised nutrients for a longer time, or b) greater microbial immobilisation of N and S in decomposing crop residues, or c) greater biochemical transformations of organic-N and -S containing decomposition products to some stabilised forms in soils amended with smaller-sized canola residues?

Hypothesis: Smaller particle size of canola residues would result in greater carbon mineralisation

Hypothesis accepted

Focus on the mechanism of stabilisation of the decomposing canola litter in soil

Physical protection of decomposing organic matter would be small in a sandy soil and thus, any physical stabilisation with clay and silt particles (i.e. due to adsorption) would be negligible or short-lived, or alternatively initial microbial respiration could be affected by certain inhibitory substances such as phenolics released during decomposition of lignin, which could be greater in finer-sized than in larger-sized canola residues, or there could be greater biochemical stabilisation of decomposing organic matter

Hypothesis: No difference

Slower C mineralisation

Determinations?

Yes

No

Hypothesis rejected. Slower C mineralisation may be due to limitation on microbial growth

Yes

No

Carbon mineralisation

Nutrient (N, S) mineralisation

Hypothesis: No difference

Hypothesis accepted

Determinations?

Figure 1: An overview of the project with proposed hypotheses and probable reasons that could explain any differences in decomposition and nutrient release dynamics from added crop residues of different particle sizes.

Incubation

One-week after preconditioning, ground as well as chopped canola residue particles were homogenously mixed with the wet soil to a concentration of 3.6 g plant material (air-dry basis) per kg soil, corresponding to 5.4 tonne residues per hectare (approximately 5 tonne per hectare on dry-weight basis) to a depth of 10 cm (BD = 1.5 Mg/m³) and the containers were refilled after mixing. A control treatment with soil, but without additions of the plant material, was also kept. Following amendments, containers were placed back in the same 5-L sealed plastic bucket containing 100 mL of distilled water to maintain a water-saturated atmosphere and 30 mL of 2 M NaOH to trap microbial respired CO₂. The sealed buckets were then wrapped with aluminium foil and placed in a dark room for incubation at 20±1 °C. The weight of the container + soil + canola residues was recorded and adjusted for moisture loss whenever noticed.
Three different-sized canola stems and one control, three replicates for each treatment, and 7 harvest times (0, 1, 4, 10, 28, 56, 183 days) during 6-month long incubation made 84 buckets to be managed during the study. Additionally, two extra buckets for each replicate containing no soil and plant material (but two sealed empty plastic containers, 100 mL water and a CO2 trap) were also kept to account for CO2 in the enclosed space. The three replications for each treatment were spaced in time (i.e. replicate 2 and replicate 3 were set up 2 days and 14 days, respectively, after the replicate 1) such that at each time, 4 buckets were harvested. At each harvest time, a part of the soil from each treatment and replication was removed for soil moisture determination after drying at 105 °C for 48 h. The remaining soil was kept at 4 °C for up to 7 days for various analyses (see below). All results are expressed as a mean of three replicate determinations on oven dry basis (105 °C for 48 h for soil and soil + canola stubble samples and 60 °C for 96 h for the plant material).

**Analyses**

Soil respiration was monitored by changing NaOH in the sealed buckets on 1, 2, 4, 7, 10, 15, 22, 28, 42, 56, 77, 100, 120, 141, 162, 183 days and measuring trapped CO2. The HCO3– and CO32– ions in the 1 mL of 2 M NaOH were precipitated with 10 mL of 1 M BaCl2 solution and total respired CO2-C was determined by titrating the residual NaOH with 0.1 M HCl using phenolphthalein as indicator.

Microbial S was determined using 1:2 ratio of soil to 0.01 M CaCl2 extractant (Wu et al. 1994). Briefly, about 22 g moist samples of non-amended and non-fumigated soils were fumigated for 24 h in the presence of chloroform containing amylene (0.006% v/v) as a stabiliser, followed by extraction with 40 mL of 0.01 M CaCl2. Total-S in the 0.01 M CaCl2 extracts (of both fumigated and non-fumigated soils) was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), and sulphate-S in the CaCl2 extract from the non-fumigated soils by a BaCl2-geletin turbidimetric method of Tabatabai (1974). Biomass-S was calculated by dividing the difference between the total-S extracted from the fumigated and non-fumigated samples by 0.31, as recommended by Wu et al. (1994) for soil-to-extractant ratio of 1:2 (w/v).

**Statistical**

A two-way analysis of variance (ANOVA) in a randomised complete block design was performed on the data using GenStat 7.1, with treatments and time as independent and interactive source of variance. When significant F-tests were observed (P < 0.05), means separation was obtained using a Least Significant Difference (LSD) test at the 0.05-probability level. The difference in the net cumulative respiration from differently treated soils at each point in time was tested by the two-tailed t-test (P < 0.05) and assuming equal variances.

**Results and discussion**

There was no difference in the net cumulative respiration (presented as % of added C after subtracting CO2-C from the non-amended soil) from the soil amended with 5–7 mm and 20–25 mm particle size of canola residues (Figure 2). However, the respiration from the soil amended with <1 mm residues was significantly smaller (P < 0.05) than that amended with the 20–25 mm residues between days 7–28, and with the 5–7 mm canola residues between days 7–10, of incubation. In total, 33–35% of the added C in canola residues was respired from the soil during 183 days of incubation (Figure 2). These results (i.e. slower decomposition of ground residues) are surprising because by reducing the size of canola residues, we expected an increase in C mineralisation due to their greater distribution in the soil and hence a greater accessibility to microbial attack (Sims and Frederick 1970; Angers and Recous 1997). As physical protection of decomposing organic matter would be small in a sandy soil (Sims and Frederick 1970) due to lack of enough adsorptive surfaces offered by the small proportion of clay and silt content in the soil, we did not expect a decrease in C mineralisation due to reduction in the particle size of canola residues.

Microbial-S was greater (P < 0.001) in the amended treatments compared to in the non-amended soil during incubation. Among the residue particle size treatments, microbial-S was about the same. Although standard errors associated with the microbial-S data were large (Figure 3), which could be due to greater solubility of canola-tissue S in CaCl2 and thus to errors in sub-sampling representative soil for fumigation and non-fumigation, it appears that microbial-S in soil built up rapidly upon addition of canola stubble (during first 1–4 days). The microbial-S then decreased by day 56 and increased again between day 56 and day 183 in both the amended and non-amended soils (P < 0.01). About 75% of added S in canola residue was immediately (i.e. on day zero following incorporation) extracted as sulphate-S in 0.01 M CaCl2, thus increasing the
sulphate-S level in all the amended soils by 4-fold. This indicates that canola residues could serve as a good source of readily-available S for uptake by microbes. The rapid flush of canola-tissue S early on day zero following incorporation suggests that much of the canola S released existed in soluble inorganic sulphate form. Wu et al. (1993) have also shown, using rape leaves as plant residues, that plant-tissue S was rapidly released as sulphate-S (within 5 days) following incorporation. However, they extracted soil + rape leaves residue on day 5 after incorporation and hence, it was not possible to know the initial level (i.e. on day zero) of sulphate-S in the residue-amended soil. Janzen and Kucey (1998) also found that most of the mineral-S released from wheat, lentil and rape straw appeared in the initial extract of 0.001 M CaCl₂ on day 14 following incorporation into soil.

Figure 2: Carbon mineralisation of canola residues mixed with soil for 183 days. The CO₂-C evolved for each treatment was calculated from total CO₂-C evolved from the amended minus CO₂-C from the non-amended soil. Bars on the data points are ±SE of means of three replicates.

The sulphate-S in the amended and non-amended soils decreased steadily (**P** < 0.001) to about 65% of the initial level during 183 days of incubation. This suggests that the S released was subsequently immobilised by microbes, or it may have been re-incorporated into decomposing canola residues through microflora, or S associated with microbial decomposition products may have been transformed to stable soil organic-S. The decrease in microbial-S during the first 56 days of incubation was not accompanied by an increase in sulphate-S in the amended and non-amended soils, thus suggesting that microbial-S was transformed to stable soil organic-S fractions. This observation is in agreement with the findings of Wu et al. (1993). However, between days 56–183, an increase in microbial-S was associated with the decline in sulphate-S in the both amended and non-amended soils (**cf**. Figures 3 and 4), suggesting microbial immobilisation of sulphate-S in the soil occurred. This may have occurred, because microbial demand for S increases over time, as microbes would switch from labile to relatively less-labile C substrates such as cellulose and lignin (Chapman 1997). A further examination of water-soluble, neutral detergent soluble, hemicellulose, cellulose and lignin components of the decomposing canola residues would provide information on the type of substrates that were turned over at different times during incubation. A particulate organic matter (POM) separation technique that could successfully recover decomposing crop residues from soil would be useful for this purpose. The increase or decrease of nutrients (e.g. N, S, P) associated with POM would also provide information on any microbial immobilisation of these nutrients that would occur in the decomposing residues.
Conclusions

Out of the three particle sizes of canola residues used, only <1 mm treatment showed significantly slower decomposition between days 7–28 after incorporation; but such differences were compensated afterwards, and thus would not have practical relevance for build up of organic matter in soil through alteration of particle size of crop (canola) residues. Canola residue application can significantly increase the size of the microbial-S and sulphate-S pools in a S-deficient soil, thus improving the S-supplying potential of the soil.
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