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## Amount and incorporation of plant residue inputs modify residue stabilisation dynamics in soil organic matter fractions



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### ABSTRACT

Carbon sequestration in agricultural soils has been promoted as a means to reduce atmospheric concentrations of greenhouse gases (GHG) whilst improving soil productivity. Although there is broad agreement on practices that increase carbon (C) stocks, uncertainty remains on how agricultural management affects the stability of these gains. The fate of above-ground residue into soil organic matter (SOM) was tracked using isotopically labelled (<sup>13</sup>C and <sup>15</sup>N) residue over 12 months in a pasture soil in sub-tropical Australia. Agricultural residue management was simulated by (1) altering the rate of residue input and (2) incorporating residue with topsoil or leaving on soil surface. Increased input and incorporation of residue increased residue-derived SOM content, with the majority of residue-derived SOM accumulating as particulate organic matter (POM) (65%) with more modest gains in mineral-associated fractions. Rapid accumulation of residue-derived SOM in the mineral-associated fractions in the initial stages of decomposition, coinciding with a high loss of labile residue components, indicate an important role for soluble OM inputs in providing an immediate and long-term sink for C and N. However, this must be considered alongside high rates of accumulation in the more readily mineralised POM fraction, particularly when a soil is approaching saturation, which is likely to lead to greater mineralisation of SOM.

#### 1. Introduction

Soils constitute the largest terrestrial organic C pool ( $\sim$  1500 Pg C to a depth of 1 m) (Batjes, 1996), which is three times the amount of CO<sub>2</sub> currently in the atmosphere and 240 times the current annual fossil fuel emissions (Ciais et al., 2014). As such, soil organic carbon (SOC) sequestration has been viewed as an important climate change mitigation strategy as increasing net soil C storage by even a few per cent represents a substantive C sink potential (Paustian et al., 2016). Soil carbon management is the basis of the 4 per 1000 initiative, a voluntary action plan under the Lima-Paris Action Agenda to ensure food security and mitigate climate change through the increase of soil carbon stocks (http://4p1000.org/).

Improved agricultural management practices have been shown to increase SOC content by decreasing soil disturbance and increasing C input to the soil. However, uncertainty remains on the stability of these gains (Powlson et al., 2014; Janzen, 2015) with some studies indicating that the additional C accumulated is concentrated in particulate organic fractions, which can be readily mineralised, with only modest gains in more persistent C pools (Bhattacharyya et al., 2011; Stewart et al.,

#### 2012; Brown et al., 2014).

The retention time of sequestered C in soils can range from short term (immediately released back to the atmosphere) to long-term (millennia) storage (Trumbore, 2000). Soil organic C, though intrinsically susceptible to decay, can be protected by the mineral matrix (Six et al., 1999, 2002; Baldock and Skjemstad, 2000; Krull et al., 2003; von Lützow et al., 2006; Kögel-Knabner and Amelung, 2014). Substrates may become encapsulated within aggregates, physically shielded from microbial activity (Tisdall and Oades, 1982), or they may be sorbed to mineral surfaces, rendered more immune to microbial enzymes (Janzen, 2015). Soil organic carbon is closely associated with total N with strong biological links and consistent stoichiometry (Cleveland and Liptzin, 2007) meaning any changes in SOC will also affect soil total N which is dominated by the organic fraction (Pringle et al., 2014). If N resides in more persistent mineral-associated N pools it is less susceptible to microbial mineralisation and subsequent leaching and gaseous losses (Kelley and Stevenson, 1995). As the global N cycle accelerates, the capacity for ecosystems to retain N will become an increasingly important ecosystem service (Castellano et al., 2012).

The study aimed to determine how the amount and placement of

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residue inputs affects the fate of residue-derived C and N in functionally relevant SOM fractions that vary in their degree of protection from decomposition. We traced the fate of above-ground residue into SOM fractions through the use of isotopically labelled plant material (<sup>13</sup>C and <sup>15</sup>N), which allowed C and N inputs from residue to be differentiated from existing SOM (e.g. Bird et al., 2008; Cotrufo et al., 2015). SOM fractions were isolated using the approach of Zimmermann et al. (2006) obtaining; (1) a light particulate organic matter fraction (POM), comprised primarily of identifiable plant material that is chemically similar to its source, characterised by a relatively short turnover time due to its lack of protection. (2) a sand-sized fraction (SA) containing OM physically protected within microaggregates, and (3) a silt and clay sized fraction (SC) where OM is chemically associated with mineral surfaces. This study determined whether residue input and placement influenced the partitioning of residue among unprotected POM and mineral associated pools. We hypothesised that increasing the rate of residue input and residue incorporation would increase residue-derived SOM formation. At higher input levels we expected the saturation of mineralassociated pools, resulting in the accumulation of residue-derived SOM in the more labile unprotected pool (POM).

#### 2. Materials and methods

#### 2.1. Experimental site

The experiment was conducted on a long-term grassland soil (100 + years) on a farm in Crows Nest, Queensland, Australia (27°16′S 152°03′E). Livestock were excluded from the study site by a temporary fence prior to the start of the experiment. The climate is subtropical with warm wet summers and dry winters with a mean annual temperature of 17 °C. Annual precipitation averages 630 mm with the highest levels of rainfall received in the summer months. The soil is a vertosol (Isbell, 2002) with selected soil properties shown in Table 1.

Soil bulk density (BD) in the experimental area was determined on 4 replicates by the soil core (10 cm) method, for the 0–5 cm (BD =  $1.4 \pm 0.1$ ) and the 5–10 cm (BD =  $1.4 \pm 0.2$ ). Temperature was measured using a data logger (Onset, HOBO) placed at a depth of 10 cm. Daily rainfall was collected manually (total rainfall in experimental period = 588 mm) (Fig. 1).

#### 2.2. Isotopically labelled residue production and analyses

To trace residue-derived C and N in soils <sup>13</sup>C and <sup>15</sup>N labelled Rhodes grass tops (*Chloris gayana*) were used. The grass was grown within a continuous labelling chamber under controlled conditions as described in Mitchell et al. (2016). Once the Rhodes grass had reached maturity, the chamber was opened and plants were cut at height of 10 cm. Residue was air-dried, cut to 10 cm pieces and homogenised. Residue moisture content was measured on three oven-dried (60 °C) subsamples for dry weight correction. The oven-dried subsamples were mill-ground and used for the determination of C (44% ± 1.2) and N (3.1% ± 0.1) concentrations and their isotopic composition (<sup>13</sup>C = 3.8 atom%; <sup>15</sup>N = 5.7 atom%) by elemental analysis and isotope ratio mass spectrometry (EA-IRMS, Sercon Limited, UK).

#### 2.3. Experimental design

On 13th February 2014, the air-dried labelled residue was

incubated on the surface of the grassland, inside PVC collars (10 cm in diameter) which were inserted to a depth of 10 cm (with 5 cm remaining above the soil surface). Above-ground vegetation was previously removed from inside the collars by clipping to soil level. Collars were covered by a 2 mm polyethylene mesh to prevent loss of the labelled residue or input of external plant material.

To explore the effects of residue management on SOM formation, we established two experiments. The first experiment tested the effect of varying residue input level. It used a two factor design, with input level: Control =  $0 \text{ t ha}^{-1}$  dry matter (DM), LO =  $5 \text{ t ha}^{-1}$  (224 g C m<sup>-2</sup>), MED =  $10 \text{ tha}^{-1}$  (448 g C m<sup>-2</sup>), HI =  $15 \text{ tha}^{-1}$  (672 g C m<sup>-2</sup>), and time: four harvests occurred on day 95 (T1), day 197 (T2), day 286 (T3) and a final harvest on day 378 (T4), as the two factors in a fully randomised block design with 4 replicate blocks. The second experiment examined the effect of incorporating the residue within the top soil, with residue incorporation being either present (MIX, at a rate of 10 t ha<sup>-1</sup>) or not (Control). The MIX treatment was applied at the same rate to the MED surface applied treatment (10 t ha<sup>-1</sup> input), to evaluate effects of residue incorporation. The experiment examined the effect over time using the same sampling intervals and replicate block design described above. In order to incorporate the residue with the soil for the MIX treatment, the surface 10 cm of soil was removed, mixed with the labelled residue in a plastic bag and returned to the PVC tube at the same field bulk density. For the Control, no residue was added but the top 10 cm of soil was mixed as in the MIX treatment. At each harvest, a Control, a LO, a MED, a HI and a MIX collar from each of the four replicate blocks were sampled.

#### 2.4. Residue and soil collection

At the four harvesting intervals described above (T1 to T4), all recognisable residue on the soil surface (LO, MED, HI treatments) within each collar of experiment one were carefully picked by hand, dried at 60 °C, weighed and pulverized for further analyses.

All the intact soil cores (depth 10 cm) from both experiments were excavated by shovel, placed in pre-labelled plastic bags and kept refrigerated (4 °C). In the laboratory, cores were divided into 0-5 cm and 5-10 cm depth layers as it was expected that the isotopic signal at depths > 5 cm would be minimal in the surface applied treatments (experiment one) as found in Mitchell et al. (2016). Samples were then sieved to 2 mm with any residue > 2 mm analysed separately as coarse organic matter (OM). In the MIX treatment, undecomposed residue (> 2 mm) was removed from the soil prior to fractionation by sieving and was used as a fraction comparable to residue remaining on the soil surface for the surface applied treatments. The contribution of applied residue to the > 2 mm fraction was determined using IR-MS analysis and the isotopic mixing model for all treatments (Section 3.6). The decline in this fraction over time was used to determine residue decay rates T1 to T4 (Section 3.6). A representative subsample from each soil sample was dried in an oven at 60  $^\circ \rm C$  , pulverized and used for elemental and isotopic analyses.

Soil was fractionated by size and density to separate its primary components, using the same process described in Mitchell et al. (2016) using Zimmermann et al. (2006) approach. Briefly, thirty grams of soil (< 2 mm) were added to 150 ml water and dispersed using a weak ultrasonic treatment (output energy of  $22 \, J \, ml^{-1}$ ) to disrupt macroaggregates leaving more stable microaggregates intact (Amelung and Zech, 1999). Low energy sonication should also act to preserve fragile

Table	1
Selecte	ed

Selected soil characteristics 0–10 c	m, Crows Nest, QLD, Australia.
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Depth (cm)	Sand (%)	Silt (%)	Clay (%)	BD (g cm <sup>-3</sup> )	pН	EC ( $\mu$ S cm <sup>-1</sup> )	Total C (%)	Total N (%)	POM-C (% of TOC)	Sand and aggregates-C (% of TOC)	Silt and clay-C (% of TOC)
0-10	31	30	39	1.4	5.2	180	3.4(0.3)	0.29(0.02)	24	10	66

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