



# Greater soil carbon stocks and faster turnover rates with increasing agricultural productivity

Jonathan Sanderman<sup>1,2</sup>, Courtney Creamer<sup>1,3</sup>, W. Troy Baisden<sup>4</sup>, Mark Farrell<sup>1</sup>, Stewart Fallon<sup>5</sup>

<sup>1</sup>Agriculture & Food, CSIRO, PMB 2, Glen Osmond SA 5064, Australia

<sup>2</sup>current address: Woods Hole Research Center, Falmouth MA 02540, USA

<sup>3</sup>current address: USGS, Menlo Park CA, USA

<sup>4</sup>National Isotope Centre, GNS Science, Lower Hutt, New Zealand

<sup>5</sup>Australian National University, Canberra ACT, Australia

10 *Correspondence to:* Jonathan Sanderman (jsanderman@whrc.org)

**Abstract.** Devising agricultural management schemes that enhance food security and soil carbon levels is a high priority for many nations. However, the coupling between agricultural productivity, soil carbon stocks and organic matter turnover rates is still unclear. Archived soil samples from four decades of a long-term crop rotation trial were analysed for soil organic matter (SOM) cycling relevant properties: C and N content, bulk composition by NMR spectroscopy, amino sugar content, short term C bioavailability assays, and long-term C turnover rates by modeling the incorporation of the bomb-spike in atmospheric <sup>14</sup>C into the soil. After >40 years under consistent management, topsoil carbon stocks ranged from 14 to 33 Mg C ha<sup>-1</sup> and were linearly related to the mean productivity of each treatment. Measurements of SOM composition demonstrated increasing amounts of plant- and microbially-derived SOM along the productivity gradient. Under two modelling scenarios, radiocarbon data indicated overall SOM turnover time decreased from 40 to 13 years with increasing productivity; twice the rate of decline predicted from simple steady-state models or static three-pool decay rates of measured C pool distributions. Similarly, the half-life of synthetic root exudates decreased from 30.4 to 21.5 hours with increasing productivity indicating accelerated microbial activity. These findings suggest that there is a direct feedback between accelerated biological activity, carbon cycling rates and rates of carbon stabilization with important implications for how SOM dynamics are represented in models.

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## Key Words

soil organic matter; carbon sequestration; carbon models; radiocarbon; amino sugars; NMR spectroscopy; microbial activity



## 1 Introduction

Sequestration of carbon as soil organic matter (SOM) through shifts in land use and improvements in land management is seen as having the potential to offset a significant fraction of current greenhouse gas (GHG) emissions (Smith et al., 2008; Paustian et al., 2016), and also as an important stop-gap measure as nations transition to a low carbon economy (Read, 2008). A major reason for the prominent position soil carbon sequestration has in national GHG reduction agendas is because of the purported “win-win” situation of mitigating GHG while improving food security through improved soil health and cropland fertility (Tiessen et al., 1994).

What has been mostly lost in the debate is the concept that the much of the biological utility of SOM in agriculture comes not from its accumulation but rather from its decay and subsequent release of nutrients and energy (Albrecht, 1938). Janzen (2006) eloquently argued that there may be a trade-off between hoarding and using soil organic carbon (SOC), what he termed “the soil carbon dilemma.” The argument goes that while sequestering carbon as SOM there are nutrients and energy that must remain non-available to plants and microorganisms in association with this new C because of stoichiometric constraints (Manzoni et al., 2010). These nutrients are only then released upon decomposition of the SOM. Some researchers (e.g., Lam et al., 2013; Kirkby et al., 2014) have gone as far as putting a monetary nutrient cost on SOC sequestration.

At the heart of this argument is the fundamental question of how carbon flow in soils is conceptualized and whether or not microbial physiology needs to be explicitly represented to accurately model SOC dynamics (Allison and Martiny, 2008; Lawrence et al., 2009). Microbes and the exoenzymes that they produce are the primary agents of decomposition. It would be logical to think that the activity and abundance of microbes and exoenzymes would be critical components of a SOC model, yet many SOC models can successfully simulate C dynamics at coarse scales by only implicitly representing microbial activity as static decay constants (Schimel, 2001). While these linear models generally work well, their predictive capacities may be limited (Sanderman et al., 2014) as evidenced by the large range of SOC responses (-70 to +86 Pg C change over the past century) reported for different terrestrial biosphere models by Tian et al. (2015). Microbe and enzyme-based SOC models produce greatly diverging predictions in response to major perturbations as compared to traditional linear models assuming implicit microbial physiology (Allison et al., 2010; Weider et al., 2013; Lange et al., 2015).

In addition to the elevated importance of soil microbes in SOC turnover models, there is mounting evidence from biochemical studies that the byproducts of microbial activity (i.e., microbial necromass) are dominant components of the stable SOM pool (Liang and Balser, 2008; Miltner et al., 2012; Cotrufo et al., 2013, 2015). If soil microbial activity is critical for both the decomposition and stabilization of SOM, then a better understanding of the microbial response to a major perturbation such as changing plant productivity would be critical to assessing the validity of the soil carbon dilemma.



In this study, we test whether or not there is a trade-off between sequestering and using SOC by utilizing a unique set of samples from a long-term agricultural trial in southern Australia. In particular, we exploit the spike in atmospheric  $^{14}\text{CO}_2$  due to aboveground nuclear weapons testing to determine if intrinsic SOC cycling rates (defined as the decay constants) differed among agricultural management regimes that make up a large productivity gradient. A change in SOC decay rates in response to management would indicate a biological feedback to SOC decomposition that is currently omitted from traditional C cycling models. The  $^{14}\text{C}$ -based soil carbon turnover rates are then supported by measurements of SOM chemistry and short-term soil microbial activity. Finally, we discuss the results in terms of biological controls on SOC cycling and how soil microbial processes are represented in carbon models.

## 2 Materials and methods

### 2.1 Trial description

The Permanent Rotation Trial at the Waite Agricultural Research Institute, South Australia, was established on a former open Eucalyptus woodland in 1925 to study the agronomic effects of 10 different cropping rotations including the use of long fallows and pasture phases. The site has a Mediterranean climate typical of the region. Mean annual rainfall is 626 mm, with 487 mm falling in the April-October growing season. Maximum and minimum mean annual temperatures are  $21^\circ$  and  $12^\circ\text{C}$ , respectively. The soil is classified as a Rhodoxeralf (USDA; Soil Survey Staff, 1999) or Chromic Luvisol (WRB; FAO, 1998) with a fine sandy loam texture in the upper soil horizons. Soil in the upper 10 cm had an average pH ( $\text{H}_2\text{O}$ ) of 5.9 and a clay content of 18% (Grace et al., 1995). As most Australian soils are typically deficient in phosphorous (Richardson et al., 2009), a basal dressing of superphosphate fertilizer was applied annually across all managements. No nitrogenous fertilizers were used in the trial. Cultivars, cultivation and stubble management were consistent with typical district practice throughout the trial and have been detailed in Grace et al. (1995). Full agronomic production and climatic data records can be downloaded from the CSIRO Data Access Portal (DOI: 10.4225/08/55E5165EC0D29).

In this current study we have chosen to analyze archived soil samples from five plots representing a gradient in productivity: permanent pasture (Pa), two years wheat followed by four years of pasture (2W4Pa), continuous wheat (WW), wheat-oats-fallow rotation (WOF) and wheat-fallow rotation (WF). While every phase of each rotation was represented annually, we randomly chose one plot for each of these rotations (WF, WOF, 2W4Pa) for analysis. Detailed records of grain yield, aboveground dry matter production, pasture production and composition were kept for the duration of the trial. In 1963, 1973, 1983 and 1993 soil samples from the 0-10 cm layer were collected by compositing 20 soil cores taken along the 90 m length of each plot prior to sowing. Samples were dried in a forced fan oven at  $40^\circ\text{C}$  for at least 48 hours before being stored in glass jars.



## 2.2 Soil analyses

All soil samples were analyzed for OC content ( $\text{mg C g}^{-1}$ ) via high temperature combustion (LECO CNS-2000, LECO Corp., St. Joseph, MI). Soil OC stocks ( $\text{Mg C ha}^{-1}$ ) were then calculated by multiplying measured TOC values ( $\text{mg C g soil}^{-1}$ ) with bulk density measurements ( $\text{Mg soil m}^{-3}$ ) adjusted for sampling depth. No gravel correction was necessary. Bulk density (BD) was only measured in all rotations in 1997 (Baldock, 1998). To overcome this data limitation, we have developed a pedotransfer function using 132 samples from the 1997 dataset (see Appendix A) recognizing that since soil texture was similar across the trial, the main variation in BD will be due to changes in TOC content. This pedotransfer function ( $\text{BD} = -0.109 \times \text{TOC (\%)} + 1.543$  (Fig. A1)) was then applied to the measured TOC values for the 1963-1993 samples. Given the small range in measured BD (1.25 – 1.58) across a 5-fold gradient in TOC, this approximation for BD appears to be the best of a range of options to minimise uncertainty and bias in calculated SOC stocks .

Mid infrared spectroscopy in combination with partial least squares regression (MIR-PLSR) was used to estimate the distribution of OC into three biologically meaningful pools, the particulate (POC), humus (HOC), and resistant (ROC) organic carbon fractions (Baldock et al., 2013a, b), using the Unscrambler X software package (CAMO Software, Oslo, Norway). Prediction statistics (Hotelling's T-square and Mahalanobis distance) suggested that this soil type was well represented in the calibration set of Baldock et al. (2013b). Given that the three fractions were predicted independently, the sum of the fractions did not always equal the measured TOC values (mean carbon mass balance =  $101 \pm 5\%$  (s.d.)). Thus, the predicted fraction data were adjusted to sum to the measured TOC values by calculating the fractional distribution of the MIR-PLSR predicted fractions (i.e.,  $\text{POC}/(\text{POC}+\text{HOC}+\text{ROC})$ ) and then multiplying these proportional allocations by the measured SOC stock.

Organic matter chemistry was assessed using solid-state cross-polarization magic-angle spinning  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy and by quantification of amino sugars. Solid-state  $^{13}\text{C}$  NMR spectra were obtained for the 1983 soil samples using a Bruker 200 Avance spectrometer (Bruker Corporation, Billerica, MA, USA) equipped with a 4.7 T, wide-bore superconducting magnet operating at a resonance frequency of 50.33 MHz. Soils were first demineralized using 2% hydrofluoric acid (HF) to concentrate C and remove paramagnetic interferences (Skjemstad et al., 1994). The HF procedure resulted in an average C loss of 17.4%. Operating conditions were identical to those reported in Baldock et al. (2013a). In order to quantify absolute differences in chemical composition, data were reported by normalizing the recorded signal intensity by the amount of observable C, determined following the conventions of Smernik and Oades (2000), in the analysed sample.

Amino sugars, used here as a biomarker for microbial derived OM (Liang and Balser, 2008), were extracted and derivatized to form aldonitrile acetate derivatives from 300-500 mg of ground soil following the procedure of Zhang and Amelung (1996)



as modified by Liang et al., (2012). The aldonitrile acetate derivatives were identified and quantified relative to a 6-point calibration curve containing all compounds of interest and relative to the internal standard (myo-inositol) on an Agilent 7890G GC and 5977 MS using a 30 m DB-5 column (Agilent Technologies, Santa Clara, CA, USA). The recovery standard N-methylglucamine monitored derivatization efficiency.

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Radiocarbon activity was measured by accelerator mass spectrometry at the Australian National University Radiocarbon Dating Laboratory (Fallon et al., 2010) and reported using the geochemical  $\Delta^{14}\text{C}$  notation (Stuiver and Polash, 1977). Analysis of radiocarbon data is presented in the next section.

10 To assess the short-term availability and turnover of carbon, we measured extractable OC, basal respiration, and the turnover of a  $^{14}\text{C}$ -labeled synthetic root exudate cocktail on the 0-10 cm samples from all plots and from all years with 3 analytical replicates. Extractable OC was quantified from a 0.5 M  $\text{K}_2\text{SO}_4$  extract (1:5 soil:solution) on a Thermalox TOC analyser (Analytical Sciences, Cambridge, UK). Basal respiration was measured as the  $\text{CO}_2$  accumulation in a 24 h period at 22 °C on 5 g d.w. soil in a 50 mL centrifuge tube capped with a Subaseal using an infra-red gas analyser (LiCor Li-820, LI-COR Biosciences, Lincoln, NE, USA), after pre-incubation of two weeks at 60% water-holding capacity (WHC). The mineralization of  $^{14}\text{C}$ -tagged synthetic root exudates ( $300 \mu\text{g C g}^{-1}$  soil,  $5 \text{ kBq mL}^{-1}$ ) was quantified over a period of one week in identically pre-incubated soils by liquid scintillation counting (Tri-Carb 3110 TR liquid scintillation counter and HiSafe 3 Scintillation cocktail; Perkin Elmer Inc., Waltham, MA, USA) after trapping of respired  $^{14}\text{C}$ - $\text{CO}_2$  in 1 M NaOH. The synthetic root exudate cocktail, used to approximate the soil microbial community response to inputs of labile C, consisted of 65% carbohydrates (glucose, fructose, sucrose; 2:1:1), 30% organic acids (citrate, oxalate; 6:4) and 5% amino acids (glutamate, alanine, glycine; 2:1:1) and was based on the chemistry of *Zea mays* root exudates (Krafczyk, 1984). Root exudate C not taken up by the microbial community was quantified as  $^{14}\text{C}$  remaining in 0.5 M  $\text{Na}_2\text{SO}_4$  extracts of the soil at the end of the incubation; on average 5.1 % of the added  $^{14}\text{C}$  was recovered in the extract, indicating near-complete microbial uptake.

25 Root exudate-C mineralization was modelled by fitting a single first-order exponential curve (Paul and Clark, 1996) to the cumulative  $^{14}\text{CO}_2$  data (curve fits shown in Fig. B1):

$$\text{Mass loss} = A + C_{\min} e^{(-rt)};$$

30 where  $A$  is the asymptote,  $C_{\min}$  is the size of the modelled pool that was mineralized and  $r$  is the decay rate of the root exudate-C ( $\text{hr}^{-1}$ ). Curve fitting was performed in SigmaPlot 12.3 (Systat Software Inc., San Jose, CA). We justified the use of a single-pool model because the size of the modelled pool of mineralized root exudate-C ( $57 \pm 2.3$  % of total root exudate-C added) did not vary significantly with management ( $P = 0.492$ ). The half-life ( $t_{1/2}$ ) of this modelled pool was calculated as  $\ln(2)/r$ .



## 2.3 Bomb-spike $^{14}\text{C}$ turnover modelling

Atmospheric nuclear weapons testing in the middle of the 20<sup>th</sup> century nearly doubled the  $^{14}\text{C}$  content of the atmosphere. When time series samples are available, this excess  $^{14}\text{C}$  can be utilized as a highly sensitive tracer of carbon fluxes in terrestrial ecosystems (Trumbore, 1993; Baisden et al., 2013). Here, we have applied a steady state soil carbon turnover model to estimate SOC turnover times ( $\tau$ ) in the five rotations. The model is nearly identical to the two-pool model presented by Baisden et al. (2013) with the exception that inputs ( $C_{in}$ ) are partitioned into three pools (active, slow and passive) each with a characteristic decay rates ( $k_{pool}$ ; where  $\tau_{pool} = 1/k_{pool}$ ). Carbon entering the soil each year will have the  $^{14}\text{C}/^{12}\text{C}$  ratio of the previous year's atmospheric  $\text{CO}_2$ . Inputs are then apportioned into the three pools in proportion to their fractional distribution ( $f_{pool}$ ) in the soil and losses are determined by first order kinetics (i.e.  $k_{pool} \times C_{pool}$ ) with shifts in  $^{14}\text{C}/^{12}\text{C}$  ratio of each pool also effected by radioactive decay ( $\lambda = 1.21 \times 10^{-4} \text{ yr}^{-1}$ ). Data for the Southern Hemisphere was taken from Currie et al. (2011). The overall SOC decay rate ( $k_{overall}$ ) was calculated as the pool weighted mean value and then the overall SOC turnover time ( $\tau_{overall}$ ) is the reciprocal of this value.

In this model there are five tuneable parameters: the decay constants ( $k_{pool}$ ) for the three pools and the fractional allocation of  $C_{in}$  into the pools (given  $f_{active} + f_{slow} + f_{resistant}$  must equal 1, only two of the three pools can be solved for). In order to avoid over fitting since we have only four observations per treatment, no more than three parameters could be solved for in any given scenario. We chose to take two complimentary approaches to fit the model. In the first scenario, we used the distribution of SOC into measurable fractions as determined by MIR-PLSR to fix the  $f$  parameters and solved for the  $k_{pool}$  values by minimizing the sum of squared errors between observed and predicted SOC  $\Delta^{14}\text{C}$  values. In the second scenario, the  $k_{pool}$  values were fixed at 0.33, 0.02 and 0.001  $\text{yr}^{-1}$  for the active, slow and resistant pools and the model was solved for  $f_{active}$  and  $f_{slow}$ . These  $k_{pool}$  values were chosen because they are the default values in the Rothamsted Soil Carbon (RothC) model (Jenkinson, 1990) with the resistant pool being equivalent to that found in the CENTURY model (Parton et al., 1987).

Models were evaluated using root mean square error (RMSE) and standard error (SE) between measured ( $y_i$ ) and predicted ( $\hat{y}$ ) values:

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y})^2}{n}};$$

$$SE = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y} - Bias)^2}{n-1}} \text{ where}$$

$$Bias = \frac{\sum_{i=1}^n (y_i - \hat{y})}{n}.$$



Lastly, because the SOC stocks are known, the annual heterotrophic respiration ( $R_h = \text{SOC}/\tau$ ) can be calculated and compared to estimates of  $C_{in}$  as an independent check on the reality of the model because at steady state inputs should equal losses. Carbon inputs ( $C_{in}$ ) to the upper 10 cm were estimated using measured aboveground dry matter production ( $DM$ ) and yield ( $Yield$ ) data as follows:

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$$C_{in} = C_{dm} * [(DM - Yield) * StubRet + DM * RtS * F_{root}];$$

where  $C_{dm}$  is the carbon content of plant residues estimated as  $450 \text{ mg C g}^{-1}$ ;  $StubRet$  is the stubble retention factor which was set to 0.40 for both crops and pasture phases based on agronomic records (Grace et al., 1995);  $RtS$  is the root to shoot ratio which was set to 0.40 for wheat and oats and to 0.55 for pasture as adopted in the Australian National GHG Accounting system (Skjemstad et al., 2004);  $F_{root}$  is the fraction of total root biomass found in the upper 10 cm which was estimated at 0.65 for crops and 0.63 for improved pastures based on root biomass distribution data of similar varieties in similar soil conditions (Siddique et al., 1990; Lodge and Murphy 2006).

## 15 2.4 Data analysis

One-way repeated measures analysis of variance (ANOVA) tests with year as subject and rotation as factor were used to determine if significant differences existed between the different rotations for carbon stocks, fractional distribution, amino sugar yields and short-term availability/activity measurements using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA). Pair-wise multiple comparisons were performed using the conservative Holms-Sidak method ( $\alpha = 0.05$ ). Aboveground dry matter production and soil carbon input data were normalized using square root or square root ( $Y + 1$ ) transformations when fallows were excluded or included, respectively, prior to testing for significant differences between mean annual data for 1963-1993 time period using a one-way ANOVA for the factor rotation.

## 3 Results

25 Mean annual dry matter production varied from 2.0 to 9.6 Mg D.M.  $\text{ha}^{-1}\text{yr}^{-1}$  and mean  $C_{in}$  varied from 0.48 to 3.24 Mg C  $\text{ha}^{-1}\text{yr}^{-1}$  from WF to Pa rotations (Table 1) with most of the within treatment variability between 1963 and 1993 being due to differences in annual rainfall (Fig. 1a). Grain yield trends are discussed in detail in Grace et al. (1995) and are not shown here. By 1963, most of the change in SOC stocks had occurred (at the commencement of the trial in 1925, Grace et al. (1995) estimated that the top 10 cm contained 2.75% C equating to 34.2 Mg C  $\text{ha}^{-1}$  using the BD pedotransfer function presented in





Fig. S1) with the exception of the WOF treatment where stocks showed a near-significant ( $P = 0.08$ ) negative linear trend with time between 1963 and 1993 (Fig. 1b). Averaged across this period, SOC stocks ranged from 14.4 to 32.6 Mg C ha<sup>-1</sup> in the same general order of increasing treatment production (Table 2). The C:N ratio of the bulk SOM was slightly but significantly lower in the treatments with pasture phases relative to the treatments without pastures (Table 2). Particulate OC stocks varied from 1.0 to 5.5 Mg C ha<sup>-1</sup> across the treatments resulting in a doubling of the proportional distribution of SOC into POC versus HOC + ROC fractions (Table 2).

Solid-state <sup>13</sup>C NMR spectra normalized to the amount of observable C in the measured sample also suggested more active cycling carbon in the treatments with pasture phases (Fig. 2). All treatments had a similar amount of Aryl (110-145 ppm) and O-Aryl C (145-165 ppm) but there were increasing amounts of Alkyl (0-45 ppm), N-Alkyl (45-60 ppm) and O-Alkyl C (60-95 ppm) with increasing productivity. The Alkyl to O-Alkyl C ratio, indicative of the degree of microbial processing of OM (Baldock et al., 1997), decreased with increasing productivity and SOC content suggesting less microbial processed OM in the high productivity treatments (Fig. 2 inset).

The yield of total amino sugars was significantly ( $P = 0.009$ ) impacted by the rotation, with the permanent pasture having significant more amino sugars per g of soil than most other rotations (Table 3). This trend was present in every amino sugar (glucosamine  $P = 0.008$ ; galactosamine  $P = 0.012$ , muramic acid  $P = 0.008$ ) except mannosamine ( $P = 0.70$ ). There were significant positive linear trends between individual and total amino sugar yields and SOC stocks for all sugars except mannosamine (data not shown).

The degree of uptake of the bomb-spike in atmospheric <sup>14</sup>CO<sub>2</sub> into the soil also followed an increasing trend with increasing productivity with the exception that the WOF treatment had more negative  $\Delta^{14}\text{C}$  values than the WF treatment in all years except 1963 (Fig. 3). Both modelling scenarios provided good representations of the  $\Delta^{14}\text{C}$  data (Fig. 3, Fig. C1) with scenario 1 ( $k_{\text{pool}}$  values solved for while allocations to pools were fixed using estimates from MIR-PLSR) performing slightly better (Table 4). In scenario 1, the turnover time ( $\tau$ ) for each pool decreased along the treatment productivity gradient (Fig. 4a). In scenario 2 with fixed  $k_{\text{pool}}$  values, the proportion of SOC allocated to the active and slow cycling pools generally increased while the allocation to the resistant pool decreased with increasing productivity (Fig. 4c), although these changes were overestimated relative to actual fraction distributions (Table 2). In both scenarios this resulted in a decrease in  $\tau_{\text{overall}}$  from 40-30 to 13-8 years (Fig. 4 c,d).

To complement the long-term integrative data presented above using  $\Delta^{14}\text{C}$ , assays pertinent to short-term C cycling were also conducted (Table 5). In general, trends in absolute (per g soil) terms followed the long-term integrative data, in that Pa had the





greatest amount of extractable C, the highest basal respiration rate, and the fastest turnover (shortest  $t_{1/2}$ ) of respired root exudate C, whereas the reverse was observed for WF. Other rotations were intermediate.

## 4 Discussion

5 Globally, there are ever increasing demands on soils to meet growing food demand on a finite land area. Simultaneously, there is a recognition that gains in agricultural production cannot continue to come simply by mining the soil resource. Given the additional desire to offset agriculture's GHG footprint, management that promotes the regeneration of SOM is now a high priority on many nations' research, development and extension agendas (Bustamante et al., 2014). However, there is debate as to whether soils can simultaneously mitigate climate change through carbon sequestration and provide the nutrition needed for increased crop production (Janzen, 2006). The Waite trial data demonstrate that a higher carbon return management strategy can provide both of these benefits as the results indicate significantly increased soil microbial activity and greater SOC stocks.

After nearly 70 years under consistent management representing a five-fold gradient in plant productivity (Table 1), a gradient in SOC stocks ranging from 14 to 33 Mg C ha<sup>-1</sup> was established. It is important to acknowledge here that the range of observed SOC stocks was a result of greater SOC losses in the low productivity treatments relative to the 1925 initial stocks, with most of this loss occurring between 1925 and 1963 (Grace et al., 1995). The levelling off in the loss rates suggests that these treatments were close to a steady state SOC value with respect to the long-term management. The strong linear relationship between inputs and SOC is well supported by findings in other agricultural trials (e.g., Rasmussen and Parton, 1994; Paustian et al., 1997; Kong et al., 2005) and forms one of the principle tenets of most conceptual (Jenny, 1941) and numerical models of SOC dynamics (e.g. Parton et al., 1987; Jenkinson 1990).

There were strong correlations between nearly all of the measured, estimated and modelled parameters (Table 6). With increasing annual C inputs, a greater proportion of SOC in what is regarded as a readily available form (i.e., POC) was found. A more nuanced picture emerges from interpretation of the NMR data; by normalizing the NMR spectra to observable C content, all treatments appear to have a similar amount of char-like carbon but increasing amounts of both plant (i.e. POC-like) and microbial-like (i.e. highly aliphatic compounds) OM with increasing productivity, agreeing with the C fractionation data. The amino sugar results lend further support for increasing amounts of microbial-derived OM in the soils under the higher input rotations. Modelling scenario #2 supported this notion as well with an increase in proportion of SOC in the active and slow cycling pools at the expense of the resistant pool (Fig. 4c). These findings, again, are consistent with expectations from previous research across a range of ecosystems (e.g., Cambardella and Elliot, 1992; Kong et al., 2005; Lajtha et al., 2014) and would be more or less simulated by most multiple pool numerical models. For example, Kong et al. (2005) found a 5 Mg C



ha<sup>-1</sup> gradient in POC, measured as the 250-2000 µm size fraction, after 10 years under a range of management practices resulting in a tenfold gradient in C inputs.

The striking and unexpected feature of this analysis was the 3-fold increase in carbon cycling rates ( $k_{overall}$ ) with increasing productivity revealed by modelling the incorporation of the bomb-spike in atmospheric <sup>14</sup>CO<sub>2</sub>. We used the term ‘unexpected’ because this rate of increase in carbon cycling was much greater than would be predicted from simple steady-state or pool distribution considerations (Fig. 5). The most basic way to estimate  $k_{overall}$  would be to assume a single homogenous pool at steady state so  $k_{overall} = C_{in}/SOC$ . This calculation suggests  $k_{overall}$  increases 75% from the low to high productivity systems (Fig. 5). Another method is to assume that each fraction has a characteristic  $k$  (as in CENTURY or Roth C), and with the estimated distribution of C into fractions from the MIR-PLSR analysis (Table 2),  $k_{overall}$  similarly increases by 75% with increasing productivity. However, the  $k_{overall}$  determined from the more robust <sup>14</sup>C modeling increased 300% from low to high productivity (Fig 5). Importantly, the increase in  $k_{overall}$  with increasing productivity could not be explained by the RothC model (Sanderman et al., 2016). Sanderman et al. (2016) found that the decay constants in RothC needed to be adjusted in a similar manner as seen in Fig. 4a in order to explain the SOC and <sup>14</sup>C data across the five treatments.

While the Waite Trial represented a large productivity gradient, the quality of the incoming organic matter was not equal. The treatments with pasture phases contained N-fixing legumes (on average 26% of the pasture production was attributable to legumes based on pasture composition data included in the public data repository) which likely resulted in a more N-rich input material. The bulk soil C:N ratio was significantly lower in the two pasture treatments compared to the cropping rotations (Table 2). By providing more N to a likely N-limited system (no nitrogenous fertilizer was used in the trial), input quality may have acted in concert with increased inputs to further increase the SOC cycling rates seen in the Pa and 2W4Pa treatments (Fig. 4b). However, higher quality (i.e. lower C:N) litter will also likely be used more efficiently by the microbial community thus potentially stabilizing more of this litter as SOC (Lange et al., 2015).

Although long- and short-term C turnover rates were well correlated with  $C_{in}$  and SOC stocks, the mechanisms behind the observed increases in both C stocks and overall decomposition rates ( $k_{overall}$ ) are not clear. Increased C inputs frequently increase the size and the activity of the soil microbial community and can result in the accelerated decomposition or ‘priming’ of more stable, nutrient rich SOM (Fontaine et al., 2004; Dijkstra and Cheng 2007; Blagodatskya and Kuzyakov, 2008). Given that microbial biomass is typically positively associated with SOC levels (Wardle et al., 1999), with higher  $C_{in}$  and SOC stocks, overall decomposition rates ( $k_{overall}$ ) would increase as long as decomposition rate per unit microbial biomass stayed constant (Manzoni and Porporato, 2009). However, in typical linear donor-control models (e.g. RothC), microbial biomass size is uncoupled from SOC decomposition, which renders any changes in microbial biomass size irrelevant for SOC turnover, and therefore (in the instance of increasing microbial biomass), potentially underestimates  $k_{overall}$  (Fang et al., 2005). An emerging class of nonlinear soil carbon models (e.g., Allison et al., 2010) allows coupling between microbial biomass size and SOC



turnover, effectively representing the relationship between biomass size and  $k_{overall}$ , although the form of this relationship remains unclear. Our results indicate that this approach is required at the Waite trial soils to accurately model SOC dynamics, and should produce better projections of SOC in response to change in management. Similarly, Lange et al. (2015) postulated that with increasing plant diversity, SOC stocks increased because a larger microbial community was driving a more efficient transfer of SOC from a fast to slow cycling C pool which effectively decreased the overall turnover rate. Importantly, the acceleration in carbon cycling ( $k_{overall}$ ) seen in the Waite trial data was not greater than the size of the imbalance between inputs over losses; thus, we observed a gradient of higher SOC stocks with increasing productivity.

The modelling work presented here and in all of the references discussed above assumes a static land surface yet erosion and its associated C loss is known to be quantitatively important in agricultural settings (Van Oost et al., 2007; Chappell et al., 2015). Chappell et al. (2015) estimated that at the Waite Trial erosional loss of SOC could account for approximately 10% of the annual C flux from the soil on decadal time scales with lower estimates for the treatments with pasture phases and higher estimates for those with fallows. While we have not attempted to account for soil redistribution in this study, our findings and conclusions would not change if we had because the short-term microbial assays are independent of any erosion and the amount of erosion that has occurred (estimated as 3.0 – 7.7 mm per decade from Chappell et al., 2015) would be included as a minor component of the apparent C turnover rate for the upper 100 mm of soil using time-series  $^{14}\text{C}$  measurements. This minor component would be within error margins.

In contrast to paradigms underlying most ecosystem soil carbon models, the acceleration in microbial cycling may itself be leading to increased SOM stabilization rates, as microbial activity has been shown to contribute to SOM formation through the production of microbial biomass residues (i.e. necromass) (Miltner et al., 2012; Cotrufo et al., 2013). These residues can constitute a large proportion of the more stable slow cycling OM pool, and persist much longer than plant necromass (Kiem and Kögel-Knaber, 2003; Liang and Balser, 2008). Yet, where enhanced rates of stabilization occur, we find they are matched by enhanced turnover of the SOM pool. The fate, formation and stabilization of microbial biomass has therefore been highlighted as a major unknown in advancing the scientific understanding of soil carbon stabilization and turnover (e.g. Allison et al., 2010; Weider et al., 2013). Our results, showing increased plant productivity along with higher C stocks, faster C turnover and a greater abundance of amino sugars, suggest, in line with the conclusions of Lange et al. (2015), that the production and stabilization of microbial residues is important for building and maintaining adequate SOM levels.

## 5 Conclusions

The data from the Waite trial portray a nuanced and dynamic picture whereby increasing carbon flow to the soil leads to greater SOC stocks but because of feedbacks to the microbial community there can be an acceleration of SOM mineralization beyond



what would be expected based on simple steady-state and carbon pool distribution shift arguments. These feedbacks have important implications for how soil carbon dynamics are represented in conceptual and numerical models. From an agronomic standpoint, a simple but important picture emerges whereby a higher carbon return management system, in the case of the Waite Trial that means greater number of pasture phases and no fallows, results in a soil with more soil carbon that is also  
5 supplying more nutrients back to the crop.

### **Data availability**

The Full agronomic, production and climatic data and measured/predicted soil properties from selected trials can be downloaded free of charge from the CSIRO Data Access Portal (DOI: 10.4225/08/55E5165EC0D29).

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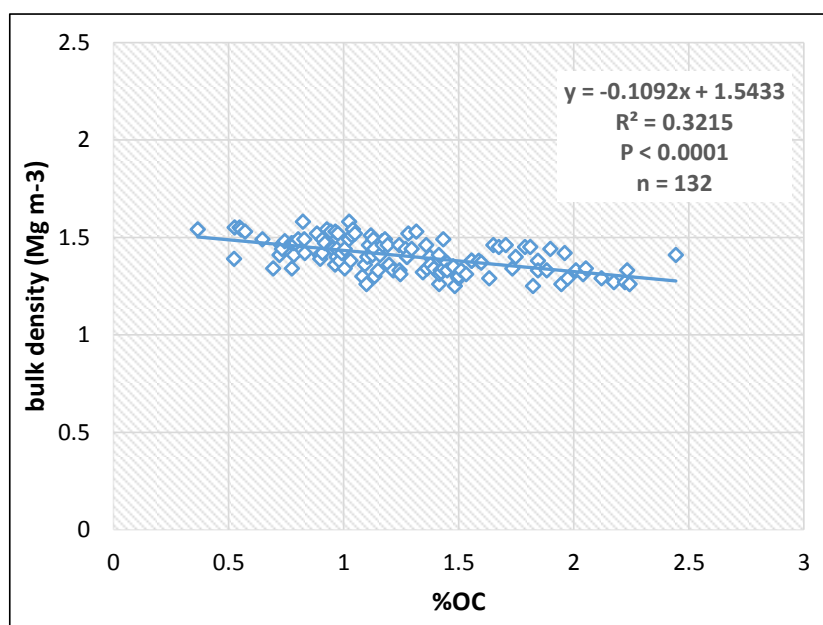


## Appendices

### Appendix A. Bulk density pedotransfer function

- 5 Bulk density was not measured during the trial years of 1963-1993 but it was measured in detail in 1997 at all of the Waite Trial plots (Baldock, 1998 – data available upon request from either J. Sanderman or J. Baldock) not just the 5 selected for this current study. Plots were split and two full profiles were collected in 10 cm increments. Here we use the data collected from the 0-10 and 10-20 cm horizons to develop a relationship between bulk density and percent organic carbon which is then applied to the soil samples collected in prior years.

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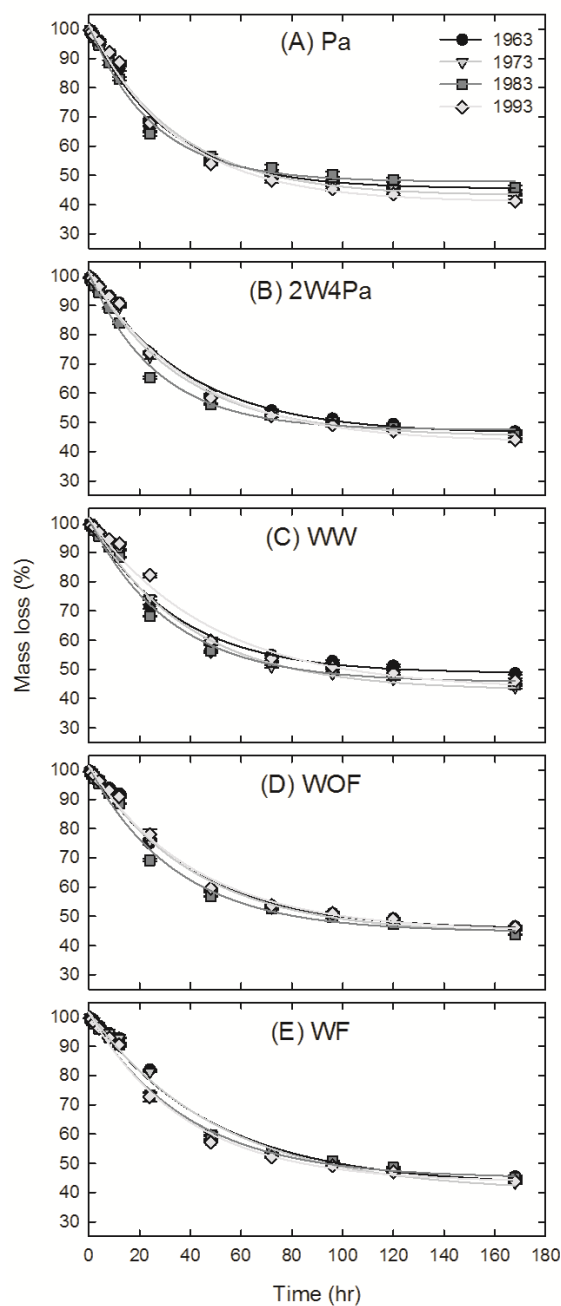


**Figure A1.** Relationship between %OC and bulk density for the 0-10 and 10-20 cm soil layers collected in 1997 from all trial plots.

15



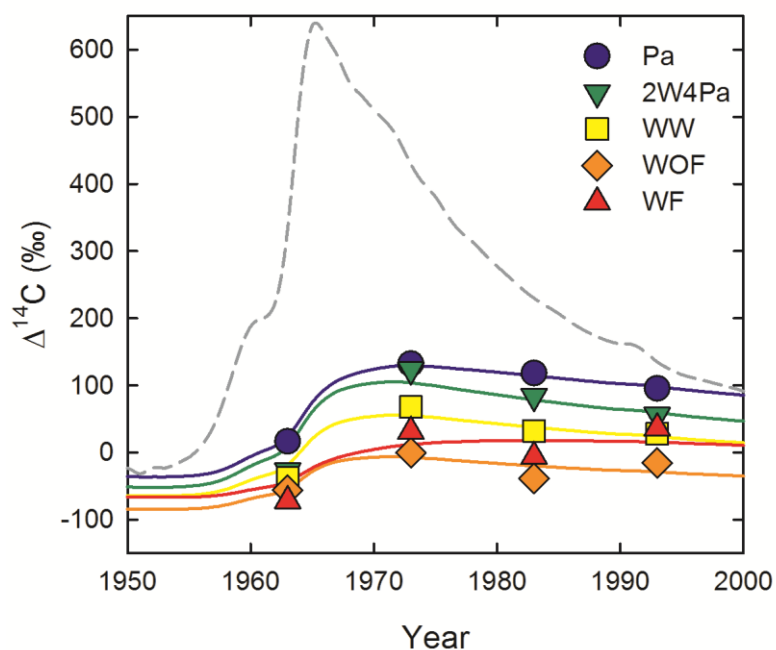
## Appendix B. Root exudate decay curves



**Figure B1.** Root exudate incubation results presented as percentage mass loss. Each point represents mean  $\pm$  1 s.e.m. ( $n = 3$ ). Error bars are smaller than symbols. Best fit single-pool exponential models are also shown (adjusted  $R^2 > 0.98$  for all curves).



## Appendix C. Alternate modelling scenario results



**Figure C1.**  $\Delta^{14}\text{C}$  values of SOC in upper 10 cm along with best fit model solutions for scenario 2 (constant  $k_{pool}$  values solving for best distribution into C pools). Scenario 1 is shown in Fig. 3. Southern Hemisphere atmospheric  $^{14}\text{CO}_2$  record is shown for reference (dashed line).





## Author contribution

SF performed the radiocarbon analyses. JS and WT performed the modeling and analysis. JS performed the NMR analyses. CC and MF conceived the tracer  $^{14}\text{C}$  experiment and CC performed amino sugar analyses. MF performed and interpreted the  
 5 tracer  $^{14}\text{C}$  experiment. JS conceived the study and wrote the first draft of the manuscript, with all authors contributing ideas and revision.

## Competing interests

The authors declare that they have no conflict of interest.

## 10 Acknowledgements

We would like to thank our predecessors at the Waite Research Institute for having the foresight to archive soil samples from this long term trial, T Carter for laboratory assistance, J McGowan for assistance in running NMR analyses. Funds for this research were provided by the CSIRO Sustainable Agriculture National Research Flagship and New Zealand public research funding through GNS Science (540GCT82). MF was supported by a CSIRO Julius Career Award.

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**Table 1.** Mean treatment production and soil C input data during 1963-1993. Standard deviation given in parentheses. Significant differences (one-way ANOVA,  $\alpha < 0.05$ ) between rotations are indicated with different letters in each column.

Rotation	Dry Matter (Mg D.M. ha <sup>-1</sup> )		C inputs (Mg C ha <sup>-1</sup> )	
	Annual mean*†	Cumulative	Annual mean*†	Cumulative
Pa	9.63 (3.11) <sup>a</sup>	298.63	3.24 (1.05) <sup>a</sup>	100.32
2W4Pa	6.49 (3.28) <sup>b</sup>	201.10	2.07 (1.17) <sup>b</sup>	64.23
WW	2.66 (1.28) <sup>cd</sup>	82.52	0.63 (0.30) <sup>c</sup>	19.66
WOF	3.10 (2.81) <sup>c</sup>	96.01	0.70 (0.67) <sup>c</sup>	21.84
WF	2.02 (2.31) <sup>d</sup>	62.76	0.48 (0.59) <sup>c</sup>	15.02

\*Annual mean includes a value of 0 for fallow years

†data transformed using square root (value + 1) for ANOVA



**Table 2.** Soil carbon data summary. Mean across time and standard deviation (in parentheses) given for each rotation. Significant differences (one-way repeated measured ANOVA,  $\alpha < 0.05$ ) between rotations are indicated with different letters in each column.

Rotation*	<u>Measured</u>		<u>MIR-PLSR predicted data</u>						<u><math>\Delta^{14}\text{C}</math> value</u>
	SOC	C:N	POC†	HOC†	ROC†	f(POC) ‡	f(HOC) ‡	f(ROC) ‡	SOC
	(Mg C ha <sup>-1</sup> )		----- (Mg C ha <sup>-1</sup> ) -----						(‰)
Pa	32.6 (1.6) <sup>a</sup>	10.9 (0.4) <sup>a</sup>	5.2 (0.7) <sup>a</sup>	17.8 (0.7) <sup>a</sup>	9.4 (0.7) <sup>a</sup>	0.16 (.01) <sup>a</sup>	0.55 (.01) <sup>a</sup>	0.29 (.01) <sup>a</sup>	90.8 (51.7) <sup>a</sup>
2W4Pa	25.1 (1.0) <sup>b</sup>	10.9 (0.3) <sup>a</sup>	2.8 (0.3) <sup>b</sup>	14.4 (0.5) <sup>b</sup>	8.0 (0.5) <sup>b</sup>	0.11 (.01) <sup>b</sup>	0.57 (.01) <sup>b</sup>	0.32 (.01) <sup>b</sup>	59.1 (63.8) <sup>ab</sup>
WW	20.6 (1.4) <sup>c</sup>	11.9 (0.3) <sup>b</sup>	1.9 (0.2) <sup>b</sup>	11.9 (0.8) <sup>b</sup>	6.6 (0.6) <sup>bc</sup>	0.09 (.01) <sup>b</sup>	0.58 (.00) <sup>bc</sup>	0.32 (.01) <sup>bc</sup>	22.7 (44.3) <sup>bc</sup>
WOF	18.8 (2.5) <sup>d</sup>	11.7 (0.3) <sup>ab</sup>	1.3 (0.3) <sup>c</sup>	11.0 (1.5) <sup>c</sup>	6.5 (0.8) <sup>c</sup>	0.07 (.01) <sup>c</sup>	0.58 (.01) <sup>bc</sup>	0.35 (.01) <sup>c</sup>	-27.4 (24.8) <sup>c</sup>
WF	14.4 (1.5) <sup>e</sup>	12.1 (0.7) <sup>b</sup>	1.1 (0.3) <sup>c</sup>	8.6 (0.7) <sup>d</sup>	4.8 (0.5) <sup>d</sup>	0.07 (.01) <sup>c</sup>	0.60 (.01) <sup>c</sup>	0.33 (.01) <sup>bc</sup>	-2.2 (50.0) <sup>c</sup>

\*Pa = permanent pasture, 2W4Pa = 2 years wheat followed by 4 pasture years, WW = continuous wheat, WOF = wheat-oat-fallow rotation, WF = wheat-fallow rotation

†POC = particulate organic carbon, HOC = humic organic carbon, ROC = resistant organic carbon

‡fraction as a proportion of bulk soil carbon





**Table 3.** Amino sugar data (mean  $\pm$  1 s.d. reported). One-way repeated measured ANOVA summary given below each data column and pairwise comparisons between rotations are indicated with different letters in each column when significant ( $\alpha < 0.05$ ).

Rotation	Glucosamine		Mannosamine		Galactosamine		Muramic acid		Total yield	
	(mg amino sugar g soil <sup>-1</sup> )									
Pa	0.84 ± 0.18	a	0.022 ± 0.017		0.32 ± 0.08	a	0.059 ± 0.016	a	1.24 ± 0.30	a
2W4Pa	0.47 ± 0.34	ab	0.016 ± 0.013		0.14 ± 0.12	ab	0.026 ± 0.032	b	0.66 ± 0.50	ab
W	0.32 ± 0.22	b	0.021 ± 0.025		0.11 ± 0.06	b	0.026 ± 0.013	b	0.48 ± 0.31	b
WOF	0.27 ± 0.10	b	0.008± 0.002		0.10± 0.04	b	0.015 ± 0.006	b	0.40± 0.17	b
WF	0.42 ± 0.14	ab	0.012 ± 0.001		0.15 ± 0.04	ab	0.029 ± 0.018	ab	0.63 ± 0.22	ab
F statistic	5.667		0.554		5.183		5.687		5.566	
P value	0.008		0.700		0.012		0.008		0.009	



**Table 4.** Turnover model goodness of fit statistics.

Rotation	Scenario 1: Fit $k$ with $f$ fixed			Scenario 2: Fit $f$ with $k$ fixed		
	RMSE*	SE†	R <sub>h</sub> ‡	RMSE*	SE†	R <sub>h</sub> ‡
Pa	0.25	0.29	3.35	4.74	5.39	2.45
2W4Pa	7.63	8.81	3.18	21.08	23.91	1.78
WW	15.08	17.42	0.95	12.26	13.95	1.20
WOF	12.61	14.56	0.47	11.93	13.78	0.66
WF	19.35	22.34	0.43	23.48	26.88	0.29

\*RMSE = root mean square error

†SE = standard error

‡R<sub>h</sub> = steady state heterotrophic respiration (tC ha<sup>-1</sup> yr<sup>-1</sup>)



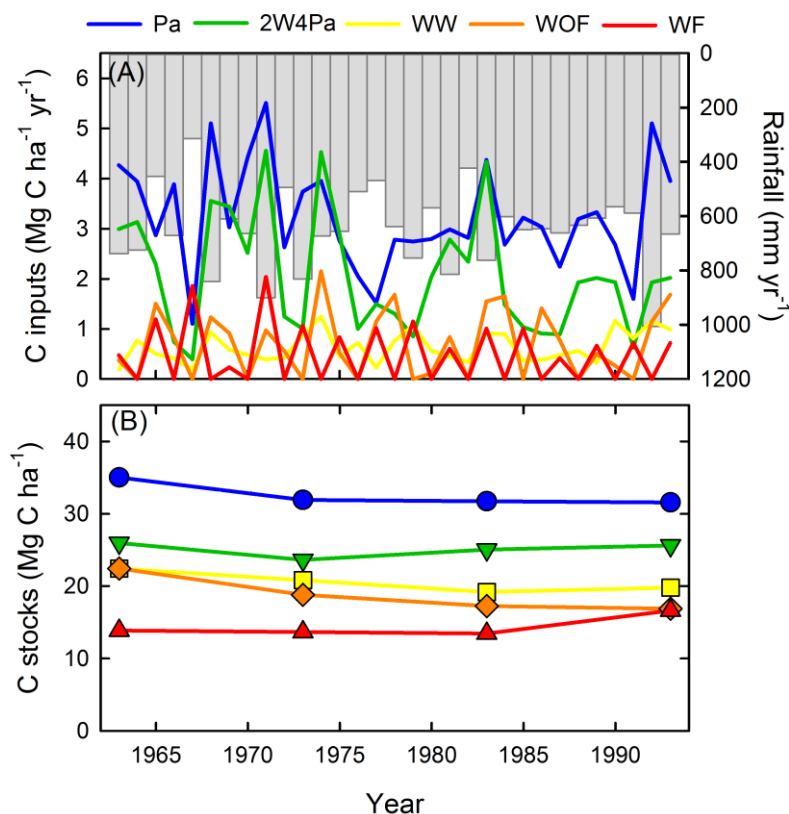
**Table 5.** Short term microbiological activity assays (mean of sampling times  $\pm$  1 s.d. reported). One-way repeated measured ANOVA summary given below each data column and pairwise comparisons between rotations are indicated with different letters in each column when significant ( $\alpha < 0.05$ ).

					Root exudate curve fit parameters				
	Extractable OC		Basal respiration		Size of				
Rotation	(μg C g soil <sup>-1</sup> )		(μmol CO <sub>2</sub> d <sup>-1</sup> )		mineralized pool				
				(%)		Decay rate of pool (h <sup>-1</sup> )		Half-life of pool (h)	
Pa	207 ± 26	a	7.1 ± 0.4	a	57.2 ± 3.2	0.033 ± .005	a	21.5 ± 3.0	a
W4PA	132 ± 22	b	5.6 ± 0.6	a	55.8 ± 2.0	0.029 ± .006	ab	24.4 ± 4.0	ab
WW	67 ± 7.1	c	3.5 ± 0.2	b	56.6 ± 2.8	0.027 ± .005	ab	26.5 ± 4.8	ab
WOF	64 ± 12	c	3.4 ± 0.4	b	56.6 ± 0.6	0.026 ± .002	b	27.3 ± 2.4	ab
WF	55 ± 22	c	3.1 ± 0.6	b	58.8 ± 2.2	0.023 ± .003	b	30.4 ± 4.4	b
F statistic	30.84		17.97		1.64	7.01		5.61	
P value	<0.001		<0.001		0.227	0.004		0.009	

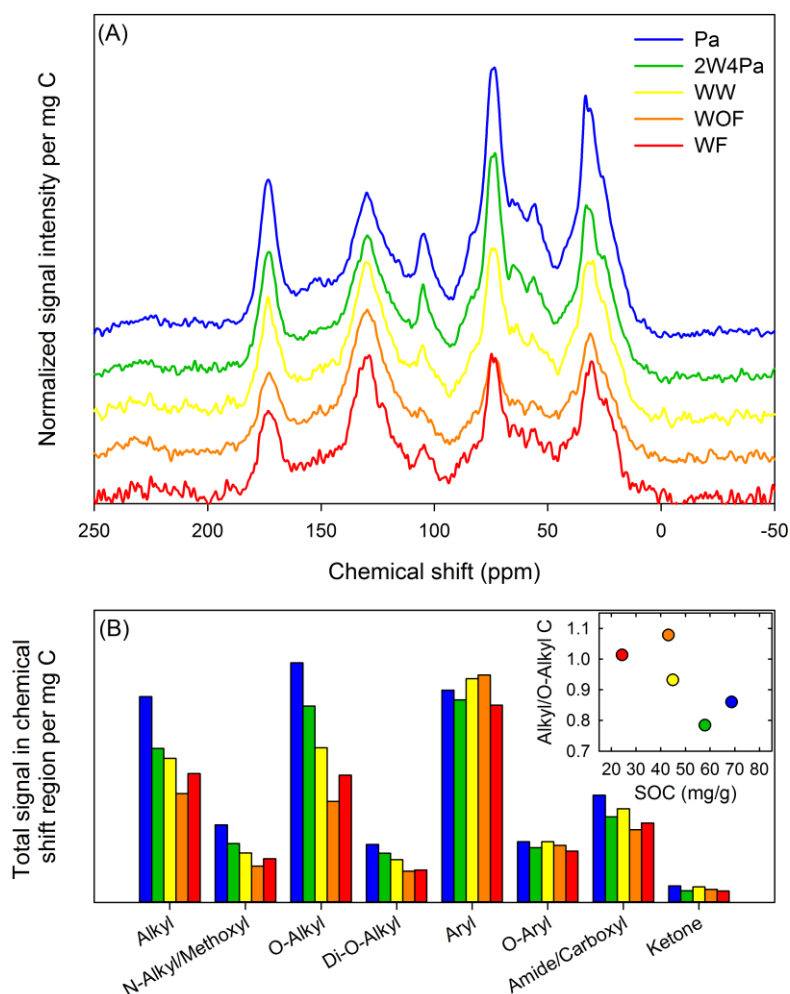


**Table 6.** Pearson's correlation coefficients between mean annual C input ( $C_{in}$ ), SOC stock, C:N ratio, fraction of SOC as POC ( $f_{POC}$ ), radiocarbon activity ( $\Delta^{14}C$ ), overall turnover time (given as the average of the two modelling scenarios,  $\tau_{mean}$ ), half-life of added root exudates cocktail ( $t_{1/2}$ ), the Alkyl/O-Alkyl C ratio from NMR data (A/O-A), and the total yield of amino sugars ( $\Sigma AS$ ).

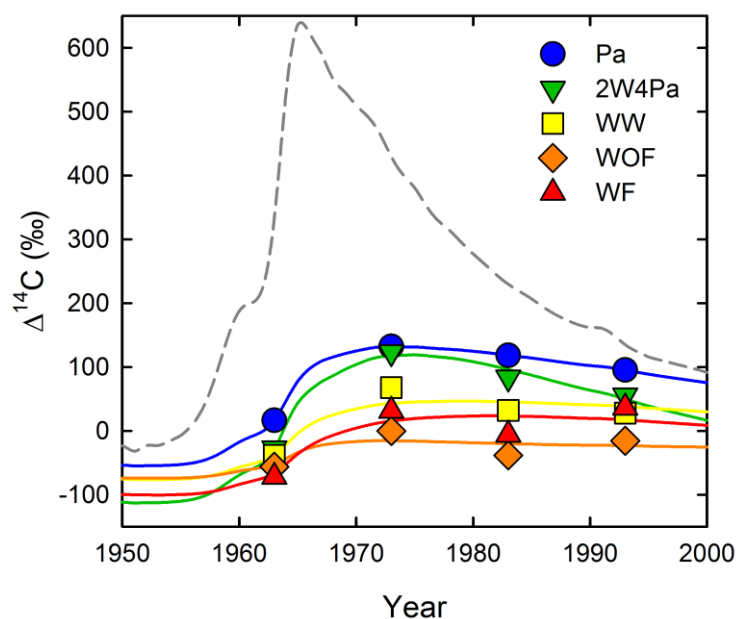
	SOC	C/N	$f_{POC}$	A/O-A	$\Sigma AS$	$\Delta^{14}C$	$\tau_{mean}$	$t_{1/2}$
$C_{in}$	0.93**	-0.94**	0.95**	-0.72	0.87*	0.90**	-0.83*	-0.94**
SOC		-0.83*	0.96**	-0.67	0.80	0.87*	-0.86*	-0.99**
C/N			-0.81*	0.76	-0.67	-0.80*	0.86*	0.90**
$f_{POC}$				-0.72	0.91**	0.95**	-0.83*	-0.93**
A/O-A					-0.54	-0.89**	0.93**	0.71
$\Sigma AS$						0.85*	-0.57	-0.74
$\Delta^{14}C$							-0.89**	-0.86*
$\tau_{mean}$								0.90**
Significance (n = 5): * P < 0.10, ** P < 0.05								



**Figure 1.** Annual precipitation and carbon inputs for the five cropping systems (A), and measured SOC stocks to 10 cm at the four sampling dates in these cropping systems (B). Cropping systems are as follows: Pa = permanent pasture, 2W4Pa = 2 years wheat followed by 4 pasture years, WW = continuous wheat, WOF = wheat-oat-fallow rotation, WF = wheat-fallow rotation.

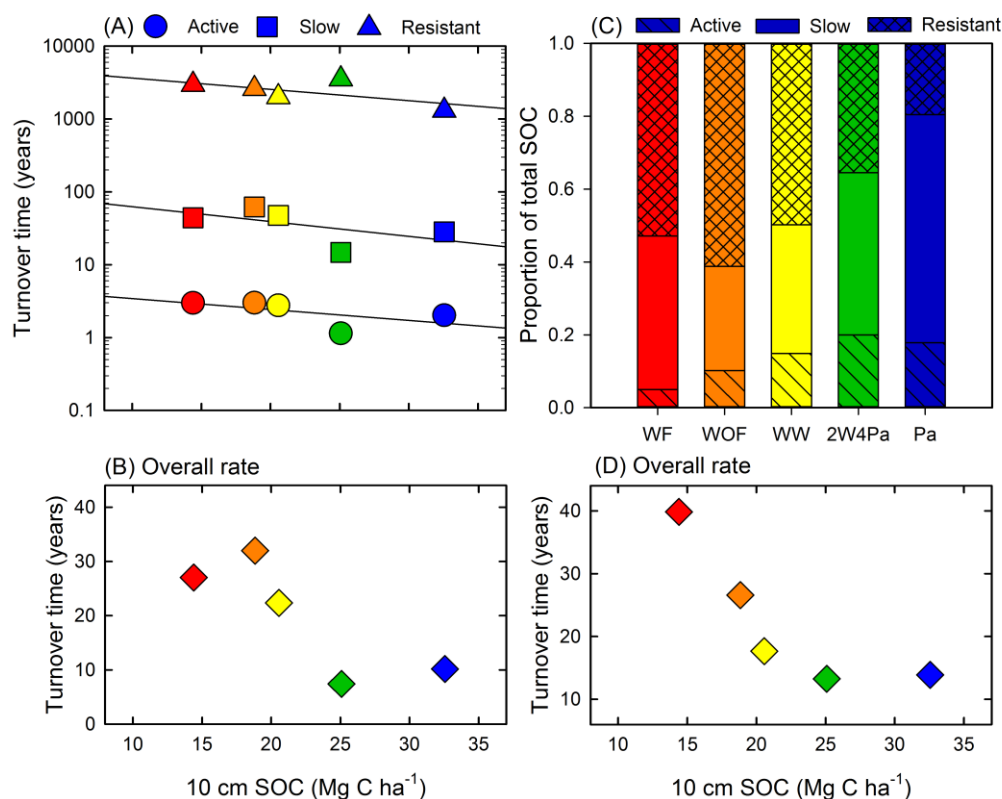


**Figure 2.** Solid-state  $^{13}\text{C}$  NMR spectra (A) and total signal intensity integrated into eight major chemical shift regions (B) for the 1983 soil samples from the five treatments. Signal intensity has been normalized for number of scans,  $nc\_proc$ , C observability and C content in NMR rotor (Y-axis units are irrelevant). Inset in (B) shows the Alkyl C to O-Alkyl C ratio plotted as a function of SOC content ( $R^2 = 0.51$ ,  $P = 0.17$ ).

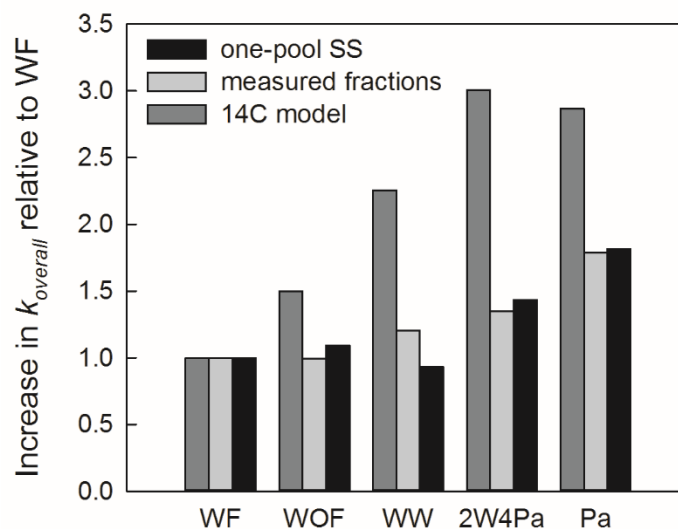


**Figure 3.**  $\Delta^{14}\text{C}$  values of SOC in upper 10 cm along with yearly output from the best fit bomb-spike model solutions for scenario 1 (curve fits for scenario 2 shown in Fig. C1) where the allocation of C to pools was fixed using the estimated fractions from MIR-PLSR analysis and the model was solved for  $k$  values. Southern Hemisphere atmospheric  $^{14}\text{CO}_2$  record is shown for reference (dashed line).





**Figure 4.** Turnover time for SOC pools (A) and inventory-weighted SOC turnover time (B) for best fit model solutions when allocation of C to fractions from MIR-PLSR predictions was used (scenario 1). In (C), best fit model solutions for allocation of SOC to pools when turnover times were fixed at 3, 50 and 1000 yrs for active, slow and resistant pools, respectively (scenario 2), with the inventory-weighted overall turnover time given in (D). Colours representing the five treatment sites as in Fig. 1.



**Figure 5.** Comparison of different approaches for estimating changes in carbon cycling rates. Increases in  $k_{overall}$  relative to the WF treatment is given for a simple one-pool steady-state (SS) calculation ( $k = C_{in}/SOC$ ), the predicted  $k$  based on measured fractions assuming  $k_{pool} = 0.33, 0.02$  and  $0.001 \text{ yr}^{-1}$  in the POC, HOC and ROC fraction, respectively, and the mean  $k_{overall}$  value from modelling the incorporation of  $^{14}C$  into the soil (scenario 1).