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Article *in* Soil Biology and Biochemistry · October 2016

DOI: 10.1016/j.soilbio.2016.07.008

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The influence of above-ground residue input and incorporation on GHG fluxes and stable SOM formation in a sandy soil

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ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
29 June 2016
Accepted 9 July 2016

Keywords:
Decomposition
Isotopes
Stable SOM
GHG fluxes
Agricultural residue management

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, there is a lack of understanding on the stability of these gains and how changes in soil organic carbon (SOC) pools can influence GHG fluxes. We tracked the fate of above-ground residues into functionally different SOC pools and GHG fluxes using isotopically labelled residues (^{13}C and ^{15}N) 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) mixing residue with topsoil. GHG fluxes were significantly greater at high residue input levels due to the priming of existing SOC and elevated N_2O losses, fuelled by a greater availability of labile substrate. There was evidence of an asymptotic relationship between C input and residue-derived C accumulation in stable soil C pools at higher input levels, indicating that the soil was reaching its protective capacity. Mixing of residues contributed to a 40% increase in GHG fluxes in comparison to surface applied treatment, most notably from residue-derived C and N. This can be attributed to (i) the physical disruption of soil, particularly that of aggregates, which changed the microenvironment stimulating microbial activity, and (ii) greater residue-soil contact. Greater residue-soil contact through mixing also contributed to a 2 fold increase in the residue-derived C recovered in the mineral soil with the majority (56%) in the active C pool. Over a 12 month period, C sequestration was outweighed by GHG fluxes at high rates of input and when residues were mixed with the topsoil. C sequestration policies and associated management approaches must be assessed holistically under a range of conditions and in the long-term to ensure that detrimental practices are not promoted.

1. Introduction

As the largest terrestrial carbon (C) pool (Schlesinger, 1995) soils hold tremendous potential to capture and store increased volumes of C in soil organic matter (SOM), thereby reducing greenhouse gas (GHG) concentrations in the atmosphere (Walthall et al., 2012). This process, referred to as C sequestration, can be achieved through changes in soil management that are also beneficial to the multitude of ecosystem services that soils provide (Dungait et al., 2012).

In agroecosystems, soil C levels are governed by the balance between input of C through plant residues and losses of C, primarily through decomposition i.e. heterotrophic soil respiration (Paustian et al., 2000). Agricultural management practices that increase inputs (e.g. stubble retention) and/or reduce decomposition rates (e.g. no tillage) should increase soil organic carbon (SOC) stocks. A linear relationship between C input and C content has been observed across many long-term trials (Larson et al., 1972; Paustian, 1997; Huggins et al., 1998; Kong et al., 2005) and this is represented in most SOM models (e.g.Parton et al., 1996; Grace et al., 2006). However, in some soils mineral-associated SOC shows little or no increase with increasing C inputs, indicating that soils have a finite capacity to store C within relatively stable pools in the mineral soil matrix (e.g. Hassink, 1996; Huggins et al., 1998; Six et al., 2002; Stewart et al., 2007; Gulde et al., 2008; Castellano et al., 2015).

Tillage is a management practice that increases C losses due to an increased rate of decomposition. This is primarily attributed to

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http://dx.doi.org/10.1016/j.soilbio.2016.07.008

the physical disturbance of soil structure, particularly that of aggregates, which exposes previously inaccessible C to microbial activity (Six et al., 2000). Incorporation of crop residues through tillage also results in greater residue-soil contact, changing the microsite conditions and enhancing microbial access to residue inputs. In contrast no-tillage minimises physical disturbance and leaves residues on the soil surface, thereby restricting microbial access to residues (Helgason et al., 2014).

Whilst there is broad agreement on agricultural practices that increase SOC content, little is known about the stability of these gains. In the context of C sequestration, it is the augmentation of the stable C pools, with the longest mean residence time, that will optimise C storage. Soil C can be stabilised relative to total SOM due to physicochemical protection by mineral association and microaggregate occlusion (Six et al., 2002; von Lützow et al., 2006; Stewart et al., 2008). In order to trace the decomposition of aboveground residues into stable SOC pools, isotopically labelled plant material was used (^{13}C and ^{15}N), which allowed C and N inputs from residues to be differentiated from existing SOM. Rates of isotopically labelled crop residue decomposition have been estimated in a wide variety of plant materials and soil types in both laboratory and natural environmental conditions (e.g. Smith and Douglas, 1971; Jenkinson and Ayanaba, 1977; Ladd et al., 1983, 1985; Amato et al., 1984; Voroney et al., 1989; Bird et al., 2003, 2008; Helgason et al., 2014; Cotrufo et al., 2015). However no previous study has integrated weekly GHG fluxes with stable SOM formation and their response to residue management measures using *in situ* field measurements. SOC pools were isolated through density and size separation in order to obtain (1) an active C pool (particulate organic matter), which is predominantly plant derived and is expected to have a greater sensitivity to land management changes due to its lack of protection (Del Galdo et al., 2003), and (2) stable C pools, which consisted of either C occluded within microaggregates or C associated with silt and clay particles, where C is protected from the decomposer community and expected to have a longer residence time in the soil.

In addition to driving changes in SOC pools, residue management can affect the mineralisation of existing SOC (the priming effect) and other GHG fluxes like nitrous oxide (N_2O) and methane (CH_4), which has the potential to negate any soil C gains (Schlesinger, 1999; Fontaine et al., 2004; Li et al., 2005). Although CO_2 is by far the most abundant GHG, N_2O and CH_4 are also important because of their unique radiative properties and long residence time in the atmosphere resulting in global warming potentials (GWPs) of 298 and 34 times that of CO_2 respectively (IPCC, 2013). This study aimed to provide a holistic assessment of the influence of the rate of residue input and incorporation on the fate of above-ground residues as stable SOM and GHG fluxes. This understanding will be key in the development of soil management recommendations that promote C sequestration; both for soil productivity and to offset global climate change.

2. Materials and methods

2.1. Experimental site and design

The experiment was conducted at Samford Ecological Research Facility, Samford Valley, Queensland, Australia. The climate is subtropical with warm wet summers and dry winters with a mean annual temperature of 20 °C. Annual precipitation averages 1110 mm and is concentrated between the months of November and March. The soil is a Chromosol (70% sand, 24% silt, 6% clay) according to the Australian Soil Classification (Isbell, 2002), with selected soil properties shown in Table 1. The site was a long-term grazed pasture (50 + years), but was kept fallow for the duration of the experiment in order to isolate heterotrophic respiration. Fallow conditions on the site were initiated in May 2013 and the site was kept free of vegetation through the use of herbicides. Livestock were excluded from the study site by a temporary fence.

Soil bulk density (BD) was determined in the experimental area on 4 replicates for 0–5 cm (1.4 g cm^{-3}), 5–10 cm (1.4 g cm^{-3}) and 10–20 cm (1.5 g cm^{-3}). Soil moisture was measured from 0 to 10 cm continuously using a MP406 standing wave soil moisture probe (ICT International Pty Ltd, Armidale, NSW, Australia) that was calibrated for the soil at the research site. Temperature was measured using a data logger (Onset, HOBO) placed at a depth of 10 cm. Soil mineral N content (0–10 cm) was measured at fortnightly intervals over 9 months (September 2013–May 2014) according to van Delden et al. (2016).

PVC collars, 10 cm in diameter, were inserted to a depth of 10 cm (with 5 cm remaining above the soil surface) in August 2013. Plants were previously removed from inside the collars through clipping and herbicide. On 5 September 2013, the dried isotopically labelled residue was placed into PVC collars, in direct contact with the soil surface. Collars were covered by a polyethylene net (mesh size 2 mm) to prevent loss of the labelled residue or input of external litter. A 0.5 cm screened hole was placed in the side of the PVC tube close to the soil surface to prevent the pooling of water during heavy rainfall, whilst limiting residue loss.

The experiment was a single factor design with four replicates which explored the effect of altering the rate of residue input to mimic agricultural residue management strategies such as residue retention (LO = 5t ha^{-1}, MED = 10 t ha^{-1}, HI = 15 t ha^{-1}). This experiment also included a control (zero input) treatment. A second experiment examined the effect of mixing these residues to mimic tillage (MIX). In the mixed residue treatment, the surface 10 cm of soil was removed, mixed with the labelled residues in a plastic bag and returned to the PVC tube. Residues were mixed at the same input rate as MED treatment (10 t ha^{-1}) and a control was established were no residues were added but the top 10 cm of soil was mixed.

2.2. Isotopically labelled residue production and analyses

To trace residue-derived C and N in soils and gaseous effluxes, 3.8 atom % ^{13}C and 5.7 atom % ^{15}N labelled Rhodes grass tops *(Chloris gayana)* were used. The grass was grown within a continuous labelling chamber with controlled conditions, similar to that described in Soong et al. (2014).

Briefly, a demountable unit was used to house a sealed plexiglass chamber measuring 2 × 2 × 3 m. Temperature was maintained at 28 °C by an air conditioner and humidity with a dehumidifier. Temperature and humidity sensors were installed to continually monitor conditions inside the chamber and ensure optimal growth conditions. The $^{13}C\text{-}CO_2$ enrichment was achieved by reacting $^{13}CNaCO_3$ ($^{13}C = 99$ atom %) with HCl (10%). The $^{13}C\text{-}CO_2$ was then pumped into the chamber. CO_2 levels were monitored by drawing chamber air through an Infrared Gas Analyser (Licor LI-800) and then back into the chamber, maintaining a closed system. An injection of $^{13}C\text{-}CO_2$ was triggered if CO_2 levels within the chamber fell below 380 ppm. Rhodes grass were grown from seed in 15 L pots within a soil-free potting mix (vermiculite (20%), perlite (80%)) and fertilised through an irrigation system. Water was pumped from an external tank (50 L) into the chamber and fed to each individual pot with drip irrigation hoses. A return system, for excess water, was created by attaching irrigation hoses to the base of the individual pots. The water was labelled with ^{15}N by using $^{15}N\text{-}KNO_3$ ($^{15}N = 98$ atom %) added to a Hoagland's nutrient solution.

Once the Rhodes grass had reached maturity, the chamber was

Table 1
Selected soil characteristics for Samford Ecological Research Facility.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	BD (g cm^{-3})	pH	EC (μS)	Total C (%)	Total N (%)
0–10	70	24	6	1.4	5.4	46	1.5	0.12
10–20	74	18	8	1.6	6.0	31	1.2	0.07

opened and plants were cut at 10 cm above the potting mix level. Residues were air-dried, cut to 10 cm pieces and well mixed in a homogenous pool. Residue water content was measured on three oven-dried (60 °C) residue subsamples, for dry weight correction. The oven-dried subsamples were mill-ground and used for the determination of C (44%) and N (3.1%) 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. GHG sampling and isotopic analyses

Soil CO_2 efflux measurements were carried out on a weekly basis for one year from September 2013–September 2014. Measurements were taken using a portable soil CO_2 flux system (LI-COR 6100, LI-COR, Lincoln, Nebraska, USA). CO_2 flux rates were calculated using the instruments software (LI-COR Viewer 1.3.0) from the linear increase in CO_2 concentration over time (chamber closure period of 2 min). To gain measurements of N_2O and CH_4 fluxes, gas samples were also taken using the static closed chamber approach (Parkin and Venterea, 2010). Manual gas sampling was carried out weekly and within 24 h of the cessation of heavy rain events to try and ensure peak N_2O fluxes were captured.

For each measurement, an air tight PVC lid was placed on the collar and a sample drawn from the chamber headspace (5 cm) through a septum in the lid after 30 and 60 min. Samples were immediately injected into previously evacuated glass vials (12 ml) (Exetainer, Labco, High Wycombe, Buckinghamshire, UK) with a double wadded Teflon/silica and rubber septa. Gas samples were analysed for absolute concentration of N_2O and CH_4 using an Agilent gas chromatograph equipped with an ECD detector for N_2O and a FID for CH_4. Flux rates of N_2O and CH_4 were calculated from the slope of the linear increase in gas concentration during the closure period as described by Scheer et al. (2014). The coefficient of determination (r^2) was used as a quality check for linearity and flux rates were discarded if r^2 was <0.85 for N_2O and CH_4. In order to calculate the $\delta^{13}C$ of the soil respiration and the $\delta^{15}N$ of the N_2O soil efflux, samples were analysed for $^{13}CO_2$ and $^{15}N_2O$ using an IRMS linked to a Sercon Cryoprep trace gas concentration system.

2.4. Residue and soil collection

After 12 months, all recognisable residues on the soil surface within each collar were carefully picked by hand, dried at 60 °C and weighed. Intact soil cores (depth 10 cm) were excavated by shovel, placed in pre-labelled plastic bags and kept refrigerated (4 °C) until further analysis. Plant and bulk soil samples were dried at 60 °C, mill-ground and analysed for elemental and isotopic analyses by EA-IRMS (Sercon Limited, UK).

2.5. Soil carbon fractionation

All soil samples were sieved to 2 mm, and dried at 60 °C. A representative sub-sample from each soil sample was mill-ground and used for elemental and isotopic analyses as above for residues.

SOM was fractionated by physical and chemical procedures using the protocol described by Zimmermann et al. (2006) and updated using recommendations by Poeplau et al. (2013). 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 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 (mA) 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. POM 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.

2.6. Data analyses

The residue-derived C and N contribution to the bulk soil, SOM fractions, CO_2 and N_2O fluxes 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, SOM fractions, CO_2 and N_2O samples. The δ_S and δ_B are the $\delta^{13}C$ (or $\delta^{15}N$) of the specific bulk soil, SOM, or gas sample from the residue (δ_S) and the control (δ_B) treatment respectively. For bulk soil and SOM fractions, the δ_B average values across 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 were obtained by multiplying the $f_{residue}$ values to corresponding C (or N) pools or fluxes. Residue-derived C and N pools in the SOM fractions were calculated for the 0–10 cm soil depth summing the respective 0–5 and 5–10 cm pool sizes. Soil-derived C and N fluxes were determined as the difference between total C and N fluxes and residue-derived C and N fluxes. The priming effect (PE) was quantified as the difference between soil-derived CO_2 (unlabelled) from SOM mineralisation with residue additions (treatment) and unlabelled CO_2 from SOM mineralisation without residue additions (control). Therefore:

$$PE = F_{soil(treatment)} - F_{soil(control)}$$

Daily soil CO_2 efflux data (F) were modelled between weekly measurements using an empirical function ($F = a\,\theta_V e^{b\,T}$), where T is the soil temperature at a depth of 10 cm, θ_V is the soil water content (0–10 cm) and, a and b are parameters derived from a non-linear curve fit using R Statistics (R version 3.2.3). Residue-derived CO_2 flux was calculated by multiplying the interpolated $F_{residue}/F$ ratio with the corresponding F value following Ngao et al. (2005). All daily F and $F_{residue}$ values were summed between September 2013 and September 2014 to determine cumulative losses.

Daily N_2O fluxes were determined by linear interpolation between weekly sampling points, except for the days directly following a major emission pulse when an exponential decay curve was used based on high resolution N_2O flux data measured at hourly intervals on the same fallow plot (van Delden et al., 2016) using the automated gas sampling system described by Rowlings et al. (2013). The curve of the decline in N_2O flux was determined as the % reduction in N_2O flux in hourly intervals from peak N_2O flux. These values were then applied to weekly manual gas sampling data.

We used IPCC (IPCC, 2013) global warming potential (GWP) factors over a 100 year time horizon to calculate CO_2-equivalents ha^{-1} yr^{-1} for the estimated differences in (1) soil organic carbon sequestered = C in all measured pools; particulate organic matter (POM), microaggregates (mA) and silt and clay (SC), (2) treatment induced primed soil C (difference between soil-derived C flux in treatment and control), and (3) N_2O flux (difference between total N_2O flux treatment and control) (4) CH_4 flux (difference between total CH_4 flux and control), using the following equations (Six et al., 2004):

$$SOC_{\ sequestered} = SOC_{\ (POM)+(mA)+(SC)} \cdot \frac{44}{12} \cdot (-1)$$

$$SOC_{\ primed} = CO_{2(soil-derived\ C\ flux\ treatment)} - CO_{2(soil-derived\ C\ flux\ control)} \cdot \frac{44}{12} \cdot (-1)$$

$$\Delta N_2O = N_2O_{(total\ flux\ treatment)} - N_2O_{(total\ flux\ control)} \cdot 298$$

$$\Delta CH_4 = CH_{4\ (total\ flux\ treatment)} - CH_{4\ (total\ flux\ control)} \cdot 34$$

The total GHG balance was calculated using the equation:

$$GHG\ balance = SOC\ sequestered + SOC\ primed + \Delta N_2O + \Delta CH_4$$

To combine uncertainties for the GHG balance calculation, we converted the individual variances into CO_2 equivalents and summed those values using the equation:

$$Var\ GHG\ balance = Var\ (SOC) + Var(SOC\ primed) + Var(N_2O) + Var\ (CH_4)$$

The square root of this sum is the estimated standard deviation. This computation of uncertainty implicitly assumes that the three components of total GWP are uncorrelated (Six et al., 2004).

2.7. Statistics

One-way analysis of variance (ANOVA) was performed on $\delta^{13}C$, C and N content values using the statistical package SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Treatment differences were considered significant when $p < 0.05$.

3. Results

3.1. Overall recovery: expressed as % of applied residue-derived C recovered

In the surface applied treatments, an average of 13% of applied residue-derived C was recovered to a depth of 20 cm, in comparison to 27% of applied residue-derived C in the mixed treatment (Fig. 1). A lower recovery of residue-C in the mineral soil in surface applied treatments corresponded to a higher proportion of applied residues (average of 34%) remaining undecomposed on the soil surface after 12 months. The proportion of residues remaining undecomposed on the surface increased at higher input levels (29% of applied residue-C in LO input treatment and 38% of applied residue-C in HI input treatment). There was no significant difference in CO_2 flux between the HI and LO input treatments (25% of applied residue-C respired in HI input treatment, versus 27% in LO input treatment). The incorporation of residues in the MIX treatment meant that there were no residues left undecomposed on the soil surface. Mixing of residues resulted in a two fold increase in the rate of mineralisation of applied residues in comparison to the equivalent surface applied treatment (44% of applied residues were mineralised in MIX treatment versus 23% in MED). In terms of the remaining residue-C that was unaccounted for, some may have been leached to soil depths >20 cm, which is likely given the relatively high sand content (70%) of the soil. Residue-derived C could also have been in fine dissolved organic carbon (DOC) (<0.45 μm) which was separated from silt and clay during fractionation. The size of this fraction was estimated by the difference between the bulk soil (whole soil measurement prior to fractionation) and the sum of the fractions (POM + mA + SC) and show that DOC only accounted for 1–3% of residue-C. The largest portion of SOC that was unaccounted for is most likely coarse organic matter fragments (>2 mm) that were removed when bulk soil was sieved to < 2 mm prior to fractionation.

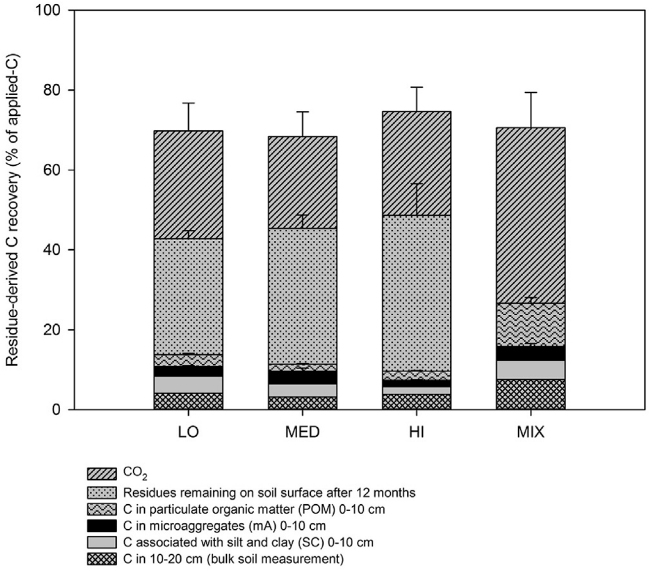

Fig. 1. Total recovery of applied residue-derived C across different treatments (expressed as % of applied residue-derived C recovered). C recovered in bulk soil 10–20 cm, C in stable C pools (C associated with silt and clay and C in microaggregates) 0–10 cm, particulate organic matter 0–10 cm, residues remaining on the soil surface after 12 months (only applicable to surface applied treatments LO, MED, HI) and residue-derived CO_2 flux. 100% recovery was not achieved. This is most likely due to the removal of coarse fragments of organic matter when the soil was sieved <2 mm prior to fractionation.

3.2. The contribution of applied residues to GHG fluxes

Carbon dioxide dynamics over 12 months were primarily influenced by variations in soil moisture (Fig. 2a, Fig. 2b). The cumulative CO_2 flux over 12 months was greatest from MIX treatment (20.2 t C ha^{-1}) followed by HI (16.9 t C ha^{-1}) > MED (15.6 t C ha^{-1}) > LO (12.4 t C ha^{-1}) (Fig. 4). Cumulative C losses were significantly greater than control plots in all treatments ($p < 0.05$)

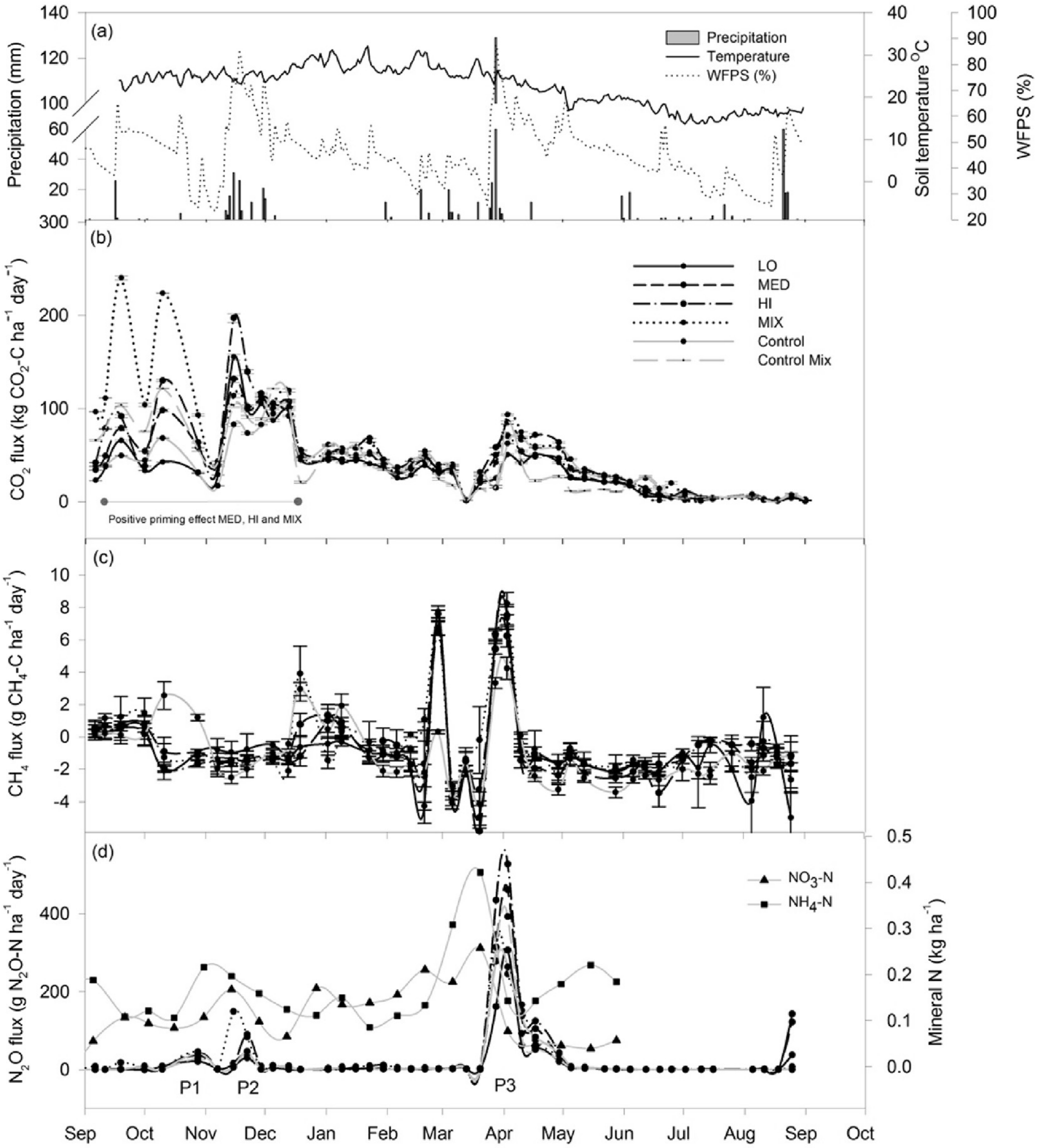

Fig. 2. Temporal dynamics of GHG fluxes (CO_2, CH_4 and N_2O), Samford, September 2013–September 2014 for input treatment (LO, MED, HI) and tillage (MIX). LO = low input (5t ha^{-1}), MED = medium input (10 t ha^{-1}), HI = high input (15 t ha^{-1}), MIX = mixed treatments where residues were mixed with top 10 cm of soil at same input as MED (10 t ha^{-1}). (a) Temperature at 10 cm depth (solid line), precipitation (bars), and WFPS % (dotted line). (b) Total CO_2 flux in all treatments in comparison to Control. Horizontal line indicate the length of time over which the priming effect was most significant for MED, HI and MIX treatments (c) CH_4 flux in all treatments in comparison to Control. (d) N_2O flux in all treatments in comparison to Control, indicating 3 major emissions pulses during experiment (P1–P3) and soil mineral N levels; nitrate (triangles) and ammonium (squares). The values are means of four replicates (±SE).

except LO input where there was no significant difference. The greatest variation in CO_2 flux between treatments was seen from week 2 – week 13 (September–December) in Fig. 2b. On average, this period accounted for 52% of the annual CO_2 flux in the surface input treatments (LO, MED, HI) and 61% in MIX treatment. Differences in CO_2 flux between residues input levels were most apparent during heavy rainfall events. For example, the first major rainfall event in November, when 82 mm was received over 7 days, resulted in CO_2 fluxes ranging from 155 kg CO_2-C ha^{-1} day^{-1} in LO input treatment to 197 kg CO_2-C ha^{-1} day^{-1} in HI input treatment.

One day after the application of residues, the contribution of residue-derived C to heterotrophic respiration (*f* residues) was 33% of the total CO_2 flux in MIX treatment, in contrast to an average of 3% in surface applied treatments (LO, MED, HI) (Fig. 3). The contribution of residue-derived C to CO_2 flux increased considerably during rainfall events. For example, in mid-November, when 54 mm rain fell over 5 days, *f* residues (measured on 15 November) contributed to 30% of LO input treatment, 54% MED and 59% of HI input treatment CO_2 flux.

The addition of residues resulted in a greater soil-derived CO_2 flux than the control (i.e. a priming effect) in MED, HI and MIX treatments, which remained significant for four months after the application of residues coinciding with the early stages of decomposition (Table S1). Over 12 months, the priming effect was only significant in the MIX treatment, where primed CO_2 contributed to 15% of the total CO_2 flux (Fig. 4).

The greatest N_2O fluxes occurred when heavy rainfall events coincided with elevated soil mineral N levels (Fig. 2d). Major

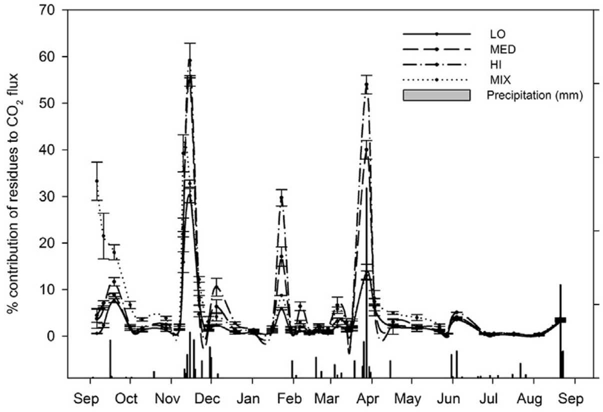

Fig. 3. The variation in the relative contribution of residue-derived C to total CO_2 flux (expressed as a % of total CO_2 flux, *f* residues) over 12 months in residue input (LO, MED, HI) and mixed (MIX) treatment showing an increase in residue-derived CO_2 flux corresponding to periods of heavy rainfall (shown in bars). The values are the mean of four replicates (±SE).

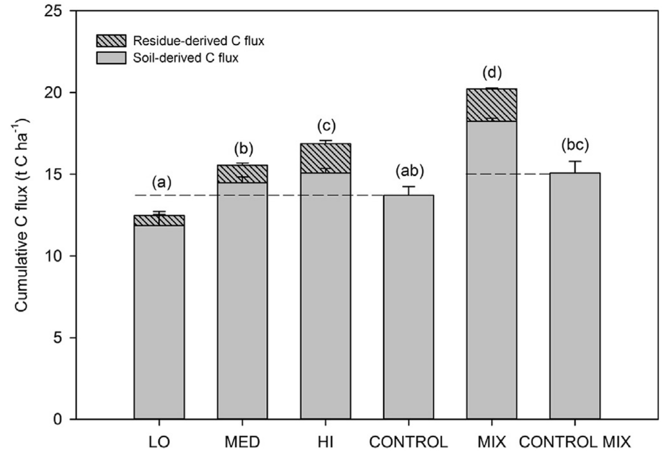

Fig. 4. An increase in cumulative C flux with increasing input of residues (LO → HI) and with mixing (MIX). Cumulative C loss over 12 months is partitioned into residue-derived C loss and soil-derived C loss. Soil-derived C loss was higher than the Control in MED, HI and MIX treatments, indicated by the dotted line. This difference (i.e. the priming effect) was only significant in MIX treatment over 12 months. The values are means of four replicates (±SE). SE is shown for residue and soil-derived C flux for treatments, and for Controls is SE of total C flux. Letters indicate significant differences across treatments for total CO_2 flux.

rainfall events during the experimental period resulted in 3 main emission pulses (P1–P3) that contributed to an average of 89% of annual N_2O flux for all treatments. Cumulative N_2O fluxes were significantly greater in MIX treatment (5.6 kg N_2O-N-ha^{-1}) followed by HI (5.1 kg N_2O-N-ha^{-1}) and MED (3.8 kg N_2O-N-ha^{-1}), with LO input treatment (2 kg N_2O-N-ha^{-1}) showing no significant difference to the control (Fig. 5). The largest emissions pulse (P3 in Fig. 2d) corresponded to a significant rainfall event in March where 169 mm of rainfall was received over 6 days with a maximum of 129 mm received in one day on the 28th March 2014 and coincided with high mineral N levels (0.26 kg NO_3-N ha^{-1} and 0.42 kg NH_4-N ha^{-1} 0–10 cm layer).

Residue-derived N flux was greatest from the MIX treatment (1.6 kgN_2O-N ha^{-1}), where it contributed to 29% of total N_2O flux, which was significantly higher than surface applied treatments (LO, MED, HI) where residue-derived N flux contributed to an average of 10% of total N_2O flux (Fig. 5). Residue-derived N_2O losses

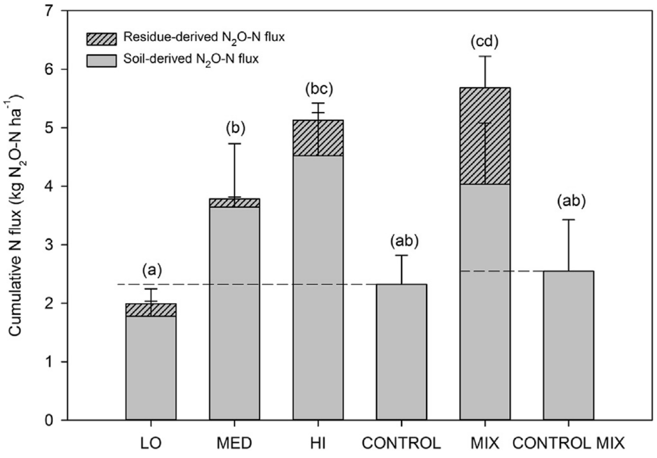

Fig. 5. An increase in cumulative N_2O flux with increasing input (LO → HI) and with mixing (MIX). N_2O flux is partitioned into residue-derived N_2O flux and soil-derived N_2O flux. Difference between soil-derived N flux and N flux in control is shown by horizontal dotted line (i.e. priming effect). A positive PE is shown in MED, HI and MIX treatments, but is only significant in HI input treatment. A lower soil-derived N flux than control is shown in LO input treatment, but this difference is not significant. The values are means of four replicates (±SE). SE is shown for residue and soil-derived N flux for treatments, and for Controls is SE of total N flux. Letters indicate significant difference between treatments.

represented between 0.1 and 0.4% of added N with the highest proportion of added N lost in the MIX treatment. The N priming effect (additional soil-derived N_2O flux from treatment plots in comparison to control plots) was only significant in the HI input treatment over 12 months where it contributed to 43% of the total N_2O flux. The mixing of residues with the topsoil (MIX) did not affect the magnitude of the PE as there was no significant difference between MIX and MED treatments.

There was a net uptake of CH_4 in all treatments over 12 months, with the greatest uptake corresponding to dry periods (May–August) and net release during wet periods (February–April) (Fig. 2 c). There was a significantly lower net uptake of CH_4 in MIX treatments (276 g CH_4-C ha^{-1}) with no significant difference in surface applied treatments with an average uptake of −456 g CH_4-C ha^{-1}.

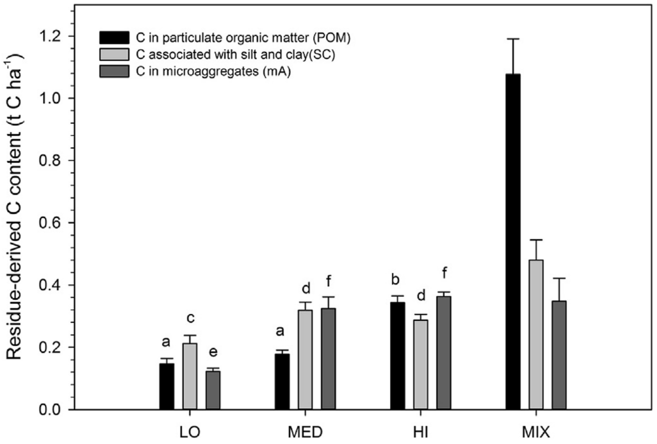

Fig. 6. The recovery of residue-derived C across residue input (LO, MED, HI) and mixed treatment (MIX) in SOC fractions (bars). Active C fraction = particulate organic matter (POM), stable C fractions = silt and clay associated C and C in microaggregates. Letters indicate significance (one-way ANOVA) between fractions across different input levels (LO, MED and HI). Values are means of 4 replicates (±SE).

3.3. *C input from surface residues to SOM*

The amount of residue-derived C recovered in the soil increased with an increasing level of input (0.48 t C ha^{-1} in LO input and 0.99 t C ha^{-1} in HI input, 0–10 cm) (Fig. 6). However, there was no significant difference in the proportion of residue-C recovered (expressed as % of applied C) in bulk SOM between LO and HI input (8% and 12% of applied residue-derived C recovered respectively to a depth of 10 cm). The mixing of residues resulted in over twice as much residue-derived C being recovered in bulk SOM (22% and 10% of applied residue-C recovered and MIX and MED treatments respectively).

There was a significant increase in residue-derived C content in both stable C fractions (C in microaggregates and C associated with silt and clay) from LO → MED input but no significant increase from MED → HI input (Fig. 6). This indicates that there was a decline in the efficiency at which C inputs accumulated in stable C fractions at higher input levels (stabilisation efficiency = ΔC inputs/ΔC stabilised). There was no significant increase in POM from LO → MED but a significant increase from MED → HI ($p < 0.05$). There was a significantly higher amount of POM recovered in MIX treatment than surface applied treatments, with over 3 times as much POM recovered in MIX than MED input treatment. POM therefore accounted for a much larger % of residue-derived C recovered in MIX treatment in comparison to the equivalent surface applied treatment, MED (13% and 2% respectively) (Fig. 6). Mixing resulted in a higher amount of residue-derived C recovery in the silt and clay associated fraction ($p = 0.05$), but there was no significant different in residue-C recovery in microaggregates.

4. Discussion

The highest GHG fluxes were experienced in the MIX treatment due to its impact on soil biophysical properties as mixing affects soil structure, aeration, soil temperature, water content and placement of residues i.e. their proximity with the soil matrix. All of these affect microbial activity, which in turn affects the rate of decomposition and N mineralisation (Mangalassery et al., 2014).

A significant C priming effect, whereby the addition of residues accelerated the decomposition of existing organic matter, was observed in MED, HI and MIX treatments in the four months immediately following residue application. The C priming effect is most likely due to an increased availability of substrate for microorganisms, which induced enzyme production or increased enzyme activity leading to a co-metabolic decomposition of organic matter (Kuzyakov, 2000). An increase in the magnitude of the priming effect at higher substrate input levels is in line with other studies (e.g. Dumontet et al., 1985; Mary et al., 1993; Guenet et al., 2010). C priming was compounded when residues were incorporated with the top soil as mixing disrupted the soil structure exposing previously inaccessible C to microbial activity. Mixing also resulted in an improved movement of water and air further ‘fuelling the fire’ of decomposition (Ball et al., 1999; Udawatta and Anderson, 2008).

Significant losses of N_2O occurred during periods of prolonged and heavy rainfall where WFPS exceeded 70%. This indicates that N_2O fluxes were primarily through denitrification under anaerobic conditions. N_2O fluxes increased at higher input levels suggesting that the supply of labile organic C was a key control on the rate of denitrification as C provides energy for denitrifying bacteria (Firestone and Davidson, 1989; Aulakh et al., 1991; Dorland and Beauchamp, 1991). However, we cannot discount that higher N fluxes under higher input levels may also be due to; (1) higher initial N input under HI input treatment which resulted in a higher accumulation of mineral N, or (2) that higher rates of microbial respiration under HI input treatment lead to the creation of anaerobic microsites making denitrification more likely (Baggs et al., 2000). We can discount a mulching effect (whereby an increase in the rate of residue addition could result in a higher soil moisture content and conditions more conducive to denitrification) as there were greater N fluxes from MIX (residues incorporated) versus MED (residues on surface).

The mixing of residues with the topsoil resulted in greater N_2O emissions than when residues were applied at the surface, particularly of residue-derived N. Elevated N_2O emissions due to mixing is in contrast to a number of studies which show a reduction of N_2O emissions with tillage as mixing improves soil aeration meaning that there is less potential for anaerobic conditions to develop which promote denitrification (MacKenzie et al., 1998; Ball et al., 1999). Our result could be indicative of soil texture at the experimental site (sandy and well-drained on minor slope) as Rochette et al. (2008) demonstrated that no-till only resulted in increased N_2O emissions in poorly aerated soils. Greater N_2O fluxes in MIX treatment may have also resulted from greater microbial activity as residues were brought into direct contact with soil microbes, resulting in the formation of O_2 limited microsites (Chantigny et al., 2002; Mutegi et al., 2010). The increase in N_2O flux under MIX treatment was particularly notable for residue-derived N, where it was 12 times greater than MED, demonstrating the effect of greater residue contact with the soil matrix on the rate of mineralisation of applied residues.

Mixing of residues resulted in an overall lower net uptake of CH_4 than surface applied treatments, which may have been due to the reduced activity of methanotrophs (CH_4 oxidising bacteria) (Ussiri et al., 2009) that are negatively affected by soil disturbance (Mosier et al., 1997).

Higher rates of residue addition resulted in an increase in residue-derived C content in all measured C pools (POM, mA, SC). However, the rate of residue-derived C increase differed between fractions, with the highest rate of accumulation in the POM fraction (active C) and the lowest in the stable C fractions (C in microaggregates and C associated with silt and clay, mA and SC), suggesting that there was an asymptotic relationship between C input and SOC content in stable C pools. This is aligned with a growing body of evidence from a diverse array of ecosystems that indicates soils have a finite capacity to store C within relatively stable pools in the mineral soil matrix (Castellano et al., 2015).

In the silt and clay fraction (SC), there was only a significant increase in residue-derived C content from LO → MED. It is likely that at lower input levels the soil matrix had the capacity to stabilise C through associations with silt and clay particles (e.g. ligand exchange, hydrogen bonding). As the input level increased from MED → HI the residue-derived C content did not increase significantly indicating that the amount of C that could associate with silt and clay particles was reaching an upper limit in this sandy soil, comparable to findings by Hassink (1997). However, there was a higher proportion of residue-derived C recovered in MIX versus MED ($P = 0.054$) suggesting that there may be some (although limited) capacity for further stabilisation if the timeframe of the experiment was extended beyond 12 months and residues in HI input treatment were able to reach a similar level of decomposition as incorporated residues. The application of residues on the surface limited the residue-soil contact, particularly in the HI input treatment, which acted as a rate-limiting step to their decomposition and eventual incorporation into <53 μm fraction. A higher rate of accumulation of residue-derived C in MIX treatment in silt and clay fraction occurred due to residues being brought into direct proximity with mineral surfaces, in line with findings by Stemmer et al. (1999). Therefore, a decline in the accumulation of residue-derived C at higher input levels in the silt and clay

Table 2
The magnitude of the C priming effect and N_2O and CH_4 fluxes in relation to SOC sequestered (kg CO_2 eq ha^{-1}) over 12 months. Both stable (microaggregate-C and silt and clay-C) and active SOC (POM) considered as sequestered. The balance shows that GHG emissions offset soil C sequestration in HI and MIX treatments over 12 months.

Treatment	Total SOC sequestered (POM, mA and SC) (kg CO_2 eq ha^{-1})	CO_2 (priming effect) (kg CO_2 eq ha^{-1})	N_2O (kg CO_2 eq ha^{-1})	CH_4 (kg CO_2 eq ha^{-1})	GHG balance (kg CO_2 eq ha^{-1})
LO	−1764	0	152	−1.2	−1612 (883)
MED	−3008	182	211	−1.6	−2616 (575)
HI	−3199	7606	1292	3.6	5702 (589)
MIX	−6983	7405	1443	6.1	1872 (703)

fraction was likely due to a combination of (i) a low capacity for matrix stabilisation in a sandy soil and (ii) a limit to the rate at which residue-C can be decomposed and form associations with silt and clay particles in surface applied treatments due to restricted residue-soil contact.

Similarly, residue-derived C protected within microaggregates only increased significantly at lower input levels (LO → MED), suggesting that C content within microaggregates may also have reached an upper limit. This is likely given the low level of aggregation in this sandy soil and that microaggregates reach their C saturation potential before larger aggregate classes (Kool et al., 2007). It was expected that mixing would result in a lower accumulation of residue-derived C in the microaggregate fraction, due to the physical disruption of the soil and an increase in aggregate turnover (Six et al., 1999, 2000). However, our results showed that there was no significant difference between MIX and MED treatments indicating that (i) disruption of aggregates did not have an effect on the turnover of residue-derived C in this sandy soil (where aggregation was limited) and (ii) mixing, which brought the residues into closer proximity with the mineral soil, did not increase the rate of incorporation of residue-derived C into microaggregates. This supports the idea that there was C saturation of microaggregates, as increased contact with the soil matrix and microbial population did not act to increase residue-derived C incorporation into microaggregates.

In contrast to the stable C fractions, there was no evidence of an asymptotic relationship between C input and residue-derived C content in the POM fraction as there was a significantly higher amount of POM in HI versus MED input treatment. There was a 2 fold increase in POM from MED → HI input and no significant change in the stable C fractions (microaggregates and silt and clay associated C), demonstrating that the accumulation of C in POM was responsible for almost all of the increase in the SOC content from MED → HI input treatment. This demonstrates that there was no limit to the accumulation of POM under these experimental conditions, in agreement to findings by Liao et al. (2006). The higher rate of accumulation of POM at higher input rates is likely to have resulted from the saturation of stable C fractions, meaning that any further C input accumulated as POM rather than being stabilised. This finding is significant in terms of C sequestration; in a soil with a low saturation deficit (Castellano et al., 2015) a large proportion of above-ground C input will accumulate in the active C pool which is relatively easily decomposed due to its unprotected status, meaning that it is readily lost from the system (Cambardella and Elliott, 1992).

Overall, if SOC gains (in all C fractions measured; POM, mA and SC) are balanced with GHG fluxes and converted to CO_2 equivalents, we note that there is the potential for GHG fluxes to outweigh SOC gains in the short term in the HI and MIX treatments (Table 2). However, whilst the impact of residue application on GHG fluxes is likely to have been captured in the 12 month measurement period, changes in SOC will take place over a much longer timeframe. This precludes an overall GWP assessment of residue application strategies as there is no certainty on the fate of the remaining undecomposed surface residues (in surface applied treatments) and the POM fraction beyond 12 months, highlighting the need for longer term experimentation. Nevertheless, the C balance gives some indication of the sequestration potential during the implementation phase of residue application.

5. Conclusion

A process level assessment of different residue application strategies has shown that increased soil GHG emissions can potentially offset SOC gains at high C input levels and when residues are incorporated with the top soil over a 12 month period. An increase in the rate of residue addition and mixing of residues with the top soil increased GHG losses, particularly through the priming of existing SOC and N_2O by denitrification. Mixing amplified the loss of residue-derived C and N, in comparison to surface applied treatments, illustrating the importance of greater physical contact of residues with soil on their decomposition. Whilst mixing increased the loss of residue-derived CO_2 and N_2O, it also resulted in a more residue-derived C being recovered in the mineral soil than the equivalent surface applied treatment. However, the majority of these SOC gains were in the active C pool, which is not conducive in the context of C sequestration as it is rapidly mineralised and lost from the system. A similar trend was noted as the level of C input increased, due to the decline in stabilisation efficiency of residues at higher input levels. A decline in the stabilisation efficiency of C in stable C pools highlights the need to consider the C stabilisation capacity of soils (matrix stabilisation) and their level of existing C saturation prior to the implementation of any management changes. Further scrutiny of residue management practices and their impact on stable SOM formation and GHG fluxes is required under a range of conditions and in the long-term to ensure the success of C sequestration policies.

Acknowledgements

This project was financially supported by the Australian Government's Department of Agriculture, and Water Resources as part of the National Soil Carbon Programme (Filling the Research Gap, FtRG) (project number 01203.073). The data reported in this paper were obtained at the Central Analytical Research Facility operated by the Institute of Future Environments (QUT). We would like to thank Alice Strazzabosco and Johannes Friedl for their work on IR-MS; and Sarah Carrick, Stephen Leo, Charles Winchester and Christopher Chandler for their work on soil fractionation. Access to CARF is supported by generous funding from the Science and Engineering Faculty (QUT).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.07.008.

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