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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 ($^{13}C$ and $^{15}N$) 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 (~1500 Pg C to a depth of 1 m) (Batjes, 1996), which is three times the amount of $CO_2$ 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 ($^{13}C$ and $^{15}N$), 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 mineral-associated 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 ± 0.1) and the 5–10 cm (BD = 1.4 ± 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 $^{13}C$ and $^{15}N$ 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 ($^{13}C$ = 3.8 atom%; $^{15}N$ = 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 t ha$^{-1}$ dry matter (DM), LO = 5 t ha$^{-1}$ (224 g C m$^{-2}$), MED = 10 t ha$^{-1}$ (448 g C m$^{-2}$), HI = 15 t ha$^{-1}$ (672 g C m$^{-2}$), 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$^{-1}$) or not (Control). The MIX treatment was applied at the same rate to the MED surface applied treatment (10 t ha$^{-1}$ 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 °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**
Selected soil characteristics 0–10 cm, Crows Nest, QLD, Australia.

| Depth (cm) | Sand (%) | Silt (%) | Clay (%) | BD (g cm$^{-3}$) | pH | EC (μS cm$^{-1}$) | 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 |

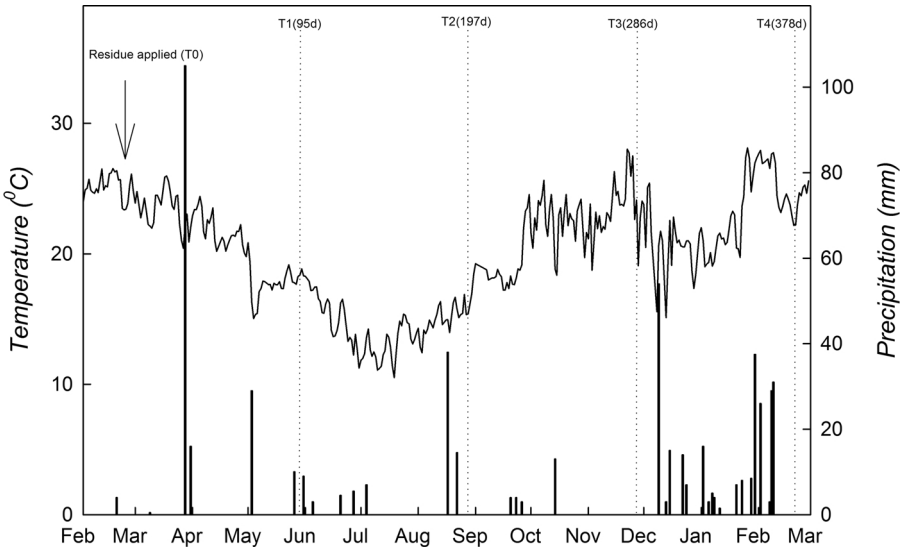


**Fig. 1.** Temperature (line), precipitation (bars) and sampling time intervals (T1 to T4) over 12 month experimental period (February 2014 February 2015) at Crows Nest, QLD, Australia. The highest rates of SOM formation were experienced in T1 and T4 when climatic conditions were warmer and wetter.

particulate organic matter (POM) from artificial spreading within the size fractions (Stemmer et al., 1999). The dispersed suspension was then wet sieved over a 53 µm aperture sieve until the rinsing water was clear. The fraction > 53 µm, containing the sand and microaggregates (SA) together with POM, was dried at 40 °C and weighed. The suspension < 53 µm was filtered through a 0.45 µm aperture nylon mesh and the material > 0.45 µm (silt and clay fraction, SC) was dried at 40 °C and weighed. The filtrate (< 0.45 µm) containing soluble organic carbon was not directly measured, but was estimated as the difference between total $^{13}C$ content in the bulk soil and the sum of $^{13}C$ recovered in the three measured soil fractions (POM, SA, SC). Particulate organic matter was isolated by stirring the fraction > 53 µm with sodium polytungstate (SPT) at a density of 1.8 g cm$^{-3}$. The mixture was centrifuged at 1000*g* for 15 min and the light fraction (POM) was decanted, washed with deionised water to remove all SPT, dried at 40 °C and weighed. All fractions were pulverized and analysed for C and N elemental and isotopic concentrations by EA-IRMS, as described above for the residue.

### 2.5. Chemical analysis of remaining residue on soil surface

Residue remaining on the surface was characterised on the initial (day 0), day 197, day 286, and day 378 samples. Changes in chemical composition over time were assumed to be the same across treatments with residue from MED input treatment taken as the representative sample. We analysed for% C and% N on an elemental analyser (LECO). We determined the% acid-unhydrolyzable residue (AUR) and cellulose using the acid detergent fibre (ADF) digestion method (Van Soest, 1963). It is important to note that the AUR does not correspond to pure lignin because it may include some other hydrolysis resistant organic structures, such as cutin, waxes, and condensed tannins at various proportions (Berg and McClaugherty, 2008; Preston et al., 2009).

All remaining residue for the different treatments were characterised by NMR (Agilent Technologies) in solid state under the same conditions allowing a quantitative comparison among NMR spectra. Residue samples were analysed at all sampling intervals (T1–T4). Spectral regions were selected and C types identified as reported in Bonanomi et al. (2013) and Preston et al. (2009). Spectra were divided into standard chemical shift regions as follows: 0–50 ppm, alkyl C; 50–60 ppm, methoxyl and N-C; 60–93 ppm, O-alkyl C; 93–112 ppm, aromatic C; 140–165 ppm, phenolic C; 165–190 ppm, carboxyl/carbonyl C. These are abbreviated as: ALKYL, METHOX, O-ALKYL, DI-O-ALK, AROM, PHEN, CARBOX. Areas of these chemical shift regions were expressed as percentages of the total area (the relative intensity). The relative shift in the different classes of organic C compounds was used to assess changes in the surface residue as decomposition progressed.

### 2.6. Data analyses

The residue-derived C and N contribution to the bulk soil and SOM fractions was assessed for the residue-added plots as compared to the control plots. The isotopic mixing model was applied as follows:

$$f_{residue} = (\delta_S - \delta_B)/(\delta_{residue} - \delta_B)$$

Where $f_{residue}$ is the fraction of the residue-derived C (or N) contributing to the bulk soil or SOM fractions. The $\delta_S$ and $\delta_B$ are the $\delta^{13}C$ (or $\delta^{15}N$) of the specific bulk soil or SOM from the residue ($\delta_S$) and the control ($\delta_B$) treatment respectively. For bulk soil and SOM fractions, the $\delta_B$ average values across the respective bulk soil or SOM fractions from all control plots are used. The $\delta_{residue}$ is the $\delta^{13}C$ (or $\delta^{15}N$) of the initial residue. The amount of residue-derived C and N in these pools was obtained by multiplying the $f_{residue}$ values to corresponding C (or N) pools. Residue-derived C and N pools in the SOM fractions were calculated for the 0–5 cm and 5–10 cm soil depth, using the respective measured BD values.

The decay constants of surface-applied and MIX residue were determined through the application of a single negative exponential decay constant (*k*) calculated according to Berg and McClaugherty (2008) using Olson (1963). Mass loss was determined by collecting undecomposed residue (> 2 mm) at each sampling interval. The residue-derived fraction was determined using IR-MS analysis. The model equation was $M_t = M_0\, e^{-kt}$, where $M_0$ is the initial mass, $M_t$ is the mass at a certain time, *t*, and *k* is the decay rate constant. The mean transfer rates of residue-derived C to SOM (bulk soil and fractions) were calculated by the slope of regression lines between each sampling interval (T1toT4) (Liao et al., 2006). This assumed a constant rate of residue-derived C transfer between sampling intervals (Bimüller et al., 2012).

Estimates of C saturation deficit was determined using Stewart et al. (2007). The conceptual model of C saturation (Six et al., 2002) implies that the further a soil is from saturation (i.e. the greater the saturation deficit), the greater its capacity and efficiency to sequester added C, whereas a soil approaching saturation will accumulate a smaller amount of SOC at a slower rate and efficiency (Hassink, 1996). Changes in soil C protective capacity (g C kg$^{-1}$ soil) was calculated using the relationship between soil texture and mineral (silt + clay) C content (g kg$^{-1}$ soil) developed by Six et al. (2002) as follows:

*Protective capacity = 0.21 * (silt + clay content) + 14.76.*

**Table 3**
Lignin and cellulose content (%) of residues remaining on the soil surface (LO, MED, HI input treatments) at 0 days i.e. before residue was applied, and subsequent sampling intervals (95, 197, 286, 378 days). NMR spectra analysis indicates the relative distribution of different C compounds (ppm) at different stages of decomposition. Data was not available for sampling interval T1 (95 days).

| Decomposition period (days) | Cellulose (%) | Lignin (%) | NMR spectra analysis relative intensity distributions (% of total area) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | ALKYL-C 0 – 50 | METHOX 50–60 | O-ALKYL 60–93 | DI-O-ALK 93–112 | AROM 112–140 | PHEN 140–165 | CARBOX 165–190 |
| 0 | 35.3 ( ± 1.2) | 3.9 ( ± 0.4) | 11.1 | 8.5 | 47.1 | 13.7 | 7.7 | 2.0 | 5.5 |
| 95 (T1) | No data | No data | No data | No data | No data | No data | No data | No data | No data |
| 197 (T2) | 13.5 ( ± 0.9) | 12.2 ( ± 0.8) | 20.3 | 11.4 | 27.6 | 10.6 | 13.0 | 3.3 | 7.3 |
| 286 (T3) | 14.2 ( ± 1.2) | 13.0 ( ± 1.3) | 21.5 | 10.1 | 26.2 | 10.1 | 13.4 | 3.4 | 7.4 |
| 378 (T4) | 13.5 ( ± 0.6) | 13.1 ( ± 0.4) | 21.2 | 10.6 | 24.5 | 11.4 | 11.4 | 3.0 | 6.8 |

The C saturation deficit was then calculated according to Stewart et al. (2007) based on SOC content for the site:

*Saturation deficit = 1 − (SOC content/protective capacity estimate)*

The fate of residue-derived C in SOM fractions at different sampling intervals was calculated as a percentage of total residue-derived C:

*Fate of residue-derived C = SOC gain (fraction) (mg C core$^{-1}$)/SOC gain (total) (mg C core$^{-1}$) * 100*

### 2.7. Statistics

The effect of treatment (LO, MED, HI, MIX) and time (T1, T2, T3, T4) on the residue-derived C content was tested using a two-way ANOVA using Least Significant Difference (LSD) for all parameters as post-hoc test with the statistical package SPSS 21.0 (SPSS Inc., Chicago, IL, USA).The $p$ values are indicated in Table 3 for all procedures.

## 3. Results

Carbon was lost from applied residue following a first-order exponential decay with $k$ values ranging from 1.26 (HI) to 1.36 yr$^{-1}$ (LO) ($r^2$ = 0.915) in surface applied treatments (LO, MED, HI) to 2.07 yr$^{-1}$ ($r^2$ = 0.765) in MIX treatment (Fig. 2). Mass loss was initially rapid but declined as decomposition proceeded in all treatments. After 12 months (T4), an average of 26% ( ± 1.9%) of applied residue remained on the soil surface (LO, MED, HI) with no significant variation between treatments ($p < 0.01$). When residue was incorporated with the soil surface (MIX), there was significantly less (13% ± 2.9%, $p < 0.01$) undecomposed residue recovered in the soil at T4 (considered as OM fraction > 2 mm removed prior to fractionation) than in equivalent surface applied treatment (MED) (Fig. 2).

Analysis of the chemistry of residue remaining on the soil surface revealed high rates of biochemical change in the initial stages of decomposition (T0 to T2) with little change thereafter (Table 2). NMR spectra analysis demonstrated significant changes in the relative intensity of different spectral regions. In the initial stages of decomposition (T0 to T2) there was a relative decrease in the O-ALKYL region (60–93 ppm), mainly associated with sugars and polysaccharides, thereafter displaying a continuous but much lower relative decrease. The DI-O-ALK region (93–112 ppm) showed a similar pattern, with a high relative decrease in the initial stages of decomposition, but the change was less pronounced. The ALKYL-C region (0–50 ppm), characteristic of lipid waxes and cutins and the most biologically stable forms of organic C (Paul and Van Veen, 1978), increased during the initial stages of decomposition (T0 to T2), with no change T2–T4. Small and inconsistent changes were noted in AROM and PHEN C. O-ALKYL C accounted for the highest proportion of C lost and resulted in the strongest positive coefficient ($r^2$ = 0.98) with residue decay rate. The increase of ALKYL and CARBOX-C corresponded to the chemical analysis of the acid-hydrolysable fraction (AUR) that displayed a significant increase ($p < 0.01$) from 3.9% (T0) to 13.2% (T4).

### 3.1. Residue-derived C recovery in bulk soil and SOM fractions

In the context of this paper, the term C stabilisation is used to refer to processes or mechanisms that are known to increase C persistence in the soil and is used to describe the combined effect of (1) organo-mineral interactions in the SC fraction and (2) encapsulation of OM within stable microaggregates in the SA fraction, as more labile macroaggregates were dispersed during weak ultrasonic treatment prior to fractionation. SOM formation is used more widely and refers to the overall contribution of residue-derived C to all SOM fractions (POM, SA and SC).

Increasing the rate of residue input (LO to HI) significantly increased ($p < 0.01$) (Table 3) residue-derived SOM formation with 20.4 ( ± 1.8) g C m$^{-2}$ recovered as SOM in LO input treatment (representing 12.2% of C residue applied) and 39.4 ( ± 2.6) g C m$^{-2}$ recovered as SOM in HI input treatment (representing 10.4% of residue C applied) (Fig. 2). There was a linear trend in total residue-derived SOM accumulation from LO to HI input (e.g. T4 $r^2$ = 0.97). When residue was incorporated with the topsoil (MIX) over three times as much residue-derived C was recovered; 35.7% ( ± 1.1) of residue-derived C was recovered as SOM after 12 months, in comparison to 9.9% ( ± 0.4) in the equivalent surface applied treatment (MED) (Fig. 3).

The fate of the majority of the residue-derived C in the soil was in the POM fraction, which comprised of an average of 65% (for LO, MED, HI, MIX) of residue-derived C recovery after 12 months of decomposition. The residue-derived POM fraction had the highest C: N ratio of all SOM fractions, which did not change significantly over time (Table 4). In surface applied treatments (LO, MED, HI), during the initial stages of decomposition (T1), POM slowly entered the SOM pool, accounting for an average of 20% of residue-derived C that accumulated during T1 (ΔSOC gain fraction T1/ΔSOC gain total T1) (Fig. 4). However, in the latter stages of decomposition (T4), there was a rapid increase in POM accumulation in surface-applied treatments that grew exponentially over time ($r^2$ = 0.73) (Fig. 5), representing an average of 85% of residue-derived C accumulation during this period.

Incorporation of residue (MIX) led to significantly higher ($p < 0.01$) levels of POM (in comparison to MED) at all time intervals (Fig. 5). The rate of POM accumulation in the MIX treatment was high from the outset of the experiment resulting in a linear growth function over time ($r^2$ = 0.67). The rate of POM accumulation in MIX was 8 times greater than that of MED in T1, but this vast difference in initial accumulation rates rapidly diminished over time. By T4, there was no significant difference in the rate of POM accumulation between MIX and MED.

There was only a small proportion of residue-derived C recovered in the SA fraction with an average of ~7% (across all treatments) of applied residue-derived C accumulating in this fraction in 12 months. The SA fraction displayed little temporal variation in residue-derived C content, representing the fraction with the lowest rates of residue-

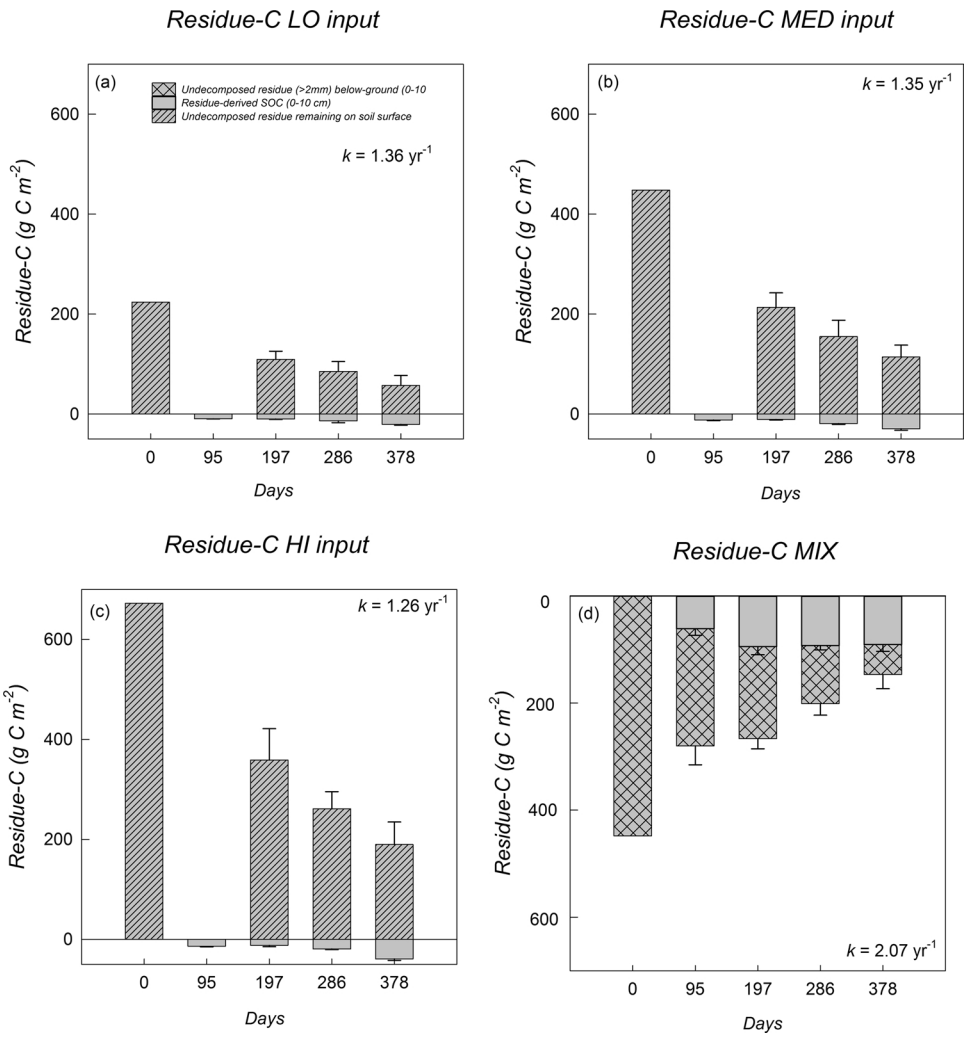


**Fig 2.** Dynamics of Rhodes grass C loss from the soil surface and recovery of residue-derived C in the mineral soil (bulk $^{13}C$ measurement i.e. total SOM = sum of fractions, 0 10 cm) at sampling intervals at 95 (T1), 197 (T2), 286 (T3) and 378 (T4) days. (a) Surface C loss and recovery of residue-derived C as SOM in LO input treatment (input rate = 5t ha$^{-1}$). (b) Surface C loss and recovery of residue-derived C as SOM in MED input treatment (input rate = 10t ha$^{-1}$). (c) Surface C loss and recovery of residue-derived C as SOM in HI input treatment (input rate = 10t ha$^{-1}$). (d) MIX treatment; undecomposed residue (> 2 mm) was separated by sieving prior to soil fractionation. Data for surface residue at 95 days (LO, MED, HI input treatments) was not available. Data are means (n = 4) with standard errors.

derived C accumulation from T1 to T4. The relationship between residue-derived C content and time was weak ($r^2$ values ranged from 0.2 to 0.3) and did not change significantly over time in any treatment (Table 3). There was no significant effect of varying the rate of residue input (LO, MED, HI) on residue-derived C content in the SA fraction at any sampling interval. The incorporation of residue (MIX) significantly increased the SA residue-derived C content with a 1.8 fold increase in residue-derived C content in T4 in comparison to MED.

The SC fraction accounted for the stabilisation of ~27% of residue-derived C across all treatments with a significant increase ($p < 0.01$) in residue-derived C content across time in all treatments (Fig. 5). Surface applied treatments (LO, MED, HI) displayed a linear increase over time ($r^2 = 0.63$) with the most rapid accumulation occurring in the initial stages of decomposition, where silt and clay associated residue-derived

**Table 2**
Summary of statistical parameters of the effect of treatment (LO, MED, HI, MIX) and time (T1, T2, T3, T4) on the recovery of residue-derived C among SOM fractions (POM, SA, SC) using a two-way ANOVA. The significance of residue-derived C accumulation is indicated by the p value for each experiment (1) the effect of variations in the amount of residue applied to soil surface on residue-C recovery (LO, MED, HI) and (2) the effect of incorporation of residue-C recovery (MED vs. MIX).

| Experiment 1 (LO, MED, HI) | | | Experiment 2 (MED vs. MIX) | | |
|---|---|---|---|---|---|
| Fraction | Factor | *p value* | | Factor | *p value* |
| SOM (total) | Time (T1–T4) | < 0.01 | | Time (T1–T4) | < 0.01 |
| | Residue-C input level (LO, MED, HI) | < 0.01 | | Residue-C incorporation (MED vs. MIX) | < 0.01 |
| | Time * residue-C input | < 0.01 | | Time * residue-C incorporation | < 0.05 |
| POM | Time (T1–T4) | < 0.01 | POM | Time (T1–T4) | < 0.01 |
| | Residue-C input level (LO, MED, HI) | < 0.01 | | Residue-C incorporation (MED vs. MIX) | < 0.01 |
| | Time * residue-C input | < 0.01 | | Time * residue-C incorporation | < 0.01 |
| SA | Time (T1–T4) | NS | SA | Time (T1–T4) | NS |
| | Residue-C input level (LO, MED, HI) | NS | | Residue-C incorporation (MED vs. MIX) | < 0.05 |
| | Time * residue-C input | NS | | Time * residue-C incorporation | NS |
| SC | Time (T1–T4) | < 0.01 | SC | Time (T1–T4) | < 0.01 |
| | Residue-C input level (LO, MED, HI) | < 0.01 | | Residue-C incorporation (MED vs. MIX) | < 0.01 |
| | Time * residue-C input | < 0.05 | | Time * residue-C incorporation | < 0.05 |

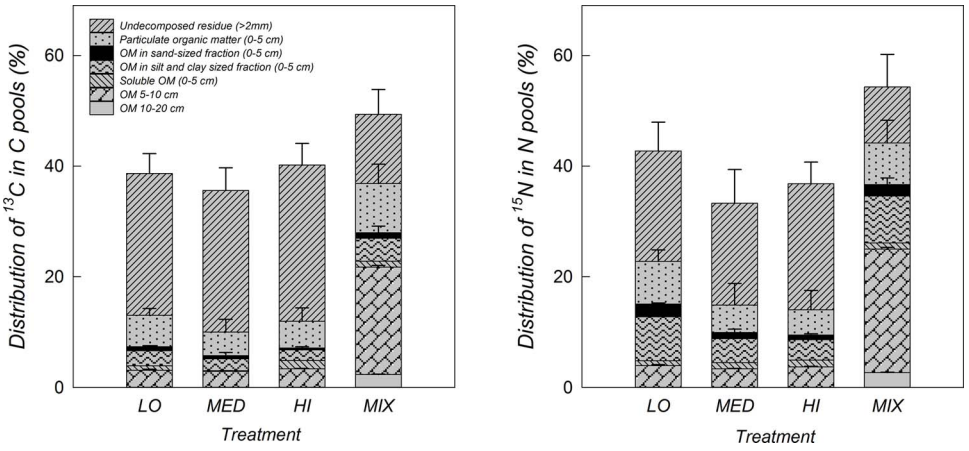


**Fig. 3.** Total recovery of applied residue-C and N across different treatments (expressed as a % of applied residue-derived C and N recovered) at sampling period T4 after 378 of *in situ* decomposition. Residue-derived C and N recovered as: undecomposed residue (> 2 mm) on soil surface in LO, MED, HI input treatments and below-ground in MIX treatment; particulate organic matter (POM), sand-sized fraction (SA) and silt and clay sized fraction (SC). $^{13}$C and $^{15}$N recovery in bulk soil (5–10 cm and 10–20 cm) is also shown.

**Table 4**
Variation in C:N ratio across sampling intervals. Average values across treatments (LO, MED, HI, MIX) are displayed as there was no significant effect of treatment on C:N ratio. Variation in C:N ratio over time was only significant in residue remaining on soil surface (significant increase) and in the SC fraction (significant decrease). Data are expressed as an average across all treatments (n = 16) ± SE.

| Fraction | Decomposition period (days) | | | | |
|---|---|---|---|---|---|
| | 0 (T1) | 95 (T1) | 197 (T2) | 286 (T3) | 378 (T4) |
| Residue remaining on soil surface | 12 (0.4) | No data | 15.6 (1.6) | 15.5 (1.8) | 16.1 (0.6) |
| | Native SOM | Residue-derived C:N ratio | | | |
| Particulate organic matter (POM) | 19 (0.4) | 11.8 (0.4) | 10.9 (0.6) | 11.1 (1.1) | 10.2 (0.9) |
| Sand-sized organic matter (SA) | 14 (0.5) | 6.4 (0.7) | 4.3 (0.5) | 5.4 (0.6) | 5.5 (0.7) |
| Silt and clay-sized organic matter (SC) | 12 (0.5) | 6.9 (0.4) | 7.1 (1.6) | 4.9 (0.4) | 5.4 (0.4) |

C contributed to 64% of residue-derived C that accumulated during T1 (Fig. 4). There was minimal accumulation of residue-derived C in T2 and T3. Residue-derived C accumulation increased significantly in T4 but only contributed to ~10% of residue-derived C accumulation in SC fraction during this period (Fig. 4). An increase in the rate of residue application (LO to HI) resulted in a significant increase ($p < 0.01$) in the amount of residue-derived C recovered in the SC fraction after 12 months of decomposition (Fig. 4). When residue was incorporated with the topsoil (MIX) this resulted in over double the amount of residue-derived C being stabilised in the SC fraction in comparison to the equivalent surface applied treatment (MED) (Fig. 5) representing 4.1% of applied residue-C in MIX and 2.1% of applied residue-C in MED.

Soil texture, or more specifically, the amount of silt and clay in the soil was used as a proxy to determine the protective capacity of the soil (Six et al., 2002). The saturation deficit expresses how far from C saturation a soil is, with a value of 0 representing the maximum amount of C a soil is capable of holding at a steady state (i.e., saturation deficit (SD) = 1 − (SOC content/protective capacity estimate)) (Stewart et al., 2007). There was a decline in the SD, although not significant, as residue input increased with the SD in T4 in the LO input treatment 0.51 ( ± 0.04) and 0.39 ( ± 0.03) in the HI input treatment. The SD in the MIX treatment was significantly lower than the surface applied treatments in T4 (0.13 ± 0.09).

### 3.2. Residue-derived N recovery

The distribution of residue-derived N recovered in SOM fractions was similar to that of C (Fig. 6) but overall residue N recovery was higher than C (Fig. 3), with an average of 10.8% of residue-derived C recovery as SOM (0–20 cm) in surface applied treatments, in comparison to 16.1% of residue-derived N recovery as SOM. The POM fraction represented the largest pool of residue-derived N recovered. After 12 months *in situ*, an average (across all treatments) of 42% ( ± 2.5) of the $^{15}$N recovered in the bulk soil was isolated as POM, 11.1% ( ± 1.9) in the sand-sized fraction, and 40.8% ( ± 0.9) in the silt and clay fraction, and a remaining 5.9% ( ± 0.8) as soluble OM.

The residue-derived C: N recovery ratio was determined for each SOM fraction (Table 4). As a reference the overall native C: N ratio for SOM fractions was compared to the residue-derived C: N recovery ratios from each fraction. As there was no effect of treatment on residue-derived C: N ratio over time (T1–T4) average values across treatments are presented. The residue-derived C: N recovery ratio was consistently lower than native C: N ratios, which was expected given the low C: N ratio of the residue applied (12:1). The C: N ratio of the residue remaining on the soil surface displayed a significant increase over time ($p < 0.01$). There was a narrowing residue-derived C: N ratio with decreasing particle size from the POM fraction (largest) to the SA and SC fractions (smallest). The C: N ratio in the POM fraction was similar to the C: N ratio of the initial residue. The C: N ratio of the POM fraction showed a slight increase T1 to T4, whilst there was a slight decrease in the C:N ratio of the SA and SC fractions − however, this relationship was only significant in the SC fraction ($p < 0.05$).

## 4. Discussion

Residue-derived C content displayed a linear increase with respect to C inputs (LO to HI) ($r^2 = 0.98$), with a linear relationship also shown in the constituent C fractions (POM, SA, SC). This verifies the accepted relationship between soil C input and SOC level at equilibrium used to model SOM dynamics. Even at the highest rate of input (HI = 15 t ha$^{-1}$ DM) there was no indication of an asymptotic relationship between C input and residue-derived SOC content. However, a large proportion of residue remained undecomposed on the soil surface (~26%) and if further decomposition had occurred saturation dynamics may have been observed.

Incorporating residues (MIX) resulted in a higher rate of residue decomposition ($k = 2.1$ yr$^{-1}$, MIX, $k = 1.3$ yr$^{-1}$ average surface applied) and SOM formation. A more advanced state of decomposition in the MIX treatment was likely due to the interaction of a number of factors including: increased accessibility of microbes to residue as a function of placement (Helgason et al., 2014) a greater abundance and activity of all microflora and fauna when residue is placed at depth (Beare et al., 1994) a less variable microclimate in the soil and a close association of soil nutrient pools (Blevins et al., 1984). This accelerated the fragmentation and comminution of residue for eventual incorporation into the SOM pool.

A more advanced state of residue decomposition in the MIX

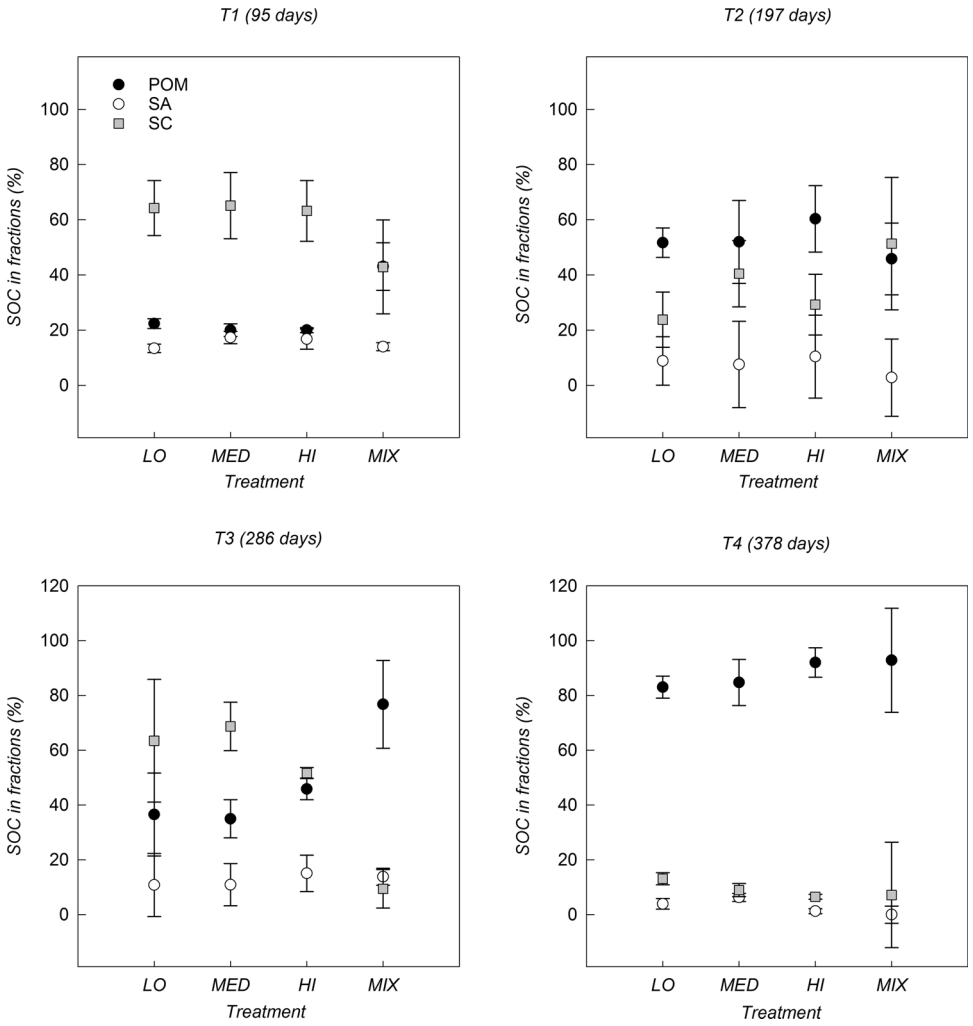


**Fig 4.** Residue-derived SOC recovery in different fractions at each sampling interval T1–T4. % SOC recovery in fractions expressed as ΔSOC gain fraction/ΔSOC gain total for each time period (T1 to T4).

treatment expedited the transfer of smaller residue particle sizes to more stable mineral-associated fractions (SA and SC). Microbial biomass and their associated by-products, which were more active and abundant in the MIX treatment, have been identified as a significant contributor to SOM formation (Sollins et al., 2009; Knicker, 2011; Mambelli et al., 2011). Microbial-derived carbohydrates have been demonstrated to initiate the formation of organo-mineral associations (Dümig et al., 2012) with continued preferential stabilisation of microbial-derived polysaccharides in comparison to plant-derived polysaccharides (Rumpel et al., 2010). Therefore a more active microbial population, combined with a closer proximity to silt and clay particles in the mineral soil, resulted in more opportunities for SOM to be stabilised in the SC fraction in the MIX treatment. In the case of the SA fraction, where it was assumed that residue-derived C was encapsulated within microaggregates, a higher availability of POM and greater contact between residue and aggregate structures (Balesdent et al., 2000) facilitated the accumulation of residue-derived C in SA in the MIX treatment.

Although the rate of SOM formation and stabilisation was greater in MIX than MED in the initial stages of decomposition, this difference diminished over time. There was evidence of an asymptotic relationship in the MIX treatment between residue input and residue-derived C content in the bulk soil, indicating that the soil was approaching saturation with respects to C inputs. An asymptotic relationship was particularly notable in the SC fraction. This corresponded to a decline in the saturation deficit (Six et al., 2002) in the MIX treatment, which did not occur in the surface applied treatments, suggesting that the SC fraction was approaching saturation due to a decline in the availability of mineral sorption sites (Hassink, 1997; Six et al., 2002; Kalbitz et al., 2005). As the capacity for silt and clay to stabilise approached zero (that is saturation), the efficiency of OM stabilisation in the SC fraction decreased. Whilst greater stabilisation of residue-derived SOM in SC occurred in MIX in the short-term, it is unlikely that this relationship would persist due to limits on the protective capacity of the soil.

Whilst the bulk soil and SC fraction displayed signs of an asymptotic relationship with respect to C input in the MIX treatment, the POM fraction displayed an exponential increase in residue-derived C content T1 to T4 ($r^2 = 0.92$). As the SOC of all fractions must sum to the SOC of bulk soil, it is likely that as the SC fraction began to saturate, the relative proportion of residue-derived C in the unprotected fraction increased exponentially to compensate (Stewart et al., 2007). The implication of the accumulation of POM is that it is largely unprotected in the soil and therefore more susceptible to mineralisation.

The greater SOM formation and stabilisation in MIX treatment, must be considered alongside the priming of existing SOM due to the mechanical disruption of soil and the accrual of new surfaces to microbial attack (Blagodatskaya and Kuzyakov, 2008). A study by Mitchell et al. (2016) demonstrated that the incorporation of residue (MIX) resulted in a 40% increase in GHG fluxes in comparison to surface applied treatments, primarily due to the priming of existing SOC, which offset SOC gains over a 12 month period. While the incorporation of residue resulted in greater SOM stabilisation in the short-term, this must be considered over longer time periods and in conjunction with changes in GHGs before drawing conclusions regarding increased C storage.

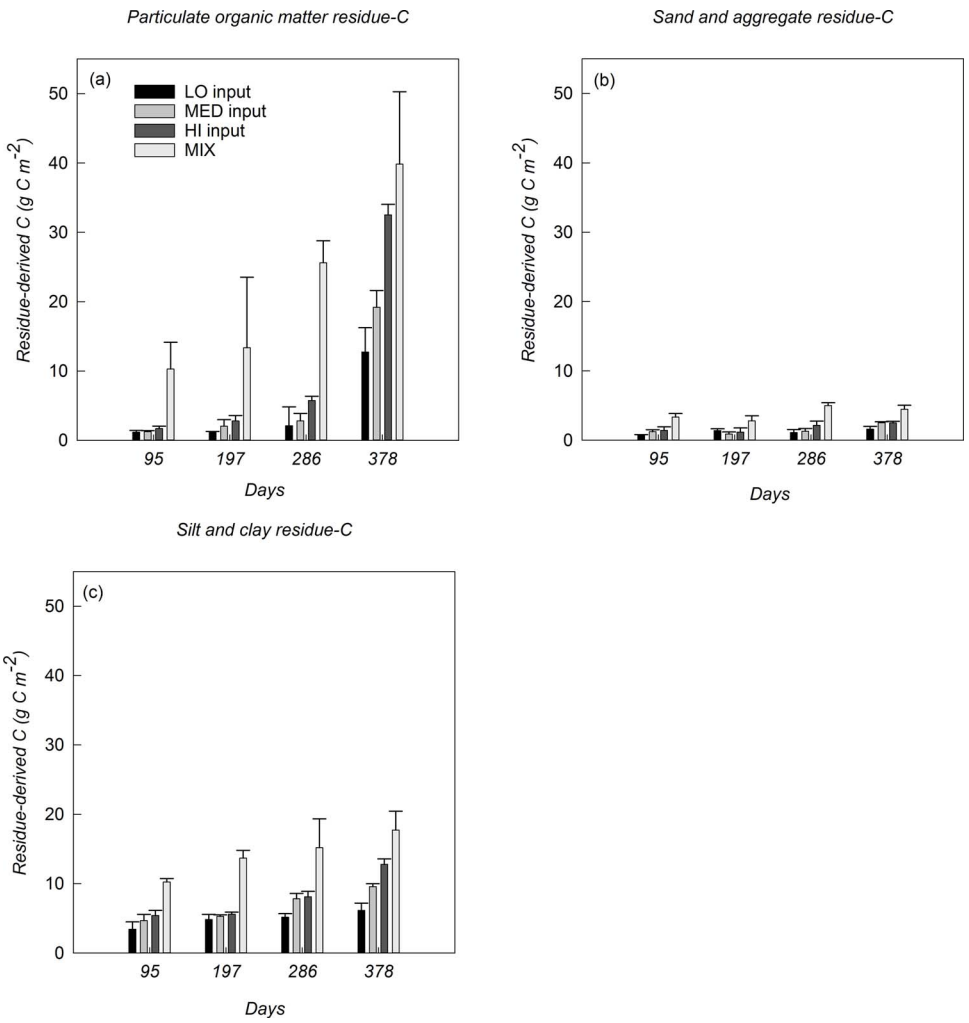


**Fig. 5.** Residue-derived C at sampling intervals T1–T4 (T1 = 95 days, T2 = 197 days, T3 = 286 days and T4 = 378 days) for three measured SOM fractions; (a) particulate organic matter (POM), (b) sand-sized OM (SA) and (c) silt and clay associated residue-C (SC). Values represent the mean of 4 replicates ( ± SE). POM fraction accumulated the largest proportion of residue-derived C, followed by SC fraction and the SA fraction.

The accumulation of residue-derived SOM displayed a marked temporal variation in all treatments. In the initial stages of decomposition (T1), in surface applied treatments, the largest proportion (SOC gain fraction/SOC gain total) of residue-derived C was recovered in the organo-mineral fraction (SC) (64%) with a smaller proportion of residue-derived C accumulating in POM (22%). These results therefore contrast with the view of SOM formation as a progressive transformation of above-ground residue inputs from lighter/coarser fractions to the finer and heavy mineral associated fractions (e.g. Guggenberger et al., 1994) whilst providing support to the model of SOM formation proposed by Cotrufo et al., (2015) that shows a DOM-microbial pathway forming fine mineral-associated OM in the early stages of decomposition.

The origin of SOM stabilised in the organo-mineral fraction (SC) early in decomposition (T1) was likely due to the leaching of labile components from a high quality residue (C:N ratio 12:1, 47% sugar and polysaccharide content, Table 2) during the heavy rainfall events experienced during this period (e.g. 105 mm on 28 March). This was supported by chemical analysis (NMR spectra) of the remaining residue on the soil surface, which displayed a rapid loss of non-structural components. This soluble material can either sorb directly to mineral soil particles (Kalbitz et al., 2005) or be used with high efficiency by soil microbes, which deposit biochemically transformed residue-derived products into the soil where they become associated with silt and clay sized minerals (Grandy and Neff, 2008; Cotrufo et al., 2013). It is unclear whether this soluble OM formed direct associations with mineral surfaces, but a reduction in C: N ratio in the SC fraction over time suggests that a greater degree of microbial processing occurred as decomposition progressed. These N rich compounds, produced as metabolites by microorganisms (Kögel-Knabner, 2002), have a high affinity for mineral surfaces (Sollins et al., 2006) and are protected from mineralisation. Therefore soluble residue inputs likely contributed to long-term C and N storage early in the decomposition process.

The input of soluble OM into the SA fraction may also have contributed to SOM stabilisation early stages of decomposition. Given that the highest rate of residue-derived C accumulation in SA occurred in early stages of decomposition for both surface applied and incorporated treatments, it seems probable that soluble residue-derived C and N penetrated the pore system of microaggregates and engaged in sorptive interactions with accessible mineral surfaces (Hatton et al., 2012). This may explain the lower than expected C: N ratio in this fraction; if the encapsulation of POM fragments in this fraction was the main process of C incorporation into microaggregates, we would expect the C: N ratio to be similar to that of POM. However, the C: N ratio was 5:1 suggesting that a DOM-microbial pathway may have been an important input pathway to this fraction.

The latter stages of decomposition (T4) were characterised by the accumulation of POM (average of 87% ± 3.3% of residue-derived C accumulation across all treatments in T4) suggesting a physical transfer of brittle residue into the mineral soil (Cotrufo et al., 2015). The POM fraction had the highest C: N ratio of all fractions, but was lower than that of unaltered residue suggesting that residue was affected by decay and colonised by microorganisms. In surface applied treatments, there was a notable lag time in the accumulation of POM, which was likely due to the time taken for biotic and abiotic agents to modify the residue to an extent that it could cross a mobility threshold and follow a

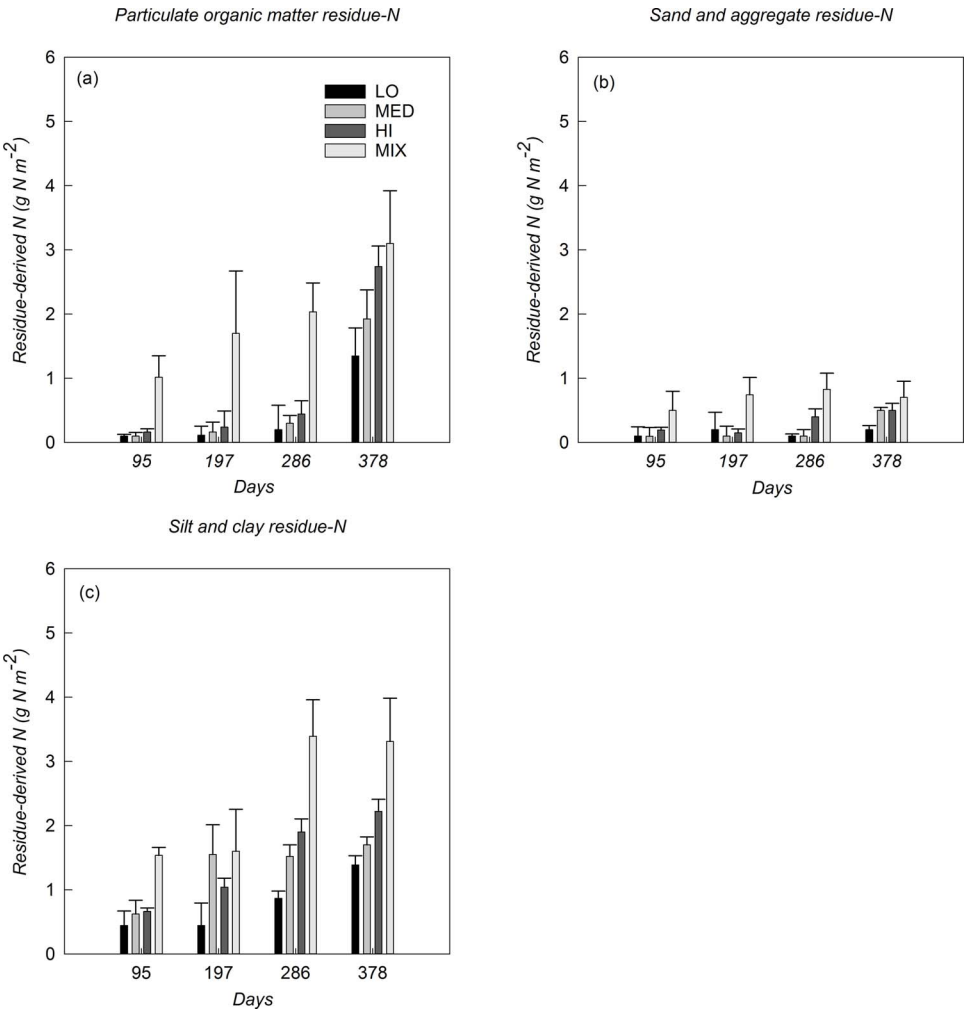


**Fig 6.** Residue-derived N recovery in different SOM fractions at sampling intervals T1–T4 (T1 = 95 days, T2 = 197 days, T3 = 286 days and T4 = 378 days) (a) particulate organic matter (POM), (b) sand-sized OM (SA) and (c) silt and clay (SC) associated residue-N. Values represent the mean of 4 replicates ( ± SE).

physical pathway into the soil profile (Hatton et al., 2012). It is hypothesised that a lower activity of fauna in surface applied residue (Beare et al., 1992) combined with greater moisture limitations (Helgason et al., 2014) during prolonged warm and dry periods experienced in the months of July and August, resulted in a lower rate of residue comminution by soil micro arthropods and decomposition by soil microbes. In contrast, when residue was incorporated, the effect of abiotic agents (e.g. rainfall regime) on the microbial use of residue-derived C was minimised, leading to steadier and higher rates of POM accumulation from the outset of the experiment.

Overall, the majority of residue-derived SOM was recovered in the POM fraction after one year *in situ* decomposition (e.g. an average of 65% ± 2.5% of residue-derived SOC across all treatments). The accumulation of POM was most pronounced in the MIX treatment, which was likely the result of greater saturation of mineral-associated fractions in this treatment. The majority of POM, if it is not encapsulated within aggregates or stabilised through inherent chemical recalcitrance (von Lützow et al., 2006) will remain unprotected in the mineral soil. The practical effect of this accumulation of POM is that this SOM pool has a higher degree of vulnerability to changes in management practice (Stewart et al., 2012).

## 5. Conclusion

This study demonstrated that increasing the rate of residue application (LO to HI) and the incorporation of residue with topsoil (MIX) increased the amount of SOM formation and stabilisation. These findings have important implications in the development of soil management recommendations to optimise SOM retention. Soil organic matter formation and retention was most pronounced in the MIX treatment due to a more advanced state of decomposition when residue was incorporated with the topsoil. Rapid accumulation of SOM in the mineral-associated fraction, coinciding with the loss of soluble residue components, indicates the importance of soluble OM in providing an immediate and long term sink for C and N. This must be considered alongside high rates of accumulation in the more readily mineralised POM fraction, particularly when a soil is approaching saturation of mineral-associated fractions, which is likely to lead to greater losses of C and N. Future research should examine whether the incorporation of residue results in the greater stabilisation of residue over longer time periods and whether increased residue-derived SOM stabilisation is offset by increased decomposition of existing SOM.

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