Geoderma 285 (2017) 151–163

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Soil with high organic carbon concentration continues to sequester carbon with increasing carbon inputs

S.E. Orgill [a,b,*], J.R. Condon [b], C.A. Kirkby [c], B.A. Orchard [a], M.K. Conyers [a,b], R.S.B Greene [d], B.W. Murphy [e]

[a] NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, NSW 2650, Australia
[b] Graham Centre for Agricultural Innovation, School of Agricultural & Wine Sciences, Charles Sturt University, Wagga Wagga, NSW 2650, Australia
[c] CSIRO, Agriculture, GPO Box 1600, Canberra 2601, Australia
[d] Australian National University, Acton, ACT 2601, Australia
[e] NSW Office of Environment and Heritage, Cowra, NSW 2794, Australia

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 27 September 2016
Accepted 27 September 2016
Available online 7 October 2016

Keywords:
Carbon saturation
Carbon sequestration
Soil incubation
Humus
Stable carbon
Clay mineralogy

ABSTRACT

Identifying soil with a large potential to accumulate organic carbon (OC) could maximise the mitigation benefits of carbon (C) sequestration and help prioritise resources to achieve increases in soil OC. The purpose of this laboratory incubation experiment was to determine if an upper limit to OC accumulation in soil was approached with increasing C input in basalt- and granite-derived soil. For each parent material, two soil layers were compared to observe OC accumulation in soil with a high OC concentration (0 to 0.10 m, A1 horizon) and soil with a low OC concentration (0.40 to 0.50 m, B2 horizon). Soil samples were incubated for up to 146 days. The experiment consisted of three soil incubation cycles, with four treatments applied at the start of each cycle: soil only (control), soil and nutrients only (nutrients), high organic matter (OM) and nutrients (approximating a field equivalent of 12.4 Mg DM/ha; HOMN) and very high OM and nutrients (31.1 Mg DM/ha; VHOMN). At the beginning of cycle one ^{13}C labelled OM was applied. There was no asymptotic behaviour between C inputs and OC accumulation in soil observed in this study. Thus, OC accumulation was not approaching an upper limit for either parent material at OM application rates ranging from field equivalents of 12.4 to 93.3 Mg DM/ha (equivalent to 5.4 to 40.6 Mg C/ha). There was no significant increase in OC concentration between cycle 2 and 3 for the VHOMN treatment in the granite-derived 0.40 to 0.50 m soil. While this is not conclusive, this may indicate the soil is approaching an upper limit to OC accumulation at a lower OC concentration due to the dominance of 1:1 clays, compared to the 2:1 clay dominated basalt-derived soil. This suggests that mineralogy rather than texture may influence OC accumulation and any potential C saturation behaviour of soil. Despite increasing microbial activity, evidenced by increasing soil respiration ($P < 0.001$) and microbial biomass C ($P < 0.05$), as well as a significant ($P < 0.05$) narrowing of the C:N ratio of soil, there was substantial ^{13}C recovery (mean between 19.8 and 25.9 (1.1 se) % for both parent material) at the end of the soil incubation. This supports the hypothesis that the increases in OC accumulation were at least partly due to the conversion of plant residues into microbial detritus which is a major component of the relatively stable pool of OC in soil.

1. Introduction

The accumulation of organic carbon (OC) in soil is important for mitigating climate change, as well as sustaining environmental services and agricultural productivity (Lal, 2004). Identifying soil with the greatest carbon (C) sequestration potential can maximise these benefits, and help prioritise resources to achieve increases in soil OC. The ability of soil to accumulate OC is a balance between organic matter (OM) supply, primarily from *in situ* net primary productivity, and OM loss, principally through decomposition and erosion (Baldock et al., 2004). However, the relationship between OM inputs and increases in soil OC are not always linear, and an upper limit to OC accumulation has been proposed (Six et al., 2002; Stewart et al., 2007; Stewart et al., 2008b).

Land management practices that maximise plant productivity and minimise physical soil disturbance are likely to increase OM supply, and where this increase in OM is greater than the rate of OM decomposition, soil OC is therefore likely to increase (Lal, 2004; Luo et al., 2010; Paustian et al., 1997a; West and Post, 2002). While there have been several field trials where increased OM supply continued to increase soil OC (Huggins et al., 1998a; Kong et al., 2005; Paustian et al., 1997b), there have been numerous long-term trials where there was no increase in

* Corresponding author at: NSW Department of Primary Industries, Wagga Wagga Agricultural Institute, NSW 2650, Australia.
E-mail addresses: Susan.Orgill@dpi.nsw.gov.au (S.E. Orgill), JCondon@csu.edu.au (J.R. Condon), Clive.Kirkby@csiro.au (C.A. Kirkby), Beverley.Orchard@dpi.nsw.gov.au (B.A. Orchard), Mark.Conyers@dpi.nsw.gov.au (M.K. Conyers), Richard.Greene@anu.edu.au (R.S.B. Greene), brian.amaroo@bigpond.com (B.W. Murphy).

http://dx.doi.org/10.1016/j.geoderma.2016.09.033
0016-7061/

soil OC with continued OM input (Campbell et al., 1991; Gulde et al., 2008; Huggins et al., 1998a; Huggins et al., 1998b; Reicosky et al., 2002). To explain disparities such as these, the concept of soil C saturation was introduced (Six et al., 2002). It has been proposed that soil approaches an upper limit of OC accumulation, C saturation, based on inherent soil properties, including: soil texture, structure, mineralogy and chemistry (Six et al., 2002; Stewart et al., 2007; Stewart et al., 2008b). This concept challenges soil OC models that are based on linearity between soil C inputs and soil OC concentration (West and Six, 2007) and has practical implications for identifying and mapping soil with C sequestration potential (Angers et al., 2011; Chan et al., 2008).

Carbon saturation is defined as the point where with increased OM supply the whole soil, or a defined soil fraction, reaches a new higher steady state of OC (Six et al., 2002). This is based on soil properties and processes that are important for OC stabilisation. A large proportion of soil OC is associated with the fine fraction of soil; that is, silt- and clay-sized particles (Baldock and Skjemstad, 2000; Kahle et al., 2002a; Kahle et al., 2002b). Clay content, mineral surface area and reactivity can affect the amount of OM that is protected through adsorption within clay mineral assemblages (Greene et al., 1973), thereby restricting microbial access (Baldock and Skjemstad, 2000; Krull et al., 2003). As clay minerals have a finite surface area, the fine fraction is suggested to be more likely to reach C saturation than the whole soil where OC can readily accumulate in the form of particulate OM (Gulde et al., 2008; Hassink and Whitmore, 1997; Stewart et al., 2008b). Kleber et al. (2007) proposed that OC compounds sorb onto mineral surfaces in a self-organising and zonal sequence, often with varying thickness of OC and discontinuous coverage on mineral surfaces. While the protection of OM is greatest in the contact zone where organo-mineral associations form, this model enables clays to stabilise OC beyond their finite surface area. Biochemical alteration of OM during decomposition is another important stabilisation mechanism, where decomposed OM and the associated microbial products may be less vulnerable to further microbial attack and more likely to bond with clay minerals (Cadisch and Giller, 1997; Six et al., 2002). Lastly, the physical occlusion of OM in soil aggregates protects OM by limiting microbial access to OM, reducing oxygen diffusion and enhancing organo-mineral associations (Golchin et al., 1994a; Golchin et al., 1994b; Golchin et al., 1995; Grandy and Robertson, 2007; Oades, 1988; Tisdall and Oades, 1982).

Thus the capacity to increase soil OC concentration is largely determined by the clay content and clay mineralogy, as well as soil OC concentration and the quantity, continuity and chemical composition of OM input to soil. The C input required to achieve soil C saturation is commonly estimated by comparing the current soil OC concentration with the storage capacity of the fine fraction (silt- and clay-sized particles) or the whole soil, thereby calculating the C saturation deficit (Angers et al., 2011; Hassink and Whitmore, 1997), or by using asymptotic regressions between increases in soil OC concentration and C input (Stewart et al., 2008b). Based on these estimates, literature indicates that some soil OC fractions will exhibit C saturation behaviour, while others may not (Chung et al., 2008; Chung et al., 2010; Gulde et al., 2008; Kong et al., 2005; Stewart et al., 2008b). However, a recent study suggested these calculations may underestimate C storage in the fine fraction of soil due to i) limitations of the regression models and inadequate representation of soils at true C saturation, and ii) not accounting for differences in the specific surface area of clay minerals (Feng et al., 2013a).

Another reason why soil may not exhibit C saturation behaviour is due to biochemical stabilisation of OC in soil. Stewart et al. (2008a) suggested that biochemically stabilised OC may be independent of texture and mineralogy. Recent literature emphasises that rather than the traditional macromolecular model of humus (Stevenson, 1994; Tate, 1987), OM in soil is instead a 'continuum of progressively decomposing organic compounds' (Lehmann and Kleber, 2015). That is, OM in soil exists with varying degrees of stability and not in discrete pools. We use the term humus to refer to an experimental, rather than operational pool of soil OC, and acknowledge that OM in soil is a continuum and ranges from macro to micro-molecules. Assuming humus is biochemically a relatively stable form of soil OC (Magid and Kjærgaard, 2001; Soil Science Society of America, 2008; Tate, 1987), and represents the largest fraction of OC in most Australian agricultural soils (Beckwith and Butler, 1983; Kögel-Knabner, 2002; Stevenson, 1994), the potential of humus to accumulate in soil warrants more attention. In an incubation experiment using different rates of wheat straw and nutrients, Kirkby et al. (2013) demonstrated that where adequate OM and soil nutrients are available, humus can be formed irrespective of soil type and OC concentration. Their study, along with others (Cadisch and Giller, 1997; Dijkstra et al., 2006; Himes, 1997; Kindler et al., 2009) support the theory that humus is largely composed of microbial detritus. Consequently, the process of accumulating microbial mass and debris may negate soil approaching C saturation. If this is the case, so long as C and nutrient inputs to the soil are maintained, then even soil with a high OC concentration should continue to accumulate OC. The limit will then become the environmental and economic feasibility of sustaining these inputs, and not just the inherent soil properties.

While studies have estimated and measured C saturation of the whole soil and soil fractions, few have assessed: i) soil under agricultural management with a high OC concentration and ii) the potential of soils with a low OC concentration, such as subsoils, to sequester C. Fewer studies have applied nutrients at the rates based on the stoichiometry of humus to promote the biochemical stabilisation of OC in soil. This study used an incubation experiment to evaluate the relationship between OM and nutrient inputs, microbial activity and OC concentration in two contrasting soil types, and assessed whether or not soil with high OC concentration approached an upper limit to OC accumulation. A regional survey was used to identify three basalt- and three granite-derived soils with high OC concentration. The two parent materials (thus, soil types) were selected to compare OC accumulation in soil with contrasting mineralogy and particle size distribution. For each parent material, two soil depths were compared to observe OC accumulation in soil with a high OC concentration (0 to 0.10 m, A1 horizon) to soil with a low OC concentration (0.40 to 0.50 m, B2 horizon). Treatments were based on 2 and 5 year pasture growth estimates for these parent materials, and nutrient rates were based on the stoichiometry of humus and achieving 30% stabilisation of OM added to soil. We hypothesised that for a given soil type, soil with a high OC concentration will continue to accumulate OC in a relatively stable form if both C and nutrient inputs are maintained.

2. Methods

2.1. Experimental design

Six permanent pasture sites, three with basalt-derived soil and three with granite-derived soil, were identified as having the highest OC concentration for their parent material class from a field survey in the Monaro region, south eastern Australia (Orgill et al., 2014). Two soil layers (0 to 0.10 and 0.40 to 0.50 m) were sampled from these sites for this experiment in September 2013. The treatment and measurement schedule for this experiment is provided in Fig. 1. The experiment consisted of three soil incubation cycles where samples were incubated in a darkened constant temperature room (CTR) at 25 °C. The six sites were divided into two groups; Group A and Group B. Group A included one basalt and one granite site, four treatments (discussed below) and measurements included: soil respiration, soil OC and N concentration and microbial biomass carbon (MBC). Group B samples (two basalt and two granite sites) had only two treatments and soil OC and N concentration measured (Fig. 1). The purpose of Group B was to ensure that the inference for soil OC from this study could be generalised to other basalt- and granite-derived soils in the Monaro region. The experiment was designed as a four replicate split-plot design with cycle randomised

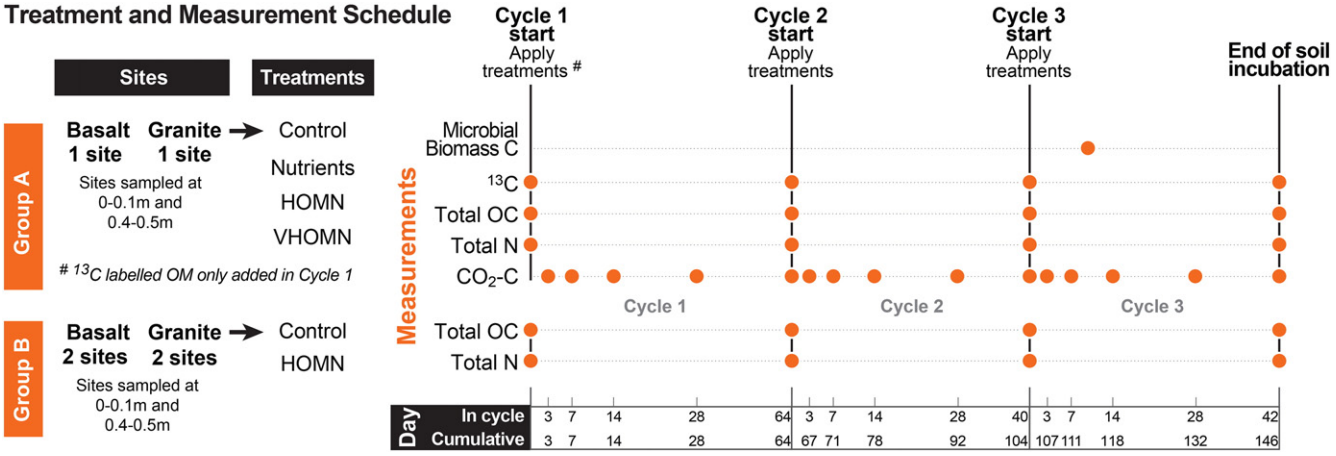

Fig. 1. Schematic representation of the soil incubation experiment, including: sites (6), parent material (basalt and granite), soil depth (0 to 0.10 and 0.40 to 0.50 m), treatments (control, nutrients, high organic matter plus nutrients; HOMN and very high organic matter plus nutrients; VHOMN), treatment applications (3), cycles (3) and measurement schedule.

to main-plots within a replicate and treatment by soil sample randomised to sub-plots (jars) within main-plots.

2.2. Soil characteristics

The basalt-derived soils had a mean soil OC concentration of 62.5 mg/g in the 0 to 0.10 m soil (A1 horizon) and 21.0 mg/g in the 0.40 to 0.50 m soil (B2 horizon) and were classified as Phaeozems (World Reference Base for Soil Resources, IUSS Working Group WRB, 2015; and Dermosols using the Australian Soil Classification Systems, ASC). The granite-derived soils had a mean OC concentration of 32.6 mg/g in the 0 to 0.10 m soil (A1 horizon) and 4.2 mg/g in the 0.40 to 0.50 m soil (B2 horizon) and were classified as Acrisols (WRB; and ASC: Kurosols). The mean bulk density (BD) for basalt-derived 0 to 0.10 and 0.40 to 0.50 m soil was 0.88 and 1.57 (g/cm^3) respectively and 0.85 and 1.58 (g/cm^3) respectively for the granite-derived soil. The basalt-derived soils were smectite dominant and >44% of particles were clay-sized, while the granite-derived soils were quartz dominant and predominately (>46%) sand-sized particles, with increasing clay (mainly kaolin) content in the 0.40 to 0.50 m soil (Table 1). The soil profile and pasture descriptions for these sites are described in Orgill et al. (2014).

2.3. Treatments

The four treatments were: 1) control (soil only), 2) nutrients (soil and nutrients only), 3) high OM plus nutrients (OM equivalent to 12.4 Mg DM/ha and referred to as HOMN), and 4) very high OM plus nutrients (OM equivalent to 31.1 Mg DM/ha and referred to as VHOMN). Treatments were applied to soil at the beginning of each of the three soil incubation cycles. Each treatment was run in four independent replicates. The rate of OM application was determined by estimating the mean annual herbage mass production in tonnes of dry matter per hectare (Mg DM/ha) for a fertilised pasture in the Monaro region using the GrassGro® pasture growth model; 6.2 Mg DM/ha (Moore et al., 1997). At the beginning of each incubation cycle, HOMN treatment had the equivalent of two years mean herbage mass production applied (12.4

Table 1
Soil mineralogy (weight %) and particle size (percent sand, silt and clay) for the three sites with basalt- and granite-derived soil. Group A (one basalt and one granite site) had all treatments and measurements applied, while Group B (two additional basalt and granite sites) had only control and HOMN treatments and total OC and N measured. Mineralogy is the weight % of the <2 mm soil and is classed according to abundance of mineral present with dominant (D > 60%), co-dominant (CD where the sum > 60%), sub-dominant (SD 20–60%), minor (M 5–20%) and trace (T < 5%) classes presented. Other minerals include: mica (Mca), calcite (Cal), dolomite (Dol), ilmenite (Ilm), anatase (Ant), rutile (Rt), gibbsite (Gbs) and amphibole (Am). Plagioclase feldspar series includes minerals from albite to anorthite.

Soil depth (m)	Mineralogy							Particle size (%)		
	Quartz	Plagioclase	Orthoclase/microcline	Smectite	Kaolin	Hematite/goethite	Other (T < 5%)	Sand	Silt	Clay
Basalt-derived soil (3 sites)										
Group A										
0–0.10	SD	SD		D	M	M	Cal, Ilm	21.9	21.4	54.8
0.40–0.50	SD	SD		D	M	M	Cal, Dol, Ilm	26.2	12.1	61.7
Group B (i)										
0–0.10	CD	SD	T	CD	T	M	Kln, Ilm	25.1	30.0	44.2
0.40–0.50	SD	SD	T	D	T	T	Kln, Hem/Gt, Ilm	23.9	15.6	59.2
Group B (ii)										
0–0.10	SD	M	T	D	SD	M	Mca, Ilm	24.6	21.2	51.7
0.40–0.50	M	T	T	D	SD	SD	Ilm, Ant, Gbs,	26.2	13.3	59.6
Granite-derived soil (3 sites)										
Group A										
0–0.10	D	T	T	T	M		Mnt, Mca, Ant	61.3	13.6	25.0
0.40–0.50	D	T		T	M	T	Mnt, Mca, Hem/Gt, Ant, Rt	46.6	4.5	48.8
Group B (i)										
0–0.10	D	SD	M		T		Kln, Mca, Am	63.6	14.1	19.2
0.40–0.50	D	SD	M		M		Mca, Am	65.2	12.2	20.7
Group B (ii)										
0–0.10	D	M	T		T		Kln, Mca, Cal	61.5	21.7	15.8
0.40–0.50	D	M	T		T		Kln, Mca, Cal	67.2	12.9	19.6

Mg DM/ha) and the VHOMN treatment had five years herbage mass production applied (31.1 Mg DM/ha).

For Group A, the OM applied at the beginning of cycle 1 was a blend (1:25) of uniformly-stable isotope-labelled (>97 atom% ^{13}C) perennial ryegrass shoots (sourced from Isolife Wageningen, Netherlands and milled to 1 mm) and field harvested (in Spring 2013) perennial ryegrass shoots (1.08 atom% ^{13}C) that were oven dried at 40 °C and milled to 1 mm. The OM applied in subsequent cycles (2 and 3) for Group A and for all cycles in Group B was solely field harvested perennial ryegrass shoots (oven dried at 40 °C and milled to 1 mm). Total C and N was measured on the prepared perennial ryegrass shoots using a LECO (CNS 2000) combustion furnace, and total acid extractable P and S measured by ICP-OES following nitric acid and hydrochloric acid (HCl) digestion (Wheal et al., 2011). Total C, N, P and S concentrations are reported in Table 2. Nutrient application rates were calculated using the concentration of nutrients required for 30% efficiency in the retention of C from the applied OM and based on the nutrient ratios of humus reported in the literature; C:N:P:S 10:0.83:0.20:0.14 (Himes, 1997; Kirkby et al., 2011) (Table 2). There was sufficient N and S in the perennial ryegrass shoot to achieve 30% efficiency in the retention of C from the applied OM, so only P was applied (as monopotassium phosphate; KH_2PO_4) prior to each cycle. The nutrient treatment (soil and nutrients only) had the same rate of nutrient application as the HOMN treatment.

2.4. Soil sample collection and preparation

At each of the sites soil samples were collected from four soil pits located in a representative area of the field. A composite sample was compiled in the field for each of the 0 to 0.10 and 0.40 to 0.50 m soil layers. Soil samples were oven-dried at 40 °C and sieved to <2 mm. Organic matter that was 0.4 to 2 mm was carefully removed using the dry sieving and winnowing procedure described by Kirkby et al. (2011). Remaining OM that was <0.4 mm is considered to be synonymous with heavy fraction OM (>1.4 g cm^3), thus relatively stable OM (Golchin et al., 1994b; Kirkby et al., 2011; Magid and Kjærgaard, 2001; Soil Science Society of America, 2008; Stevenson, 1994). The oven dried equivalent (ODE) mass of soil was measured on subsamples dried at 105 °C for 48 h. Dried and sieved samples were then stored at room temperature.

Soil samples were analysed for mineralogy by qualitative X-ray diffraction (XRD) analysis (Raven, 2013). The XRD data were interpreted using XPLOT and HighScore Plus (from PANalytical) search/match software. Soils were analysed for particle size distribution by the hydrometer method (Gee and Bauder, 1986). Results are reported as percent (%) sand (0.02 to 2 mm), silt (0.002 to 0.02 mm) and clay (<0.002 mm).

Table 2
Calculations for perennial ryegrass and nutrient applications for the high OM plus nutrients and very high OM plus nutrients treatments. Nutrient inputs required (mg) for 30% efficiency in the retention of C from the applied OM (following Kirkby et al., 2011) calculated as the nutrients required for retention less nutrients in the perennial ryegrass.

Treatment calculation	C	N	P	S
Humus ratios (Himes, 1997; Kirkby et al., 2011)	10,000	833	200	143
Nutrients per unit of humus C	1	0.0833	0.0200	0.0143
Nutrients in perennial ryegrass (%)	43.51	3.30	0.21	0.27
DM ratios (N,P,S per unit of C)		0.08	0.00	0.01
High OM plus nutrients treatment (12.4 Mg DM/ha)				
DM (mg) per 40 g soil	498			
Nutrients (mg) in added perennial ryegrass	216.66	16.45	1.07	1.36
C (mg) with 30% retention	65.00			
Nutrients (mg) required for 30% retention		5.41	1.30	0.93
Nutrients applied (mg)			0.23	
Very high OM plus treatment (31.1 Mg DM/ha)				
DM (mg) per 40 g soil	1245			
Nutrients (mg) in added perennial ryegrass	541.66	41.13	2.67	3.40
C (mg) with 30% retention	162.50			
Nutrients (mg) required for 30% retention		13.54	3.25	2.32
Nutrients applied (mg)			0.58	

2.5. Soil incubation setup and respiration

Forty g (equivalent oven dry weight) of soil was thoroughly mixed with the treatment and brought up to 70% field capacity by addition of deionised water and maintained at that moisture content throughout the experiment. The thoroughly mixed samples were placed in open 70 ml sterile plastic vials that were 60 mm in height. Vials containing samples were placed in 1 l mason jars containing a small amount of deionised water. An open scintillation vial containing 10 ml of 1 M NaOH was placed in the jar next to the vial containing the sample to trap CO_2 evolved. The 1 l jars were covered with cling film and the rubber lined lid tightly secured. Four jars were incubated as blanks containing only the 1 M NaOH trap and deionised water. The 1 M NaOH traps were collected and replaced on or near day 3, 7, 14, 28 and when respired CO_2 had plateaued (day 64, 40 and 42 for cycles 1,2 and 3 respectively). The 1 M NaOH traps were analysed on the same day as collection. For the titration, 5 ml of 0.5 M of $BaCl_2$ was added to the 1 M NaOH trap to precipitate any carbonates. Samples were centrifuged at 3000 rpm for 5 mins. Three drops of phenolphthalein were added to 2.25 ml of the solution and the solution was titrated using a Metrohm 665 Dosimat. The carbon dioxide (CO_2) evolved was calculated as described by Alef (1995). Results for this paper report CO_2 as μg CO_2-C per g soil.

2.6. Carbon and nitrogen in soil

At the conclusion of the incubation cycle, soil samples were collected and oven dried at 40 °C. Dried samples were gently hand ground using a mortar and pestle, sieved to <2 mm and any remaining recognisable plant material that was approximately 0.4 to 2 mm was carefully removed using dry sieving (Kirkby et al., 2011), static attraction and tweezers under a stereomicroscope in an attempt to remove undecomposed OM. Samples were then ground to <0.5 mm using a single puck mill head (Rayment and Higginson, 1992; Method 1B1).

Total OC and total N were determined on approximately 2 g of finely ground soil using a LECO (CNS 2000) combustion furnace (Merry and Spouncer, 1988; Rayment and Lyons, 2011; Method 6B2b). There was no inorganic C present. Results for this paper report total OC and total N as mg/g on an ODE of soil. Carbon stocks (Mg C/ha) were calculated using Eq. (1).

$$\text{C stock (Mg C/ha)} = (\text{total OC (mg/g)} \times 10) \times \text{BD}\left(\text{g/cm}^3\right) \times \text{depth (cm)} \times (1 - \text{proportion gravel}) \quad (1)$$

where, BD (g/cm^3) is the mean bulk density of the soil sample under field conditions.

2.6.1. ^{13}C isotopic soil analysis

Finely ground soil was dried at 65 °C overnight and between 1 and 15 mg (depending on total OC concentration) was weighed into tin cups. Samples were analysed on a Delta V Advantage coupled to a Conflo IV and a FlashHT in dual-reactor setup (Thermo Fisher Scientific, Bremen, Germany). Analytical precision varied between 0.08 and 0.18‰ for $d^{13}C$ and 0.001 and 0.15% for the 5 soil C standards. The atom % was calculated using Eq. (2).

$$\text{Atom\%} = \left[\left(d^{13}C/1000 + 1\right) * AR/1 + \left(d^{13}C/1000 + 1\right) * AR\right] * 100 \quad (2)$$

where; AR is the absolute $^{13}C/^{12}C$ ratio of the standard Vienna Pee Dee Belemnite (VPDB = 0.0111796).

2.6.2. Microbial biomass carbon (MBC)

Microbial biomass carbon was measured using the fumigation extraction method as described by Vance et al. (1987) on samples collected on day 10 of cycle 3. Briefly, unfumigated samples were extracted with 0.5 M K_2SO_4 and filtered (Whatman #42). Fumigated samples were incubated with ethanol-free chloroform ($CHCl_3$) in a sealed desiccator in the dark for 7 days at 25 °C as described by Amato and Ladd (1988). The fumigated samples were then treated as per the unfumigated samples. Extracts were analysed using an Elementar TOC Analyser. Microbial biomass carbon was calculated as the difference in C concentration between the fumigated and unfumigated soils. Results are reported as MBC μg C/g on an ODE of soil.

Carbon use efficiency (CUE) calculated as MBC increment per amount of consumed C-substrate was calculated according to Blagodatskaya et al. (2014) as:

$$CUE = \Delta MBC/(\Delta MBC + \Delta CO_2 - C) \quad (3)$$

where; ΔMBC is the net increase in MBC and ΔCO_2-C is the net increase in cumulative respiration.

2.7. Statistical analysis

Linear mixed models fitted using ASReml 3.0 (Gilmour et al., 2009) were used for all analyses. All models included effects for parent material (PM), soil depth (D), treatment (T) and all 2-way and 3-way interactions of these terms (PMxD, PMxT, DxT, PMxDxT) which together will be referred to as the 'PMDT terms'. Treatments refer to control, nutrients only, HOMN and VHOMN unless otherwise stated in the models detailed below. The significance of fixed effects was assessed using approximate F-tests using the techniques of Kenward and Roger (1997), the significance of random effects, other than experimental blocking and spline curvature, was assessed by comparing *d*, which equals −2 *Δlog-likelihood for nested models M0 and M1, where M0 and M1 differ only in a single random effect, with a χ^2_1distribution (P = 0.05) and the significance of spline (spl) curvature was assessed by examining 0.5(1-Pr ($\chi^2_1 \leq d$)) where *d* refers to models which differ in a single spline curvature term.

Cumulative soil respiration (μg of evolved CO_2-C per g of soil) was modelled using cubic smoothing spline regression (Verbyla et al., 1999). Mean effects for PMDT terms within each cycle were fitted as fixed effects. Linear trend in each cycle was fitted as a fixed effect by interacting PMDT terms within a cycle with 'days' where days related to a particular cycle. Random effects included spline curvature fitted as the interaction of PMDT terms within a cycle with spl(days) with days as described above. Residual variance was modelled for cycle x treatment combinations (Fig. 2).

The model for total OC for Group A included effects for cycle (1 to 3), PMDT terms and all multi-way interactions of these terms as fixed effects. Random effects included the blocking structures: replicate, main-plot and jar. The residual variance was modelled for PM × D combinations. A similar model was used for total OC for Group B, except that here there were two sub-groups (1 and 2) and only control and HOMN treatments. The fixed effects included all main effects, 2-way, 3-way and 4-way effects as described in Group A and in addition included subgroup (1 or 2) and interactions with all fixed terms. The random effects included blocking structures for each sub-group separately and residual variance was modelled as in Group A. Models for N (mg/g) and C:N were comparable to those for total OC for both Group A and B (Tables 3, 4 and 5).

The Carbon Use efficiency (CUE) model for HOMN and VHOMN was based on the 'nutrient only' mean, calculated for cycle 3 for Group A and included fixed effects for PMDT terms. Replicate was included as a random effect and residual variance was modelled for PM × D × T combinations.

The increase in total OC was compared with the C added in the HOMN and VHOMN treatments using cubic smoothing spline methods (Verbyla et al., 1999). Effects for PMDT terms with treatments being HOMN and VHOMN were fitted as fixed effects. The linear trend was fitted as a fixed effect by interacting PMDT terms with the independent variable 'C added'. Random effects included spline curvature fitted as the interaction of the PMDT terms with spl(C added), replicate and replicate interactions with C added and spl(C added) (Fig. 3).

The ^{13}C recovery model for HOMN and VHOMN, based on 'nutrient only' mean for Group A included fixed effects PMDT terms. Replicate and main-plot were included as random effects (Fig. 4).

A cube root transformation of MBC ($\sqrt[3]{MBC}$) was required to account for the mean-variance relationship in the data. The model for $\sqrt[3]{MBC}$ from cycle 3 only, included PMDT terms as fixed effects. Replicate was fitted as a random effect. All comparisons were made using 5% LSD on the cube root scale.

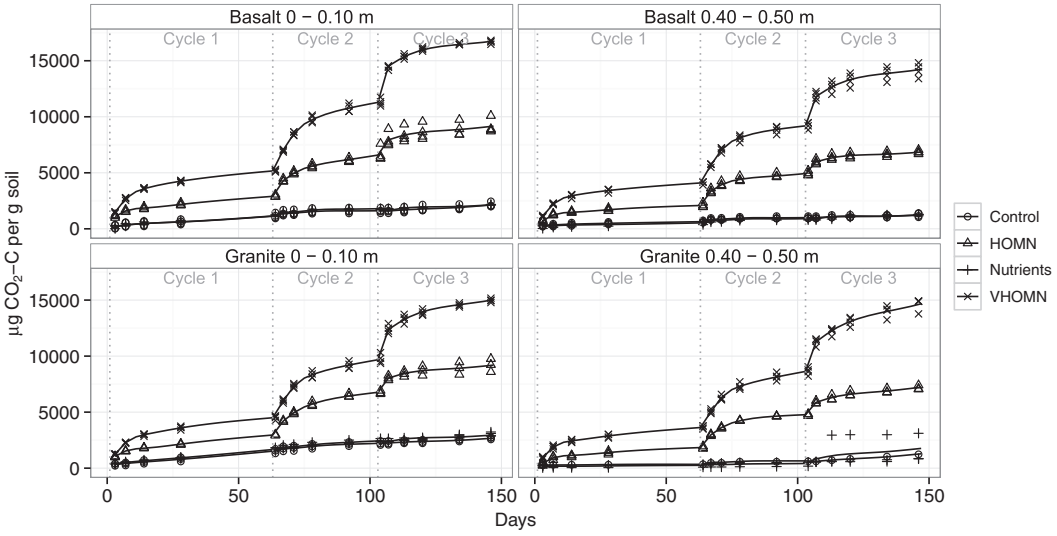

Fig. 2. Cumulative soil respiration (μg CO_2-C per g soil) for basalt- and granite-derived soil (0 to 0.10 and 0.40 to 0.50 m). Treatments applied at the beginning of each of the three incubation cycles and include: control, nutrients, high organic matter plus nutrients (HOMN) and very high organic matter plus nutrients (VHOMN).

Table 3
Mean total OC (mg/g) for basalt- and granite-derived soil (0 to 0.10 and 0.40 to 0.50 m) at the conclusion of three consecutive incubation cycles. Group A included all treatments: control, nutrients, high organic matter plus nutrients (HOMN) and very high organic matter plus nutrients (VHOMN), on the highest OC sites for basalt- and granite-derived soil. Group B included the control and high organic matter plus nutrients (HOMN) treatments on two basalt- and granite-derived soils. Calculated mean Carbon Use Efficiency (CUE %) for the HOMN and VHOMN treatments in Group A only. Standard error (SE) and least significant difference (LSD 5%) presented. Significant ($P < 0.05$) differences are marked by different letters (see footnote).

Soil depth (m)	Treatment	Group A					Group B			
		Starting OC	Cycle 1	Cycle 2	Cycle 3	CUE (%)	Starting OC	Cycle 1	Cycle 2	Cycle 3
Basalt 0–0.10	Control	72.0	71.0 a A	70.8 a A	71.1 a A		62.8	60.8 b A	61.2 b A	57.9 a A
	Nutrients		71.2 a A	70.8 a A	70.6 a A					
	HOMN		73.0 a B	73.9 a B	75.4 b B	14 a		61.8 a B	65.4 c B	63.5 b B
	VHOMN		73.2 a B	77.9 b C	84.9 c C	13 a				
Basalt 0.40–0.50	Control	26.0	26.3 a A	26.2 a A	26.5 a A		18.4	18.0 b A	18.1 b A	17.6 a A
	Nutrients		25.8 a A	25.8 a A	26.2 a A					
	HOMN		27.3 a B	29.4 b B	31.9 c B	13 a		20.9 a B	22.9 b B	24.4 c B
	VHOMN		30.2 a C	34.2 b C	39.3 c C	20 b				
Granite 0–0.10	Control	32.1	30.0 a A	30.0 a A	29.8 a A		32.9	31.6 b A	31.6 b A	30.7 a A
	Nutrients		30.6 a A	30.4 a A	29.8 a A					
	HOMN		33.4 a B	34.1 ab B	35.0 b B	16 a		34.6 a B	37.3 b B	37.8 b B
	VHOMN		34.7 a B	40.7 b C	44.5 c C	30 c				
Granite 0.40–0.50	Control	6.0	5.9 a A	6.0 a A	5.8 a A		3.3	3.8 b A	3.6 ab A	3.6 a A
	Nutrients		5.3 a A	5.4 a A	5.7 a A					
	HOMN		8.1 a B	10.0 b B	11.9 c B	15 a		6.1 a B	8.7 b B	10.3 c B
	VHOMN		11.3 a C	16.6 b C	18.4 c C	37 d				
All cycles			SE	5%LSD[a]		SE[b]		SE	5%LSD[a]	
Basalt 0–0.10			0.5	1.3		1.9		0.3	0.9	
Basalt 0.40–0.50			0.2	0.6		1.9		0.1	0.2	
Granite 0–0.10			0.5	1.3	HOMN VHOMN	1.4		0.3	0.8	
						2.2				
Granite 0.40–0.50			0.2	0.7		1.9		0.1	0.2	

[a] 5% LSD may be used to compare between cycles within treatments and between treatments within a cycle. Lower case letters refer to the former while upper case refers to the latter.
[b] SE for CUE are averages for HOMN and VHOMN when the 2 values did not differ when rounded to 2 significant figures. For CUE, the 5% LSD of 5.7 may be used for comparison across soils, depths and treatments.

3. Results

3.1. Soil respiration

There was no difference in soil respiration between the control and nutrients only treatments for either parent material or soil depth and only comparatively small increases in the CO_2 respired compared to HOMN and VHOMN treatments. These small increases occurred immediately after the start of each cycle where samples were mixed and water and/or nutrients applied, and were higher in the 0 to 0.10 m soil compared with the 0.40 to 0.50 m soil (Fig. 2). In contrast, the rate of CO_2 respired was very high for the first 3 to 10 days of each cycle for the HOMN and VHOMN treatments for both parent material ($P < 0.001$) and soil depths ($P < 0.001$), and then continued at a

Table 4
Mean total N (mg/g) for basalt- and granite-derived soil (0 to 0.10 and 0.40 to 0.50 m) at the conclusion of three consecutive incubation cycles. Group A included all treatments: control, nutrients, high organic matter plus nutrients (HOMN) and very high organic matter plus nutrients (VHOMN), on the highest OC sites for basalt- and granite-derived soil. Group B included the control and high organic matter plus nutrients (HOMN) treatments on two basalt- and granite-derived soils. Standard error (SE) and least significant difference (LSD 5%) presented. Significant ($P < 0.05$) differences are marked by different letters (see footnote).

Soil depth (m)	Treatment	Group A				Group B			
		Starting N	Cycle 1	Cycle 2	Cycle 3	Starting N	Cycle 1	Cycle 2	Cycle 3
Basalt 0–0.10	Control	4.80	5.96 b A	6.05 b A	5.65 a A	4.70	4.73 c A	4.58 b A	4.28 a A
	Nutrients		5.90 b A	6.06 c A	5.64 a A				
	HOMN		6.35 a B	6.75 b B	6.64 b B		4.97 a B	5.24 b B	5.29 b B
	VHOMN		6.78 a C	7.64 b C	7.85 c C				
Basalt 0.40–0.50	Control	1.87	1.94 a A	1.99 b A	2.04 c A	1.40	1.39 c A	1.27 a A	1.33 b A
	Nutrients		1.90 a A	1.98 b A	2.02 b A				
	HOMN		2.17 a B	2.59 b B	2.98 c B		1.73 a B	1.89 b B	2.32 c B
	VHOMN		2.66 a C	3.50 b C	4.19 c C				
Granite 0–0.10	Control	2.23	2.44 a A	2.49 a A	2.33 a A	2.54	2.59 b A	2.43 a A	2.37 a A
	Nutrients		2.53 b A	2.51 b A	2.28 a A				
	HOMN		2.92 a B	3.34 b B	3.28 b B		3.01 a B	3.22 b B	3.36 c B
	VHOMN		3.21 a C	4.07 b C	4.64 c C				
Granite 0.40–0.50	Control	0.66	0.82 b A	0.79 b A	0.68 a A	0.44	0.48 b A	0.47 ab A	0.44 a A
	Nutrients		0.77 a A	0.76 a A	0.68 a A				
	HOMN		1.07 a B	1.42 b B	1.62 c B		0.78 a B	1.10 b B	1.28 c B
	VHOMN		1.47 a C	2.36 b C	2.66 c C				
All cycles			SE	5%LSD[a]			SE	5%LSD[a]	
Basalt 0–0.10			0.05	0.14			0.03	0.08	
Basalt 0.40–0.50			0.02	0.04			0.01	0.03	
Granite 0–0.10			0.07	0.18			0.03	0.08	
Granite 0.40–0.50			0.03	0.09			0.01	0.03	

[a] 5% LSD may be used to compare between cycles within treatments and between treatments within a cycle. Lower case letters refer to the former while upper case refers to the latter.

Table 5
Mean C:N ratios for basalt- and granite-derived soil (0 to 0.10 and 0.40 to 0.50 m) at the conclusion of three consecutive incubation cycles. Group A included all treatments: control, nutrients, high organic matter plus nutrients (HOMN) and very high organic matter plus nutrients (VHOMN), on the highest OC sites for basalt- and granite-derived soil. Group B included the control and high organic matter plus nutrients (HOMN) treatments on two basalt- and granite-derived soils. Standard error (SE) and least significant difference (LSD 5%) presented. Significant ($P < 0.05$) differences are marked by different letters (see footnote).

Soil depth (m)	Treatment	Group A				Group B			
		Starting C:N	Cycle 1	Cycle 2	Cycle 3	Starting C:N	Cycle 1	Cycle 2	Cycle 3
Basalt 0–0.10	Control	12.91	11.88 b C	11.67 a C	12.60 c C	13.35	12.84 a B	13.39 b B	13.58 c B
	Nutrients		11.96 b C	11.70 a C	12.63 c C				
	HOMN		11.47 b B	10.92 a B	11.42 b B		12.43 b A	12.53 b A	12.06 a A
	VHOMN		10.85 b A	10.17 a A	10.79 b A				
Basalt 0.40–0.50	Control	13.92	13.54 c C	13.14 b D	12.97 a C	13.16	12.96 a B	14.27 c B	13.22 b B
	Nutrients		13.64 b C	13.00 a C	12.94 a C				
	HOMN		12.58 c B	11.39 b B	10.68 a B		12.09 b A	12.12 b A	10.51 a A
	VHOMN		11.39 c A	9.76 b A	9.37 a A				
Granite 0–0.10	Control	14.41	12.49 b C	11.99 a B	12.70 b C	12.99	12.23 a B	13.06 b B	12.98 b B
	Nutrients		12.30 a C	12.07 a B	13.02 b D				
	HOMN		11.37 b B	10.35 a A	10.57 a B		11.51 b A	11.63 b A	11.25 a A
	VHOMN		10.81 c A	10.06 b A	9.56 a A				
Granite 0.40–0.50	Control	9.09	7.22 a B	7.54 a C	8.60 b C	7.35	7.90 a A	7.75 a A	8.11 b A
	Nutrients		6.78 a A	7.19 b B	8.58 c C				
	HOMN		7.60 b C	6.96 a A	7.40 b B		7.88 a A	8.00 a B	8.01 a A
	VHOMN		7.70 b C	7.02 a AB	6.96 a A				
All cycles			SE	5%LSD[a]			SE	5%LSD[a]	
Basalt 0–0.10			0.04	0.10			0.05	0.15	
Basalt 0.40–0.50			0.05	0.13			0.06	0.17	
Granite 0–0.10			0.11	0.29			0.04	0.12	
Granite 0.40–0.50			0.08	0.23			0.07	0.19	

[a] 5% LSD may be used to compare between cycles within treatments and between treatments within a cycle. Lettering refers to between cycle tests. Lower case letters refer to the former while upper case refers to the latter.

declining rate during the rest of the incubation cycle. There were significantly ($P < 0.001$) greater amounts of CO_2 respired in the VHOMN treatment for all parent material and soil depths for each cycle compared with the HOMN treatment and significantly higher rates of CO_2 respired in cycle 2 and 3 compared with cycle 1 for both treatments (Fig. 2). The main effects on CO_2 respired for the HOMN treatment was soil depth, with significantly ($P < 0.001$) higher rates of CO_2 respired from the 0 to 0.10 m compared with the 0.40 to 0.50 m soil, and no difference with parent material. In contrast, for the VHOMN treatment there were significantly ($P < 0.001$) higher rates of CO_2 respired in the basalt-derived 0 to 0.10 m soil compared with other parent material and soil depths, and this increased from cycle 1 to 3.

3.2. Total OC and N concentration

3.2.1. Total OC

For Group A, regardless of parent material or soil depth, there was no difference in total OC concentration between the control and nutrients only treatments, or within these treatments with incubation cycle (Table 3). In contrast, the addition of OM and nutrients significantly

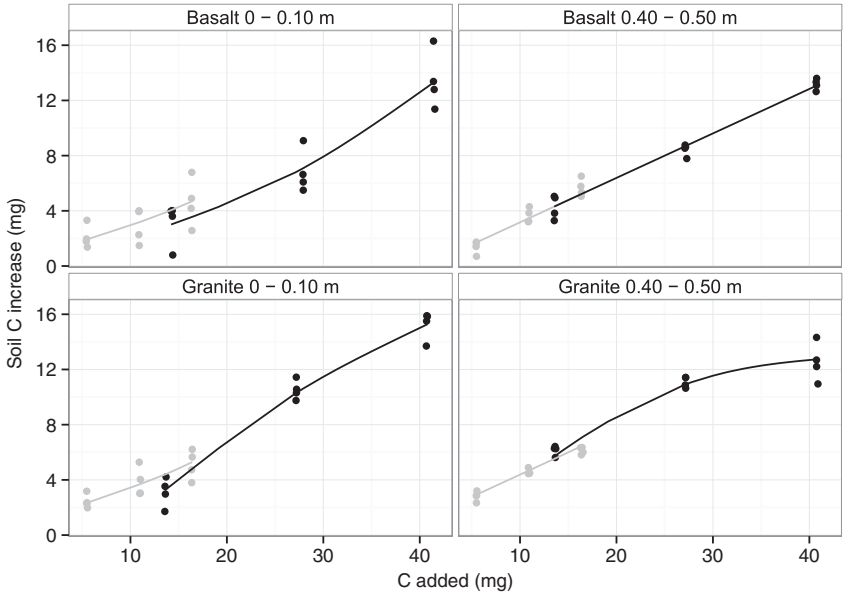

Fig. 3. Relationship between the increase in soil C (mg/g; treatment less original C concentration) and the amount of C added (mg/g based on 43.51% C in OM) for basalt- and granite-derived 0 to 0.10 and 0.40 to 0.50 m soil. Grey dots are the replicate values for cycle 1, 2 and 3 of the high organic matter plus nutrients (HOMN) treatment, and the grey line is the correlation. Black dots are the replicate values for cycle 1, 2 and 3 of the very high organic matter plus nutrients (VHOMN) treatment, and the black line is the correlation.

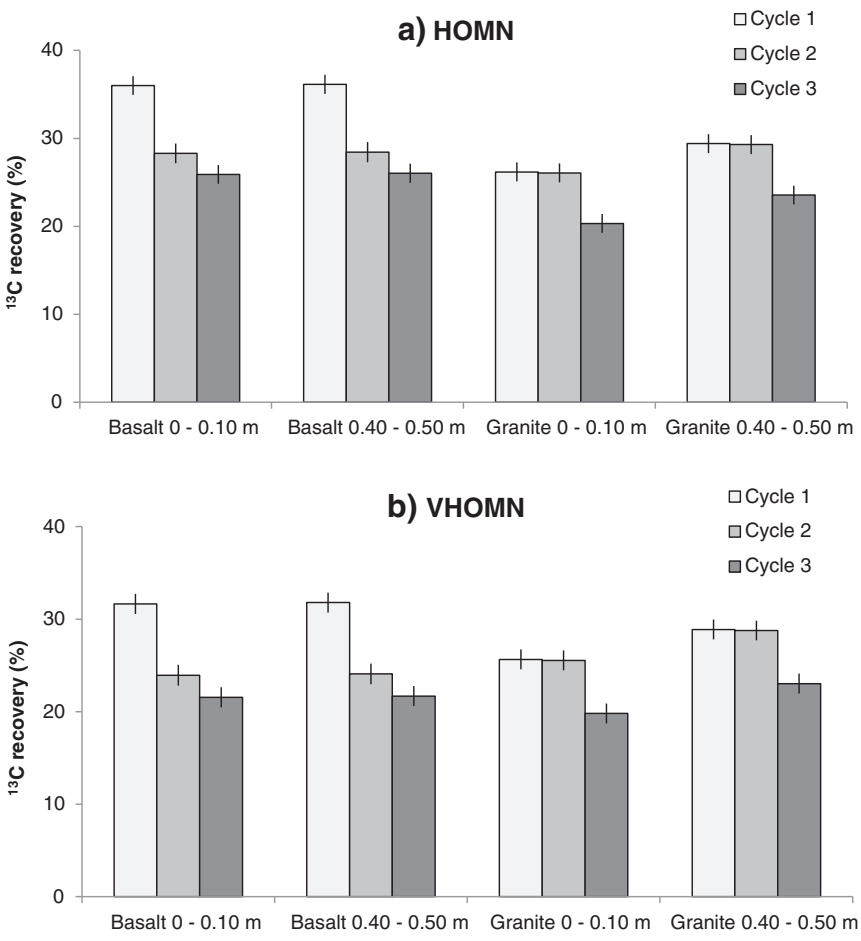

Fig. 4. The ^{13}C recovery (% of added ^{13}C labelled organic matter) for basalt- and granite-derived soil; 0 to 0.10 and 0.40 to 0.50 m soil, over three cycles of treatment application. Treatments include: high organic matter plus nutrients; HOMN (a) and very high organic matter plus nutrients; VHOMN (b). Error bars are standard error. 5% LSD is 2.9 for a) and b).

increased the concentration of total OC for both parent material ($P < 0.001$) and soil depths ($P < 0.05$) (Table 3). Total OC concentration increased with each HOMN and VHOMN treatment application. For example, in the basalt-derived 0 to 0.10 m soil the VHOMN treatment increased total OC from 72.0 mg/g to 73.2, 77.9 and 84.9 (0.5 se) mg/g in cycles 1, 2 and 3 respectively (Table 3). However, there was no significant difference detected in the concentration of total OC between cycle 1 and 2 for the HOMN treatment on the 0 to 0.10 m soil for either parent material.

The increase in total OC was compared with the C added using a regression approach across cycles with 'jar' level data on C added (Fig. 3). The slope of the underlying linear trend across C added was significantly affected by soil depth ($P < 0.001$) and the interaction of parent material and soil depth ($P = 0.002$). For the VHOMN treatment, the slope of the tangent to the regression curve at the end of cycle 3 for granite-derived 0.40 to 0.50 m soil was 0.06 per unit increase in C added (g) compared to approximately 0.35 to 0.50 per unit increase in C added for other parent material/soil depth combinations. While this is some indication that the granite-derived 0.40 to 0.50 m soil may be plateauing, it is not conclusive (Fig. 3).

For Group B, regardless of parent material and soil depth there was a significantly ($P < 0.05$) lower concentration of total OC for the control at the conclusion of cycle 3, compared with cycle 1 and 2 (Table 3). Consistent with Group A, there was a significant increase in total OC concentration with OM and nutrient application (HOMN treatment) for both parent material ($P < 0.001$) and soil depths ($P < 0.001$) (Table 3). One exception was the HOMN treatment in the basalt-derived 0 to 0.10 m soil where the increase in total OC from cycle 2 to 3 was not significant; 61.8, 65.4 and 63.5 (0.3 se) mg/g in cycles 1, 2 and 3 respectively (Table 3).

3.2.2. Total N

For Group A, there was no difference in total N concentration between the control and nutrients only treatments regardless of parent material or soil depth. The one exception was basalt-derived 0.40 to 0.50 m soil in cycle 1, where there was a difference in total N concentration within incubation cycle (Table 4). There was a significant ($P < 0.001$) increase in total N concentration with HOMN and VHOMN treatment application for both parent material and soil depths (Table 4). For example, in the basalt-derived 0 to 0.10 m soil, the VHOMN treatment increased total N from 4.80 mg/g to 6.78, 7.64 and 7.85 (0.05 se) mg/g in cycles 1, 2 and 3 respectively. However, there was no difference detected in the concentration of total N between cycle 2 and 3 for the HOMN treatment on the 0 to 0.10 m soil for either parent material (Table 4). Similarly for Group B, there was a significant ($P < 0.001$) increase in total N concentration in the HOMN treatment regardless of parent material or soil depth, and a trend of declining total N concentration under the control which was significant in some cases (Table 4).

3.2.3. Carbon to nitrogen ratio (C:N)

For Group A, there was little difference in the C:N ratio between the control and nutrients only treatments within a cycle however, in some cases there were significant differences between cycles (Table 5). There were significant differences in the C:N between HOMN and

VHOMN treatments for both parent material ($P < 0.001$) and soil depths ($P < 0.05$) in all cycles except for granite-derived, 0.40 to 0.50 m in cycles 2 and 3 (Table 5). The C:N for the HOMN and VHOMN treatments significantly ($P < 0.001$) differed from the control in all cycles for both parent material and soil depths. The C:N ratios for the VHOMN treatment on the basalt-derived 0.40 to 0.50 m soil and granite-derived 0 to 0.10 and 0.40 to 0.50 m soil significantly ($P < 0.001$) decreased from cycle 1 to cycle 3 (Table 5). For example, the VHOMN treatment on granite-derived 0 to 0.10 m decreased from 14.4 to 10.8, 10.1 and 9.6 (0.11 se) in cycles 1, 2 and 3 respectively (Table 5).

For Group B, there was a significant ($P < 0.05$) increase in the C:N ratio between cycles 1 and 3 for the control of both basalt- and granite-derived soil. For the HOMN treatment, the C:N ratio significantly ($P < 0.001$) decreased between cycle 1 and cycle 3 for the basalt-derived 0 to 0.10 and 0.40 to 0.50 m soil and granite-derived 0 to 0.10 m soil (Table 5). For example, the C:N ratio for the HOMN treatment on the granite-derived 0 to 0.10 m soil decreased from 13.0 to 11.5, 11.6 and 11.3 (0.04 se) in cycle 1, 2 and 3 respectively (Table 5). There was no difference in the C:N ratio with cycle for the HOMN treatment on the granite-derived 0.40 to 0.50 m soil.

3.3. ^{13}C recovery (% of added ^{13}C)

For each cycle, the calculated values for percent ^{13}C recovery were consistently higher for the HOMN treatment than the VHOMN treatment however, this difference was only significant ($P < 0.05$) for basalt-derived soil. For example, for the basalt-derived 0 to 0.10 and 0.40 to 0.50 m soil the mean ^{13}C recovery for the HOMN treatment in cycle 1 was 36.0 (1.1 se) and 36.2 (1.1 se) % respectively, and 31.7 (1.1 se) and 31.8 (1.1 se) for the VHOMN treatment (Fig. 4). There was a significant ($P < 0.05$) decrease in the percent ^{13}C recovery between cycle 1 and 3 for both HOMN and VHOMN for both parent material and soil depths (Fig. 4). Within each cycle, there was only a significant difference in ^{13}C recovery with soil depth for the granite-derived soil, with the 0.40 to 0.50 m soil having significantly ($P < 0.05$) higher ^{13}C recovery compared with the 0 to 0.10 m soil (Fig. 4).

3.4. Microbial biomass carbon

The granite-derived 0.40 to 0.50 m soil had significantly ($P = 0.01$) less MBC in the control and nutrients only treatments compared with the 0 to 0.10 m soil and basalt-derived soil (Fig. 5). There was no difference in MBC with parent material or soil depth for the HOMN treatment. However, there was a significantly ($P < 0.001$) higher MBC (μg/g) for the VHOMN treatment in the granite-derived 0 to 0.10 and 0.40 to 0.50 m soil; 1718 (151 se) and 1807 (156 se) μg/g respectively, compared with the basalt-derived soil; 670 (81 se) and 970 (103 se) μg/g respectively (Fig. 5).

3.5. Carbon use efficiency (CUE)

There was no difference in CUE with parent material or soil depth for the HOMN treatment. For the VHOMN treatment, the granite-derived 0 to 0.10 and 0.40 to 0.50 m soil had a significantly ($P < 0.001$) greater CUE compared with basalt-derived soil and other treatments (Table 3). The basalt-derived 0.40 to 0.50 m soil had a significantly ($P = 0.033$) greater CUE with the VHOMN treatment compared with the 0 to 0.10 m soil and the HOMN treatment (Table 3).

4. Discussion

4.1. Organic carbon in soil continued to increase with C and nutrient inputs

This experiment demonstrated that despite an increase in soil respiration ($P < 0.001$, Fig. 2), the addition of OM and nutrients to soil with high (0 to 0.10 m) and low (0.40 to 0.50 m) OC concentration significantly ($P < 0.05$) increased OC in soil (Table 3). This increase occurred regardless of parent material. By the end of cycle 3 (146 days of incubation and three VHOMN treatment applications), the application of 93.3 Mg DM/ha (equivalent 40.6 Mg C/ha) plus nutrients (N, P, and S) increased the stock of OC in basalt-derived 0 to 0.10 and 0.40 to 0.50 m soil by 11.5 and 19.14 Mg C/ha respectively, and 11.9 and 18.87 Mg C/ha respectively in granite-derived soil. The increase in soil OC with C input demonstrated in this study agrees with some field based studies (Feng et al., 2013b; Huggins et al., 1998b; Kong et al., 2005; Solberg et al., 1997). However, few studies have included soil with a high initial OC concentration, and those that have, reported little or no increase in soil OC with continuing C inputs (Campbell et al., 1991; Paustian et al., 1997b; Six et al., 2002; Stewart et al., 2009). Additionally, there are notable differences between this soil incubation experiment and field based studies. In the field, the incorporation and interaction of OM in soil, gaseous exchange and moisture availability is largely determined by soil configuration. Whereas in this experiment, large quantities of OM (up to 93.3 Mg DM/ha) were artificially incorporated into the soil and soil temperature and moisture maintained at optimum levels for OM degradation. The purpose of these conditions was to influence the dominant processes responsible for OM stabilisation, and test the hypothesis that soil with a high OC concentration will continue to accumulate OC in a relatively stable form if both C and nutrient inputs are maintained.

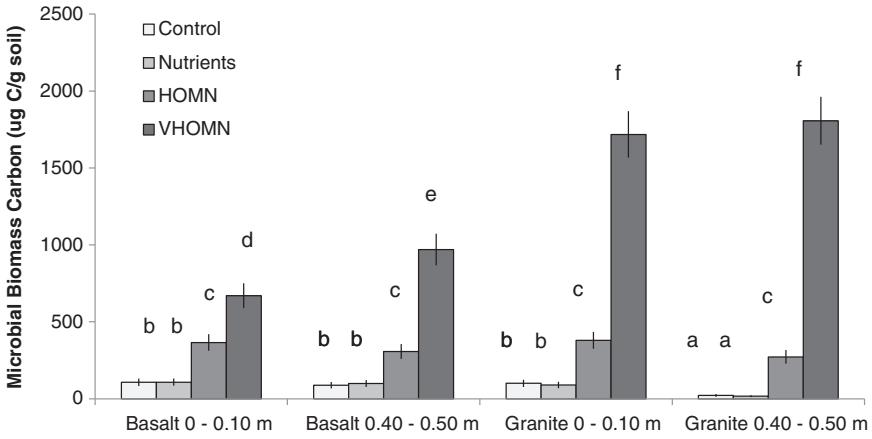

Fig. 5. Microbial biomass carbon (μg C/g soil) at day 10 of cycle 3 for basalt- and granite-derived soil; 0 to 0.10 and 0.40 to 0.50 m layers. Treatments include: control, nutrients, high organic matter plus nutrients (HOMN) and very high organic matter plus nutrients (VHOMN). Significant ($P < 0.05$) differences indicated by different letters with testing completed using cube root data (5%LSD was 1.01 on this scale). Error bars are approximate standard error as analysis was on the cube root scale.

According to the C saturation concept, the absence of an asymptotic relationship between OC concentration and C inputs indicates a C saturation deficit (Stewart et al., 2007; Stewart et al., 2009). For this reason, the low OC soil (0.40 to 0.50 m) was included in this experiment to represent soil further from any theoretical C saturation value for that parent material. However, regardless of initial OC concentration (that is, soil depth) there was no asymptotic behaviour between C inputs and OC accumulation in soil observed in this study (Fig. 3). Thus, OC accumulation was not approaching an upper limit at OM application rates ranging from 12.4 to 93.3 Mg DM/ha (equivalent to 5.4 to 40.6 Mg C/ha). Having acknowledged the differences in field and laboratory based experiments, there are a few possible explanations for not observing C saturation behaviour in this experiment. Firstly, the results of this study report OC concentration in <2 mm soil with undecomposed OM removed, not solely the silt plus clay fraction. Thus the OM applied to soil in this study may exist as uncomplexed OM (that is, particulate OM) while the silt plus clay fraction of soil may have become saturated with OC. However, following the incubation, free OM that was >0.4 mm was carefully removed using dry sieving (Kirkby et al., 2011), static attraction and tweezers under a microscope. Therefore the influence of uncomplexed OM is thought to be minimal on the observed increases in OC concentration. Secondly, the soil may have been far from C saturation and the OM inputs inadequate for the soil to approach C saturation. The sites selected for this experiment were identified from a regional survey as having the highest OC concentration for the parent material (Orgill et al., 2014). The rate of C input in this experiment exceeded the 6.0 Mg C/ha per year suggested for soils to exhibit C saturation behaviour in field based studies (Stewart et al., 2007). Hence it is unlikely that the soil was far from C saturation or that the OM input was inadequate to approach C saturation. Regardless, at rates of OM application ranging from 12.4 Mg DM/ha to 93.3 Mg DM/ha (equivalent to 5.4 Mg C/ha to 40.6 Mg C/ha), neither basalt- or granite-derived soil with high or low OC concentration exhibited C saturation behaviour (Table 3).

4.2. The role of clay content in determining a soil defined upper limit to OC stabilisation in soil

The proportion of clay-sized particles and their clay mineralogy influence OC accumulation and in most calculations one or both of these factors define the protective capacity of soil (Stewart et al., 2007). In Australia, the capacity of soil to protect OM from decomposition has been categorised based on clay content, with <10% clay indicating low protection and 10 to 25% and 25 to 45% indicating moderate and high protection, respectively (Baldock et al., 2009). Clay mineralogy also influences the extent to which this OM protection occurs (Jastrow et al., 2007). In this study the basalt-derived 0 to 0.10 m soil (Group A) was composed of 76.2% silt plus clay-sized particles and predominately 2:1 clays (mainly smectite), compared with the granite-derived soil which was composed of 38.6% silt plus clay-sized particles and predominately quartz and 1:1 clays (mainly kaolin) (Table 1). The 2:1 clay minerals such as smectite have a higher CEC and specific surface area compared with the 1:1 clay minerals such as kaolin (Churchman and Lowe, 2012); approximately 80 *vs* 15 $m^2\ g^{-1}$ (Feng et al., 2013a), and therefore have a greater capacity to absorb and stabilise OC within organo-mineral complexes (Baldock and Skjemstad, 2000; Kahle et al., 2002b; Mayer, 1994). The basalt-derived 0.40 to 0.50 m soil had a similar silt plus clay content (73.8%) and mineralogy to the 0 to 0.10 m soil. In contrast, the duplex profile of the granite-derived soil meant that the 0.40 to 0.50 m soil had higher silt plus clay content (53.3%), and specifically higher clay content than the 0 to 0.10 m soil (48.8 *vs* 25.0%). While asymptotic behaviour was not observed for any parent material or soil depth with treatment, there was no significant increase in OC concentration between cycle 2 and 3 for the VHOMN treatment in the granite-derived 0.40 to 0.50 m soil (Fig. 3). While this is not conclusive, this may indicate the soil is approaching an upper limit to OC accumulation at a lower OC concentration due to the dominance of 1:1 clays, compared with the 2:1 clay dominated basalt-derived soil.

One likely explanation for the continued accumulation of OC with increasing C and nutrient inputs is the model proposed by Kleber et al. (2007); that OC sorbs onto mineral surfaces in a zonal sequence. Under such a model, the OC coating of the mineral surface increases in thickness under increased OC accumulation in soil, not necessarily the mineral surface coverage. Thus, the capacity of soil to accumulate OC is not exclusively determined by the amount of binding sites on mineral surfaces, but instead by the C input and rates of OC change (Kögel-Knabner et al., 2008). According to the model of zonal organo-mineral association (Kleber et al., 2007), the protection of OC is greatest in the 'contact zone', where organo-mineral associations form. In this study, the greater concentration of OC in basalt-derived soil (Table 3) with high 2:1 clay content is consistent with a greater mineral surface area compared with granite-derived soil which is dominated by 1:1 clay minerals (Table 1). In contrast, OC accumulation in the outer zone is controlled by exchange kinetics and therefore OC may have a shorter residence time. For example, we did not observe a continued increase in OC concentration per unit of C input in the granite-derived 0.40 to 0.50 m soil (between cycle 2 and 3, Fig. 3) in this study. Furthermore, this model of zonal organo-mineral association acknowledges the heterogeneity of OC compounds, specifically in relation to their capacity to react with clays and self-organise in solution (Kleber and Johnson, 2010). Therefore, while the specific surface area of minerals may not define C stabilisation and upper limit to OC accumulation (Kleber et al., 2007), the OC components are likely to influence the exchange kinetics and the interactions with mineral surfaces (Kleber et al., 2007; Kögel-Knabner et al., 2008; Manzoni et al., 2012; Miltner et al., 2012). That is, influencing the OC component may also enhance its protection from microbial degradation and this may be biologically, not physio-chemically driven.

4.3. Increasing OC in soil through the accumulation of microbial detritus with C and nutrient inputs

The primary loss of soil C is through the decomposition of OM and the associated microbial respiration of CO_2 (Blagodatskaya et al., 2014). Given that humus is biochemically a relatively stable fraction of OC in soil, increasing the amount of humus through continued OM and nutrient application may increase OC accumulation. This may be an alternative reason why the high OC soils did not exhibit C saturation behaviour in this study. Humus is largely microbial detritus (Kirkby et al., 2011; Miltner et al., 2012) and as such was not directly measured in this experiment. However, the retention of the ^{13}C isotope throughout the three incubation cycles indicates the stability of accumulated OC. Despite increasing total microbial activity due to the treatments, as evidenced by both increasing soil respiration with time within each cycle (Fig. 2) and increasing MBC at one time (Fig. 5), as well as a significant ($P < 0.05$) narrowing of the C:N ratio (Table 5), there was substantial ^{13}C recovery at the end of the soil incubation (Fig. 4). This supports the hypothesis that the increases in OC accumulation were at least partly due to the conversion of plant residues into microbial detritus which is a major component of the relatively stable pool of OC in soil (Kirkby et al., 2011). Furthermore, the mean recovery of ^{13}C in soil (between 19.8 and 25.9 (1.1 se) %, Fig. 4) at the conclusion of the experiment (146 days of incubation and 3 treatment cycles) is relatively consistent with the target of 30% efficiency in the retention of C from the applied OM on which the nutrient applications were based (Himes, 1997; Kirkby et al., 2011).

4.4. Microbial capacity of soil to increase OC accumulation with C and nutrient inputs

The two microbial parameters used in this study were respiration (CO_2) and MBC. The trends in respired CO_2 were similar to trends in

soil OC concentration; highest for basalt-derived 0 to 0.10 m soil and lowest for the granite-derived 0.40 to 0.50 m soil (Fig. 2). The absence of any significant difference in soil respiration between the control and nutrients only treatment, in addition to the low cumulative respiration for these treatments, indicates that any remaining OM (that is, OM <0.4 mm) was relatively protected and that the microbial response was to the OM added in the HOMN and VHOMN treatments. By the end of each incubation cycle, the respiration rate had plateaued indicating that soil microbes had decomposed the majority of readily accessible OM. For the HOMN and VHOMN treatments, the cumulative CO_2 respired per cycle was more in cycle 2 and 3 than cycle 1 for all soil types and soil depths indicating a priming effect (Bingeman et al., 1953). By cycle 3, the application of OM and nutrients to the 0.40 to 0.50 m soil resulted in similar rates of respired CO_2 to that in the 0 to 0.10 m soil. Overall the ranking of MBC observations on day 10 of cycle 3 corresponded with the trends in cumulative respired CO_2 for the control, nutrients only and HOMN treatments. There was no difference in MBC with parent material or soil depth for the HOMN treatment (Fig. 5). This indicates that despite differences in total OC concentration and soil physio-chemical properties, the soils in this study have a similar microbial capacity to sequester C at these rates of OM and nutrient application and under these conditions. In contrast, the VHOMN treatment resulted in a significantly ($P < 0.05$) greater concentration of MBC in the granite- compared with basalt-derived soil, and in the basalt-derived 0.40 to 0.50 m soil compared with the 0 to 0.10 m soil (Fig. 5). This suggests that if adequate OM and nutrients are available, granite-derived soils and subsoils have a substantial capacity to accumulate OC in the microbial pool of soil.

The microbial capacity of soil to increase OC accumulation with C and nutrient inputs is further explained when the differences in microbial metabolism are compared in this study. Respiration (CO_2) and MBC were used to calculate CUE at day 10 (after the period of exponential growth) of cycle 3 as described by Blagodatskaya et al. (2014). The CUE values reported in this study; 13 to 20% for basalt-derived soil and 15 to 37% for granite-derived soil (Table 3) agree with the observed values (14 to 51%) for agricultural soils in the literature (Anderson and Martens, 2013; Blagodatskaya et al., 2014). For the VHOMN treatment, the granite-derived soil was significantly more efficient at converting C into microbial biomass compared with the basalt-derived soil (Table 3). This significantly greater CUE in the granite-derived 0.40 to 0.50 m soil agrees with the proportionately greater increase in total OC concentration compared with basalt-derived soil (Fig. 3 and Table 3). Furthermore, MBC is composed of both metabolically active and dormant microorganisms, while CO_2 derives primarily from active organisms (Werth and Kuzyakov, 2010). Therefore the comparable cumulative respiration in cycle 3 (Fig. 2) in the basalt- and granite-derived soils further supports the suggestion that, at rates of up to 93.3 Mg DM/ha (that is, the VHOMN treatment), the granite-derived 0.40 to 0.50 m soil accumulated a greater mass of relatively stable OC. The data from this study could also be used to calculate the efficiency of C retention given the rate of nutrient application, and as such is the subject of future research.

4.5. Implications for C sequestration in agricultural fields

The basalt- and granite-derived soil used in this study were selected for high OC concentration in the 0 to 0.10 m soil to investigate whether or not these sites had reached their maximum OC concentration. These sites were under permanent pastures and literature indicates that such sites are close to C saturation or have a small C saturation deficit (Angers et al., 2011). Despite this, the results from this laboratory study indicate that if OM and nutrient supply could be maintained at high levels, then these soils have the capacity to sequester more C. That is, at rates of up to 93.3 Mg DM/ha no C saturation occurred. It is important to acknowledge the challenges of comparing laboratory and field studies, as previously discussed (Section 4.1). We have presented a short-term (146 day) incubation experiment in a closed system. The issue of whether soil is approaching C saturation in the field or reaching equilibrium for that land use and management (West and Six, 2007) needs to be considered. To differentiate these in field based studies, climate and net primary productivity would need to be assessed. This would help to determine if constraints such as OM supply and decomposition, rather than inherent soil properties were limiting OC accumulation in soil. It is possible that the sites sampled in this study have approached, or are approaching their equilibrium OC concentration for the current climate and vegetation under field conditions, rather than C saturation. Further, it is unlikely that current agricultural management in this environment could achieve such substantial increases in OM supply. Regardless, our results support the theory that soil can stabilise OC beyond their finite surface area (Kleber et al., 2007) and that soil with a high OC concentration can continue to accumulate relatively stable OC where C and nutrient inputs are maintained.

5. Conclusions

Understanding the factors controlling OC accumulation in soil has implications for identifying soils with capacity to sequester C, determining agricultural management to achieve these increases in C sequestration and assessing the role of agriculture in mitigating climate change. This study demonstrated that with sustained C and nutrient inputs, the stable fraction of OC can increase linearly. Our results were not consistent with the soil C saturation model; instead supporting the zonal model of OC association with mineral surfaces, as well as the accumulation of relatively stable OC in the form of microbial detritus. More research is required on OC stabilisation under field conditions where soil configuration will largely determine OM degradation, and where such substantial quantities of OM addition to soil are unlikely. While there was no direct influence of parent material on OC accumulation observed in this study, our data suggests that clay mineralogy may determine any potential C saturation behaviour of soil. Therefore both the physio-chemical and biological stabilisation of OC in soil need to be considered when modelling OC dynamics in soil so that the mitigation benefits of soil C sequestration are maximised. This study suggests a large potential for C sequestration in soils under permanent pastures in southern Australia, particularly as soil nutrition is something that we can manage.

Acknowledgements

Funding for this research was provided by the Australian Government Department of Agriculture and Water Resources (National Soil Carbon Program) and NSW Department of Primary Industries. This research is part of a PhD through the Graham Centre for Agricultural Innovation, Charles Sturt University and was supported by the Future Farm Industries CRC and the Fenner School of Environment and Society, Australian National University. Dr. Alan Richardson (CSIRO) and Dr. Annette Cowie (NSW DPI) are acknowledged for their comments on the draft manuscript. Claudia Keitel and Janani Vimalathithen (Centre for Carbon, Water and Food, University of Sydney) are acknowledged for the stable isotope analysis, Albert Oates (NSW DPI) for the particle size analysis and Clive Hilliker (ANU) for assistance with the experiment schematic. The co-operation of the land holders in the Monaro regions whose properties were sampled is also acknowledged.

References

Alef, K., 1995. Estimation of soil respiration in closed jars. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, United Kingdom, pp. 215–216.

Amato, M., Ladd, J.N., 1988. Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. Soil Biol. Biochem. 20 (1), 107–114.

Anderson, T.-H., Martens, R., 2013. DNA determinations during growth of soil microbial biomasses. Soil Biol. Biochem. 57, 487–495.

Angers, D.A., Arrouays, D., Saby, N.P.A., Walter, C., 2011. Estimating and mapping the carbon saturation deficit of French agricultural topsoils. Soil Use Manag. 27 (4), 448–452.

Baldock, J.A., Skjemstad, J.O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org. Geochem. 31 (7–8), 697–710.
Baldock, J.A., Masiello, C.A., Gélinas, Y., Hedges, J.I., 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. Mar. Chem. 92 (1–4), 39–64.
Baldock, J., Grundy, M., Wilson, P., Jasquier, D., Griffin, T., Chapman, G., Hall, J., Maschmedt, D., Crawford, D., Hill, J., Kidd, D., 2009. Identification of areas within Australia with the potential to enhance soil carbon content. EP121592. St. Lucia, Queensland, Australia, CSIRO Sustainable Agriculture Flagship.
Beckwith, R.S., Butler, J.H.A., 1983. Aspects of the Chemistry of Soil Organic Matter, Soils - an Australian Viewpoint. London Academic Press, CSIRO, Melbourne, Victoria, Australia, pp. 565–581.
Bingeman, C.W., Varner, J.E., Martin, W.P., 1953. The effect of the addition of organic materials on the decomposition of an organic soil. Soil Sci. Soc. Am. J. 17 (1), 34–38.
Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. PLoS One 9 (4), e93282.
Cadisch, G., Giller, K.E., 1997. Driven by Nature: Plant Litter Quality and Decomposition. CAB International, Wallingford, Oxon, UK.
Campbell, C.A., Zentner, R.P., Bowren, K.E., Townley-Smith, L., Schnitzer, M., 1991. Effect of crop rotations and fertilization on soil organic matter and some biochemical properties of a thick Black Chernozem. Can. J. Soil Sci. 71 (3), 377–387.
Chan, C., Kay, B.D., Gregorich, E.G., 2008. Predicting a ceiling for soil carbon sequestration on variable landscapes under no-till in eastern Canada. Can. J. Soil Sci. 88 (5), 775–785.
Chung, H., Grove, J.H., Six, J., 2008. Indications for soil carbon saturation in a temperate agroecosystem. Soil Sci. Soc. Am. J. 72 (4), 1132–1139.
Chung, H., Ngo, K.J., Plante, A., Six, J., 2010. Evidence for carbon saturation in a highly structured and organic-matter-rich soil. Soil Sci. Soc. Am. J. 74 (1), 130–138.
Churchman, G.J., Lowe, D.J., 2012. Alteration, formation and occurrence of minerals in soils. In: Huang, P.M., Li, Y., Sumner, M.E. (Eds.), Handbook of Soil Sciences. Properties and Processes, 2nd edition CRC Press, Boca Raton, FL, United States of America, pp. 20.21–20.72.
Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V., Hungate, B.A., 2006. ^{13}C and ^{15}N natural abundance of the soil microbial biomass. Soil Biol. Biochem. 38 (11), 3257–3266.
Feng, W., Plante, A., Six, J., 2013a. Improving estimates of maximal organic carbon stabilization by fine soil particles. Biogeochemistry 112 (1–3), 81–93.
Feng, W., Xu, M., Fan, M., Malhi, S.S., Schoenau, J.J., Six, J., Plante, A.F., 2013b. Testing for soil carbon saturation behavior in agricultural soils receiving long-term manure amendments. Can. J. Soil Sci. 94 (3), 281–294.
Gee, G.W., Bauder, J.W., 1986. Particle-Size Analysis, Methods of Soil Analysis Part 1. Physical and Mineralogical Methods-Agronomy Monograph no. 9 (2nd edition). American Society of Agronomy-Soil Science Society of America.
Gilmour, A.R., Gogel, B.J., Cullis, B.R., Thompson, R., 2009. ASReml User Guide. Release 3. 0. Available at http://www.vsni.co.uk.
Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994a. Soil structure and carbon cycling. Aust. J. Soil Res. 32 (5), 1043–1068.
Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1994b. Study of free and occluded particulate organic matter in soils by solid state ^{13}C Cp/MAS NMR spectroscopy and scanning electron microscopy. Aust. J. Soil Res. 32 (2), 285–309.
Golchin, A., Oades, J.M., Skjemstad, J.O., Clarke, P., 1995. Structural and dynamic properties of soil organic-matter as reflected by ^{13}C natural-abundance, pyrolysis mass-spectrometry and solid-state ^{13}C NMR-spectroscopy in density fractions of an oxisol under forest and pasture. Aust. J. Soil Res. 33 (1), 59–76.
Grandy, A., Robertson, G., 2007. Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. Ecosystems 10 (1), 59–74.
Greene, R.S.B., Posner, A.M., Quirk, J.P., 1973. Factors affecting the formation of quasi-crystals in montmorillonite. Soil Sci. Soc. Am. Proc. 31, 457–460.
Gulde, S., Chung, H., Amelung, W., Chang, C., Six, J., 2008. Soil carbon saturation controls labile and stable carbon pool dynamics. Soil Sci. Soc. Am. J. 72 (3), 605–612.
Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Sci. Soc. Am. J. 61 (1), 131–139.
Himes, F., 1997. Nitrogen, sulfur, and phosphorus and the sequestering of carbon. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Soil Processes and the Carbon Cycle. CRC Press, Boca Raton, FL, United States of America, pp. 315–320.
Huggins, D.R., Buyanovsky, G.A., Wagner, G.H., Brown, J.R., Darmody, R.G., Peck, T.R., Lesoing, G.W., Vanotti, M.B., Bundy, L.G., 1998a. Soil organic C in the tallgrass prairie-derived region of the corn belt: effects of long-term crop management. Soil Tillage Res. 47 (3–4), 219–234.
Huggins, D.R., Clapp, C.E., Allmaras, R.R., Lamb, J.A., Layese, M.F., 1998b. Carbon dynamics in corn-soybean sequences as estimated from natural carbon-13 abundance. Soil Sci. Soc. Am. J. 62 (1), 195–203.
IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, Update 2015. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. FAO, Rome, Italy.
Jastrow, J., Amonette, J., Bailey, V., 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Clim. Chang. 80 (1), 5–23.
Kahle, M., Kleber, M., Jahn, R., 2002a. Carbon storage in loess derived surface soils from Central Germany: influence of mineral phase variables. J. Plant Nutr. Soil Sci. 165 (2), 141–149.
Kahle, M., Kleber, M., Jahn, R., 2002b. Predicting carbon content in illitic clay fractions from surface area, cation exchange capacity and dithionite-extractable iron. Eur. J. Soil Sci. 53 (4), 639–644.
Kenward, M.G., Roger, J.H., 1997. The precision of fixed effects estimates from restricted maximum likelihood. Biometrics 53, 983–997.
Kindler, R., Miltner, A., Thullner, M., Richnow, H.-H., Kästner, M., 2009. Fate of bacterial biomass derived fatty acids in soil and their contribution to soil organic matter. Org. Geochem. 40 (1), 29–37.
Kirkby, C.A., Kirkegaard, J.A., Richardson, A.E., Wade, L.J., Blanchard, C., Batten, G., 2011. Stable soil organic matter: a comparison of C:N:P:S ratios in Australian and other world soils. Geoderma 163 (3–4), 197–208.
Kirkby, C.A., Richardson, A.E., Wade, L.J., Batten, G.D., Blanchard, C., Kirkegaard, J.A., 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. Soil Biol. Biochem. 60, 77–86.
Kleber, M., Johnson, M.G., 2010. Chapter 3 - advances in understanding the molecular structure of soil organic matter: implications for interactions in the environment. In: Donald, L.S. (Ed.), Advances in Agronomy. Academic Press, pp. 77–142.
Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85 (1), 9–24.
Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biol. Biochem. 34 (2), 139–162.
Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: integrating biology, mineralogy, and organic matter chemistry. J. Plant Nutr. Soil Sci. 171 (1), 61–82.
Kong, A.Y.Y., Six, J., Bryant, D.C., Denison, R.F., van Kessel, C., 2005. The relationship between carbon input, aggregation, and soil organic carbon stabilization in sustainable cropping systems. Soil Sci. Soc. Am. J. 69 (4), 1078–1085.
Krull, E.S., Baldock, J.A., Skjemstad, J.O., 2003. Importance of mechanisms and processes of the stabilisation of soil organic matter for modelling carbon turnover. Funct. Plant Biol. 30 (2), 207–222.
Lal, R., 2004. Soil carbon sequestration to mitigate climate change. Geoderma 123 (1–2), 1–22.
Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528 (7580), 60–68.
Luo, Z., Wang, E., Sun, O.J., 2010. Soil carbon change and its responses to agricultural practices in Australian agro-ecosystems: a review and synthesis. Geoderma 155 (3–4), 211–223.
Magid, J., Kjærgaard, C., 2001. Recovering decomposing plant residues from the particulate soil organic matter fraction: size versus density separation. Biol. Fertil. Soils 33 (3), 252–257.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196 (1), 79–91.
Mayer, L., 1994. Relationships between mineral surfaces and organic carbon concentrations in soils and sediments. Chem. Geol. 114 (3–4), 347–363.
Merry, R.H., Spouncer, L.R., 1988. The measurement of carbon in soils using a microprocessor controlled resistance furnace. Commun. Soil Sci. Plant Anal. 19 (6), 707–720.
Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111 (1), 41–55.
Moore, A.D., Donnelly, J.R., Freer, M., 1997. GRAZPLAN: decision support systems for Australian grazing enterprises. III. Pasture growth and soil moisture submodels, and the GrassGro DSS. Agric. Syst. 55 (4), 535–582.
Oades, J.M., 1988. The retention of organic matter in soils. Biogeochemistry 5, 35–70.
Orgill, S.E., Condon, J.R., Conyers, M.K., Greene, R.S.B., Morris, S.G., Murphy, B.W., 2014. Sensitivity of soil carbon to management and environmental factors within Australian perennial pasture systems. Geoderma 214–215, 70–79.
Paustian, K., Andrén, O., Janzen, H.H., Lal, R., Smith, P., Tian, G., Tiessen, H., Noordwijk, M., Woomer, P.L., 1997a. Agricultural soils as a sink to mitigate CO_2 emissions. Soil Use Manag. 13 (4), 230–244.
Paustian, K., Collins, H.P., Paul, E.A., 1997b. Management controls on soil carbon. In: Cole, C. (Ed.), Soil Organic Matter in Temperate Agroecosystems: Long-Term Experiments in North America. CRC Press, New York, United States of America, pp. 15–49.
Raven, M.D., 2013. XRD Report - Qualitative XRD Analysis of Soil Samples for NSW Department of Primary Industries. Report No: D2878. CSIRO, Australia.
Rayment, G.E., Higginson, F.R., 1992. Australian Laboratory Handbook of Soil and Water Chemical Methods. Inkata Press, Melbourne, Australia.
Rayment, G.E., Lyons, D., 2011. Soil Chemical Methods- Australasia. CSIRO Publishing, Collingwood, Victoria Australia.
Reicosky, D.C., Evans, S.D., Cambardella, C.A., Allmaras, R.R., Wilts, A.R., Huggins, D.R., 2002. Continuous corn with moldboard tillage: residue and fertility effects on soil carbon. J. Soil Water Conserv. 57 (5), 277–284.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241 (2), 155–176.
Soil Science Society of America, 2008. Glossary of Soil Science Terms. American Society of Agronomy, Madison, WI, United States of America.
Solberg, E.D., Nyborg, M., Izaurralde, R.C., Malhi, S.S., Janzen, H.H., Molina-Ayala, M., 1997. Carbon storage in soils under continuous cereal grain cropping: N fertilizer and straw. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Management of Carbon Sequestration in Soil. CRC Press, Boca Raton, FL, United States of America, pp. 235–254.
Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. Second ed. John Wiley and Sons, New York, United States of America.
Stewart, C., Paustian, K., Conant, R., Plante, A., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86 (1), 19–31.
Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2008a. Soil carbon saturation: evaluation and corroboration by long-term incubations. Soil Biol. Biochem. 40 (7), 1741–1750.
Stewart, C.E., Plante, A.F., Paustian, K., Conant, R.T., Six, J., 2008b. Soil carbon saturation: linking concept and measurable carbon pools. Soil Sci. Soc. Am. J. 72 (2), 379–392.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2009. Soil carbon saturation: implications for measurable carbon pool dynamics in long-term incubations. Soil Biol. Biochem. 41 (2), 357–366.
Tate, R.L., 1987. Soil Organic Matter: Biological and Ecological Effects. Wiley-Interscience, New York, United States of America.
Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33 (2), 141–163.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.
Verbyla, A.P., Cullis, B.R., Kenward, M.G., Welham, S.J., 1999. The analysis of designed experiments and longitudinal data by using smoothing splines. J. R. Stat. Soc.: Ser. C: Appl. Stat. 48 (3), 269–311.
Werth, M., Kuzyakov, Y., 2010. ^{13}C fractionation at the root–microorganisms–soil interface: a review and outlook for partitioning studies. Soil Biol. Biochem. 42 (9), 1372–1384.
West, T., Post, W.M., 2002. Soil organic carbon sequestration rates by tillage and crop rotation: a global data analysis. Soil Sci. Soc. Am. J. 66, 1930–1946.
West, T., Six, J., 2007. Considering the influence of sequestration duration and carbon saturation on estimates of soil carbon capacity. Clim. Chang. 80 (1), 25–41.
Wheal, M.S., Fowles, T.O., Palmer, L.T., 2011. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Anal. Methods 3 (12), 2854–2863.