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Liming and priming: the long-term impact of pH amelioration on mineralisation may negate carbon sequestration gains.

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ABSTRACT

Acidity negatively impacts upon soil capability and conditions across approximately 50% of the world's arable land. Plant growth and nutrient cycling are known to respond positively to the addition of lime to decrease soil acidity. However, the interactions between liming and soil carbon dynamics remain incompletely understood. The nexus of soils, food security and climate change make this topic an urgent concern for investigation. This study utilised soils (Cambisols) from three long-term lime field trials (13-39 years) on farms. Soils (0-10 cm) were incubated in the laboratory for 3 months with and without ^{13}C-labelled wheat crop residue. This approach enabled direct quantification of the CO_2-C originating from three different processes; decomposition of extant soil organic carbon (SOC), decomposition of the added crop residue, and SOC priming i.e. the additional decomposition of SOC stimulated by addition of plant material. Biological and chemical soil properties were also quantified, with a novel application of measurement of the abundance of the functional genes involved in SOC decomposition. Priming was significantly increased in limed soils ($P<0.01$). The abundance of both archaeal rRNA genes, and the laccase-like functional gene involved in lignin decomposition correlated significantly and positively with SOC priming (Rho 0.46, $P<0.01$). Decomposition of extant SOC was positively correlated with pH and N (Rho 0.65, $P<0.001$). Decomposition of the added crop residue was also correlated with pH and inorganic N, albeit less strongly than for SOC (Rho 0.43, $P< 0.05$). These results support the theory that the application of lime to ameliorate soil acidity improves conditions for soil microorganisms as well as for plants. Archaea, and organisms harbouring SOC-decomposing laccases, would appear to play a key role in releasing nutrients stored in extant SOC, when priming is prompted by the addition of new plant material. This work highlights the importance of taking account of multiple response factors when assessing potential solutions within the food security-climate change nexus.

1. Introduction

Soil capability is compromised by low pH, with acid soils inhibiting plant growth across almost half of the world's arable lands (Kochian et al., 2015). Acidification is an ongoing part of current food production systems, as cations are removed from the soil in the form of plant biomass (Tang et al., 2013). Lime is well established as an ameliorant to improve soil condition in highly productive farming systems as well as in more marginal agricultural lands (Bolan et al., 2003; von Uexküll and Mutert, 1995). Lime application enhances plant growth by increasing soil pH, which increases nutrient availability while concurrently decreasing aluminium toxicity (Weil and Brady, 2017). Liming can therefore be considered an effective approach to enhance soil security and thereby food security in acid soils.

Food security and climate change are inextricably connected, with soil carbon as the nexus (Ramesh et al., 2019). Lime increases plant growth, which can be expected to increase the input of organic matter to soils (Haynes and Naidu, 1998; Page et al., 2009), both as crop residues on the soil surface and as root exudates directly into the upper soil horizons (Paul, 2016). What is the fate of this increased organic matter input? An increase in carbon input could increase soil carbon stocks (Whitmore et al., 2015), or alternatively increase microbial decomposition and thereby decrease soil carbon stocks (Fontaine et al., 2004). Reviews of the impacts of lime addition on soil carbon stocks

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(Paradelo et al., 2015) and fluxes (Page et al., 2009) report that results are mixed and inconclusive. Both studies call upon the scientific community to further investigate the processes that occur within the soil carbon cycle when lime is added to increase pH (Page et al., 2009; Paradelo et al., 2015).

The three processes within the soil carbon cycle that impact upon the stocks and fluxes of carbon are mineralisation of extant soil organic carbon (SOC), mineralisation of new organic matter added by plant residues and priming. Priming is commonly understood as the increase or decrease in mineralisation of SOC associated with the addition of external substrate. The first process, mineralisation of existing SOC, is hindered by physical protection of SOC within aggregates and physio-chemical protection via binding to multivalent cations and clay surfaces (Baldock and Skjemstad, 2000). Aggregation has long been recognised as an co-benefit of lime addition to acidic soils (Baldock et al., 1994; Haynes and Naidu, 1998). Liming alters the charge distribution in the soil via direct addition of Ca^{2+} ions and indirectly via the subsequent H^+ ion consuming reactions, thereby impacting both aggregation and binding sites on clay surfaces (Haynes and Naidu, 1998). As such, lime can be expected to hinder mineralisation of SOC by facilitating increased protection within aggregates and binding to Ca^{2+} and clay surfaces. Despite this measure of protection provided by aggregation and binding, microbial utilisation of SOC continues.

Microbial utilisation of carbon is, in fact, central to all three processes. While this has been long recognised, recent developments in soil microbiology are beginning to elucidate both the actors and more detail of the processes of the soil carbon cycle (Paul, 2016). It is widely accepted that bacteria, archaea and fungi in soil mediate mineralisation of existing SOC and plant residues through the actions of their enzymes. Cellulases and laccases are involved in the mineralisation of cellulose and lignin, respectively. Cellulose is the main structural component of higher plant cell walls and its decomposition products make up much of soil organic matter (Berg and Laskowski, 2006). The ability to degrade cellulose is widely distributed across bacteria and fungi (Lynd et al., 2002), while the role of archaea in cellulose mineralisation is currently not well described. Lignin, the second most abundant constituent of plant cell walls, is highly recalcitrant to mineralisation. Laccases produced by bacteria and fungi can catalyse the oxidation of lignin, thereby contributing to the mineralisation of SOC (Baldrian, 2006; Blackwood et al., 2007). Fungal laccases have been extensively studied and it has recently been reported that there is wide occurrence of laccase-like genes in bacteria that contribute to SOC mineralisation (Feng et al., 2019). There is, however, a gap in our understanding of how cellulose and lignin mineralisation are affected by soil pH increase.

Soil acidity is widely considered as a constraint on microbial growth and activity (Bolan et al., 2003; Page et al., 2009). However, while soil pH has been shown to affect the abundance, composition and diversity of microbial communities (Wakelin et al., 2008), the direction of the effect is varied. For example, the highest bacterial diversity is often found in neutral pH soils, with significantly lower diversity reported in more acidic soils (Fierer and Jackson, 2006; Lauber et al., 2008; Rousk et al., 2010). While neutral to slightly alkaline conditions favour bacterial growth, acidic conditions have been shown to favour fungal growth (Rousk et al., 2009). The effect of pH on archaea is still unclear, with reports of both a negative correlation between pH and archaeal abundance (Tripathi et al., 2013), and no significant relationship (Bengtson et al., 2012). The limited and mixed evidence on the impact of pH on soil microbiology, as well as the central role of microbes in mineralisation of SOC, plant residues and priming is a critical gap in our understanding of soil carbon dynamics. Combining chemical isotope tracer approaches with microbial community and enzyme studies has potential to help fill this gap (Paul, 2016).

This study was designed to investigate the effect of lime on three distinct processes within the soil carbon cycle; mineralisation of existing SOC, mineralisation of crop residue and SOC priming. Furthermore, it aimed to identify whether mineralisation was related to soil chemical and biological properties, in particular the size of the microbial community (gene abundance), key functional SOC decomposition genes (cbh1, LMCO and GH48) and soil N content. The overarching goal of this work is to support farmers to use sustainable land use practices that contribute to both food security and reduced emissions. Therefore, soils were sampled from long-term field trials in which lime was applied at rates and times commensurate with local production systems, and the soils were subsequently managed in line with the region's standard cropping system practices. Lime application has demonstrated economic productivity gains in this region and is now recommended as routine farm management (Gazey et al., 2014). To test our hypotheses over a wider range of pH values and initial soil C contents, two additional soils were included. Soils were incubated in the laboratory with ^{13}C-labelled wheat residue, which enabled the separate quantification of SOC mineralisation, crop residue mineralisation and priming. We hypothesised that: 1) the increase in pH due to liming would enhance mineralisation of both extant SOC and crop residue; 2) priming would be greater in limed than non-limed soil, due to a more favourable pH environment for microbial activity; and 3) mineralisation would be positively correlated with the abundance of key soil organic matter (SOM) functional genes. This study will yield value to land managers and policy makers considering how best to align agricultural and climate change mitigation practices.

2. Materials and methods

2.1. Soil sampled and long-term lime field trial details

Eight soils with a range of initial pH values and amounts and composition of SOC were sampled in 2013 (Table 1). Six were paired limed and non-limed soils from historical liming trials in the Western Australian wheatbelt (Tang et al., 2003; Wang et al., 2016) classified as Tenosols (Isbell, 2002) or Cambisols (WRB, 2014) and were under cropping before and after lime application. In addition to the non-limed control (0 t ha^{-1}), the following lime treatments (amounts of lime and years since application) were sampled at each trial: Wongan Hills (30°51'S 116°44'E) 5.5 t ha^{-1} applied in 1994; Kellerberrin (31°29'S 117°47'E) 5 t ha^{-1} applied in 1991 and 1 t ha^{-1} in 2000; Konnongorring (30°54'S 116°51'E) 6.2 t ha^{-1} applied in 1984 and 1.5 t ha^{-1} in 1999. Two additional soils were included to broaden the range of pH values and C contents, the subsoil (10-20 cm) from the non-limed Wongan Hills trial and a Chromosol (Isbell, 2002) or Luvisol (WRB, 2014) from Hamilton, Victoria (142°04'E 37°49'S) that had been under long-term pasture and had not been limed. For field trials, soil was sampled from all replicates of each lime treatment using exhaust pipe (5 cm diameter) and combined to form a composite sample. The field carbonate test (2 drops 1 M HCl) yielded no audible or visible effervescence, indicating no residual lime or pedogenic calcareous material. For the Hamilton site, soil was collected from 0-10 cm at several locations in the paddock with a spade. All soils were dried at 30 °C, passed through a 2-mm sieve and thoroughly mixed. Initial pH measured on air-dry soils ranged from 4.2 to 5.9, and SOC contents from 3 to 44 mg g^{-1} soil with most soils having around 10 mg SOC g^{-1} soil but the Chromosol had greater clay and SOC content (Table S1).

2.2. Carbon fluxes

2.2.1. Soil incubation experiment

Soil samples (45 g) were weighed into individual plastic vials (4.3 cm ID × 5.8 cm high), gently tapped (bulk density; 0.9 g cm^{-3} Chromosol, 1.3 g cm^{-3} Tensosol), adjusted to ~60% of field capacity with milli-Q water and pre-incubated in the dark at 25 °C for two weeks to achieve a stable soil microbial biomass. After pre-incubation, 225 mg (0.5% w w^{-1}) of ^{13}C-labelled wheat residue was thoroughly mixed through half of the vials of soil. The non-residue-amended soils were similarly mixed to ensure the same level of disturbance. Soil water content was adjusted

Table 1
Selected physical and chemical properties of the soils used in this study. All samples were collected from 0-10 cm except Wongan Hills (WH) subsoil (10-20 cm). Total C is total organic carbon as no inorganic carbon was present. POC is particulate organic carbon, HOC is humic organic carbon and ROC is resistant organic carbon.

Location	Lime history		Initial pH (0.01M $CaCl_2$)	Total	Total		MIR-predicted C fractions			Soil Texture		
	total amount (t ha^{-1})	Years		C (mg g^{-1})	N (mg g^{-1})	$\delta^{13}C$	POC (mg g^{-1})	HOC	ROC	Sand (%)	Silt	Clay
Hamilton	0		4.6	43.9	3.44	-26.54	0.3	8.4	4.2	16.8	63.6	19.6
WH subsoil	0		4.2	3.4	0.25	-26.84	0.3	4.9	2.2	74.6	19.6	5.8
Wongan Hills	0		5.0	10.9	0.79	-28.14	0.3	4.5	2.1	88.9	8.8	2.3
Wongan Hills	5.5	1994	5.9	10.4	0.78	-27.76	0.3	5.2	2.2	85.6	9.5	4.9
Kellerberrin	0		5.0	10.9	0.88	-27.33	0.2	5.3	2.4	53.8	32.4	13.8
Kellerberrin	6	1991, 2000	5.8	9.6	0.80	-27.33	0.2	4.7	2.1	54.5	34.4	11.2
Konnongorring	0		4.7	10.6	0.81	-27.48	0.5	4.6	2.2	52.5	36.5	11.0
Konnongorring	7.7	1984, 1999	5.3	7.2	0.58	-27.58	0.4	4.6	2.1	53.3	35.0	11.7

to 80% of field capacity (θ_g = 0.1 g g^{-1}) for the Tenosols and 70% of field capacity (θ_g = 0.4 g g^{-1}) for the Chromosol due to its finer texture. Soils were incubated in 1-L glass jars (Ball Wide Mouth Quart Jars, Jarden Home Brands, Fishers, USA) fitted with gas-tight lids and a water reservoir to maintain humidity. Two sets were prepared for destructive harvest at 1 week, 1 month and 3 months and incubated in the dark at 25 °C. Overall, the experiment was a completely randomized design with 8 soils × 2 residue treatments × 3 replicates × 3 sampling times.

2.2.2. Stable ^{13}C-labelled residues

The ^{13}C-labelled wheat residue used in the incubation experiment was produced as described in Butterly et al. (2015). Briefly, wheat (*Triticum aestivum* cv Yitpi) was grown in containers under field conditions and pulse-labelled with $^{13}CO_2$ 14 times through the 21-week growing period using an air-tight chamber. Shoots were collected at maturity, washed, dried at 70 °C for 48 h, grain removed, and the remaining material was milled (Retsch ZM200 centrifugal mill, Retsch GmbH, Haan, Germany) to pass through a 2-mm sieve. The C:N ratio of the residue was 42.

2.2.3. Carbon mineralisation and CO_2-C partitioning

Carbon mineralisation was measured by two methods. Firstly, an Infrared Gas Analyser (IRGA) was used to facilitate intensive measurement of C mineralisation during the initial 30-day incubation period. The headspace gas of each jar was sampled using the IRGA (Servomex 4200 Industrial Gas Analyser, Servomex Group Ltd., Crowborough, UK) at 1, 5, 10, 19 and 32 days via a septum port within the lid of each jar (Butterly et al., 2010). C mineralisation (μg CO_2-C g^{-1} soil C) was calculated following the conversion of sensor readings into CO_2 concentrations using calibration jars with known volumes of pure CO_2. After each measurement, jars were opened and flushed to exchange the headspace with ambient air. Net CO_2 release was calculated as the difference in CO_2 concentrations prior to opening the jars and when the jars were closed. Jars without soil were included as blanks. Secondly, traditional alkali absorption was used strategically to estimate C mineralisation and facilitate CO_2-C partitioning. Alkali traps (8 mL, 1 M NaOH) in each jar were sampled at 6, 27 and 91 days. The amount of CO_2 captured in each trap was quantified by titrating a 2-mL aliquot with 0.5 M HCl in the presence of excess 0.5 M $BaCl_2$ and 1 drop of phenolphthalein indicator (5% w w^{-1}) using a digital burette (BRAND Titrette, Wertheim, Germany) (Zibilske, 1994). Cumulative C mineralisation is the sum of that quantified at each of the 3 sampling times.

To estimate the CO_2 derived from the crop residue ($CO_{2\ residue}$), a 2-mL aliquot of NaOH (neutralised with 0.5 M HCl) was precipitated with $SrCl_2$, purified by repeatedly centrifuging and resuspending in milli-Q water before drying the pellet at 60 °C and determining the ^{13}C abundance ($\delta^{13}C$ Pee Dee Belemnite, PDB) using Isotope Ratio Mass Spectrometry (IRMS) (Hydra 20-22, SerCon, Crewe, UK). The proportion of CO_2 derived from residue ($\propto CO_{2\ residue}$) was calculated as

$$\alpha CO_{2residue} = \left[\left(\delta^{13}C_{soil+residue} - \delta^{13}C_{soil}\right)/\left(\delta^{13}C_{residue} - \delta^{13}C_{soil}\right)\right] \quad (1)$$

where $\delta^{13}C_{soil\ +\ residue}$ is the $\delta^{13}C$ value of precipitates from residue-amended samples; $\delta^{13}C_{residue}$ is the $\delta^{13}C$ value of the labelled residue and $\delta^{13}C_{soil}$ is the $\delta^{13}C$ value of the precipitates from non-residue-amended soil (not the $\delta^{13}C$ value of the bulk soil). Thus, the $\delta^{13}C_{soil}$ measured at each sampling time is the precise signature of the C that was mineralised. Then, the amount of CO_2 derived from residue ($CO_{2\ residue}$) was calculated as

$$CO_{2residue} = CO_{2total} \times \alpha CO_{2residue} \quad (2)$$

The CO_2 derived from soil organic C ($CO_{2\ SOC}$) was calculated as

$$CO_{2SOC} = CO_{2total} - CO_{2residue} \quad (3)$$

and primed C was calculated as the difference in $CO_{2\ SOC}$ between wheat residue-amended and non-residue-amended soils.

2.3. Soil microbiology; DNA extraction and qPCR

Duplicate DNA extractions were performed on all soils after 3 months of incubation using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, USA) following the manufacturer's instructions. DNA from pooled extractions was quantified fluorometrically (Qubit, ThermoFisher Scientific, City, USA) and diluted with sterile water to a concentration of 5 ng DNA μL^{-1} for all subsequent qPCR assays. Quantitative polymerase chain reaction (qPCR) (Applied Biosystems ViiA7, Foster City, USA) was performed on all samples in triplicate to quantify the abundance of the following genes; archaeal 16S rRNA (Biddle et al., 2006), bacterial 16S rRNA (Fierer et al., 2005), fungal 18S rRNA (Hoshino and Morimoto, 2008), laccase-like multicopper oxidase (LMCO) (Kellner et al., 2007), cellobiohydrolase (*cbh*I) (Edwards et al., 2008) and glycoside hydrolase (GH48) (de Menezes et al., 2015). Full details of the genes, primers and modifications of the qPCR cycling conditions are shown in Table 2. For bacterial and archaeal 16S rRNA genes and fungal 18S rRNA genes, each 20 μL qPCR reaction contained 10 μL of Power SYBR Green Master Mix (Applied Biosystems, Foster City, USA), 0.2 μL of the specific forward and reverse primers at a concentration of 10 μM, 2 μL of 5 mg mL^{-1} bovine serum albumin (Ambion Ultrapure BSA, ThermoFisher Scientific, Life Technologies, Carlsbad, USA), 2 μL of template DNA and 5.6 μL sterile water. The qPCR reaction for both LMCO and GH48 was the same as above except that Sso Q-PCR SYBR (Biorad, Hercules, USA) was used. For *cbh*I, each 20 μL qPCR reaction contained 10 μL of SensiFAST SYBR Lo-ROX (Bioline, Sydney, Australia), 2 μL of the specific forward and reverse primers at a concentration of 10 μM, 2 μL of bovine serum albumin at 5 mg mL^{-1}, 2 μL of template DNA and 2 μL water.

Table 2
Primers used in determining gene abundance by qPCR.

Gene	Primer	Sequences (5' – 3')	Fragment Length (bp)	Primer Reference	Cycling condition
Bacterial *16S rRNA*	Eub 338 Eub 518	ACTCCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	180	Fierer et al., (2005)	As conducted in Fierer et al., (2005)
Archaeal *16S rRNA*	Arch 915F Arch 519R	CAGCMGCCGCGGTAA GTGCTCCCCCGCCAATTCCT	421	Biddle et al., (2006)	94 °C for 10 min then 40 cycles 95 °C for 1min, 56 °C for 1 min, 72 °C for 1 min, and 78 °C for 1 min followed by a melt curve fluorescence data was collected at the 78 °C stage.
Fungal *18S rRNA*	FF390 FR1GC	CGATAACGAACGAGACCT AACCATTCAATCGGTA4T	372	Hoshino YT and Morimoto S (2008)	94 °C for 10min then 40 cycles 95 °C for 1min, 50 °C for 1min, 72 °C for 1 min, and 78 °C for 1 min followed by a melt curve fluorescence data was collected at the 78 ° stage.
LMCO	CU1AF CU2R	ACMWCBGTYCAYTGGCAYGG GRCTGTGGTACCAGAANGTNCC	142-155	Kellner et al., (2007)	95 °C for 3 min then 40 cycles of: 95 °C for 5sec, 58 °C for 30 sec, followed by a melt curve from 65 °C to 95 °C.
cbhI	fungcbhIF fungcbhIR	ACCAAyTGCTAyACngGnAA (GC[C,T] TCC CAI AT[A,G] TCC ATC	520-620	Edwards et al., (2008)	95 °C for 3min then 40 cycles of: 95 °C for 5sec, 60 °C for 30 sec, followed by a melt curve from 65 °C to 95 °C
GH48	GH48_F8 GH48_R5	GCCADGHTBGCGGACTACCT CGCCCCABGMSWWGTACCA	150	De Menezes et al., (2015)	95 °C for 3 min then 40 cycles of: 95 °C for 5 sec, 58 °C for 30 sec, followed by a melt curve from 65 °C to 95 °C.

Templates for determining gene copy numbers in the qPCR reactions were cloned plasmids. PCR amplicons of functional genes were cloned using P-GEMT (Promega, Madison, USA) and sequenced using Big Dye Terminator chemistry by the Australian Genome Research Facility Perth, Western Australia. Sequence identities were confirmed by a Blastn search on the GenBank. Bacterial and archaeal 16S rRNA genes, fungal 18S rRNA genes, LMCO, *cbh*I and GH48 genes were isolated from DNA extracted from environmental samples. The standard curves generated in each reaction were linear over seven orders of magnitude (10^2–10^8 gene copies) with $R^2 > 0.98$. Amplification efficiencies ranged from 80.3–100.7% for bacterial 16S rRNA, 86.2–99.8% for archaeal 16S rRNA, 80.8–100.0% for fungal 18S rRNA, 71.6–83.4% for LMCO, 73.0–77.6% for *cbh*I and 99.0–100.0% for GH48.

2.4. Soil chemical and physical properties

Total C and N concentrations of soil and wheat residue were determined by dry combustion on finely-ground air-dried samples (CHNS\O Analyser 2400 Series II, PerkinElmer, Waltham, USA). Soil particle size distribution was analysed with a Laser Particle Size Analyser (Malvern Mastersizer 2000, Worcestershire, UK) following dispersion of 10 g soil in 10 mL 0.164 M $(NaPO_3)_6$ in 500 mL H_2O. Soil pH was quantified at the beginning and after 1 and 3 months of incubation with a pre-calibrated electrode (HI 1053, Hanna Instruments, Woonsocket, USA) and pH meter (Thermo Orion 720A+, Beverly, USA) after end-over-end shaking for 1 hour in a solution of 0.01 M $CaCl_2$ (1:5 soil-to-solution ratio), followed by centrifugation at 2500 rev min^{-1} for 5 min.

Microbial biomass C (MBC) and N (MBN) were determined following destructive sampling at 1 month and 3 months, by the chloroform fumigation extraction (Vance et al., 1987). After fumigation with chloroform and dark incubation (25 °C, 24 h), soils were extracted with K_2SO_4 (10 g soil: 40 mL of 0.5 M solution). Paired non-fumigated soil samples were similarly extracted. The organic C concentration within filtered extracts were analysed at 600 nm using a UV-Visible Spectrophotometer (Cary 50 Bio, Varian, Palo Alto, USA) following dichromate oxidation (Heanes, 1984) using sucrose standards for calibration. Oxidation was achieved by adding 0.7 mL $K_2Cr_2O_7$ (49.0 g L^{-1}) and 1.3 mL H_2SO_4 (98%) to 2 mL of each extract and digesting at 135 °C in a pre-heated sand bath for 30 min. MBC was calculated as the difference in C concentration between fumigated and non-fumigated samples, using a k_{EC} of 0.45 (Jenkinson et al., 2004). The C contained within non-fumigated samples was considered extractable organic C (EOC). Organic N within filtered extracts was analysed by alkaline persulfate oxidation (Cabrera and Beare, 1993). Briefly, 2.5 mL of the oxidising reagent (containing 50 g L^{-1} $K_2S_2O_8$, 30 g L^{-1} H_3BO_4 and 100 mL of 3.75 M NaOH) and 2.5 mL of each extractant were autoclaved (121 °C) for 30 min. The N concentration analysed by Flow Injection Analysis (FIA, QuikChem 8500 Series II, Lachat Instruments, Loveland, USA) using urea standards for digestion calibration. MBN (MBN) was calculated as the difference in N concentration between fumigated and non-fumigated samples, using a k_{EN} of 0.54 (Joergensen and Mueller, 1996). The N contained within non-fumigated samples was considered extractable organic N (EON). Inorganic N (NO_3^- and NH_4^+) within non-fumigated non-autoclaved extracts was also determined using FIA.

Mid-infrared spectra were acquired (8000-4000 cm^{-1}) on finely-ground soils following the 3-month incubation (Nicolet 6700: Thermo Fisher Scientific Inc., Waltham, USA). According to the method of Baldock et al. (2013), the spectra were used to estimate the size of the particulate (POC), humic (HOC), and resistant organic C (ROC) fractions. The estimated C fraction data were accepted where the error statistics associated with the predicted data were below the threshold limit set by the Hotelings Ratio (Baldock et al., 2013). The fraction estimates reflect the differences in the composition/quality of SOC between soils and treatments.

Table 3
Net C mineralisation at 1 week, 1 month and 3 months, cumulative C mineralisation derived from SOC ($CO_{2\ SOC}$) and residue ($CO_{2\ residue}$) in residue-amended (residue) and non-amended (nil) Hamilton, Wongan Hills, Kellerberrin and Konnongorring topsoils (0-10 cm) and Wongan Hills subsoil (WH subsoil) (10-20 cm) with different lime histories. Primed C is calculated as the difference in $CO_{2\ SOC}$ between residue- and non-amended treatments. Least significant difference (LSD) values (P=0.05) for any two means. Not significant (ns), *, ** and *** indicate P>0.05, P≤0.05, P≤0.01 and P≤0.001 for two- or three-way analyses of variance (location × lime × depth) of paired lime trial soils.

Location	pH	Net C mineralisation						Cumulative C mineralisation			Primed C
	($CaCl_2$)	(mg CO_2-C g^{-1} soil C)						(mg CO_2-C g^{-1} soil C)			(mg g^{-1} soil C)
		1 week		1 month		3 months		$CO_{2\ SOC}$		$CO_{2\ residue}$	
		nil	residue	nil	residue	nil	residue	nil	residue		
Hamilton	4.6	3.1	12.1	6.1	15.7	14.9	23.3	24.1	29.0	22.1	4.9
WH subsoil	4.2	5.4	82.3	12.1	72.7	26.7	251.6	44.2	78.4	328.5	34.2
Wongan Hills	5.0	4.9	45.9	15.0	42.0	23.2	41.3	43.1	47.4	81.8	4.3
Wongan Hills	5.9	3.9	45.6	11.5	46.0	27.2	59.9	42.6	59.9	91.6	17.3
Kellerberrin	5.0	3.5	48.1	7.6	31.6	15.0	32.8	26.0	34.5	78.1	8.4
Kellerberrin	5.8	3.3	52.0	8.0	38.0	16.9	34.1	28.3	37.4	86.7	9.1
Konnongorring	4.7	5.6	43.3	14.5	49.0	41.1	66.2	61.2	68.0	90.5	6.7
Konnongorring	5.3	2.9	63.5	9.0	56.0	19.0	39.8	30.8	42.4	116.9	11.6
LSD (any two means)	2.6		2.4			7.2		5.0		7.2	6.3
Significance level											
location		***		***		***		***		***	ns
lime		***		**		**		***		***	**
residue		***		***		***		***		-	-
location × lime		***		*		***		***		**	*
location × residue		***		***		*		ns		-	-
lime × residue		***		***		ns		**		-	-
location × lime × residue		***		*		**		ns		-	-

2.5. Statistical analyses

A three-way ANOVA was applied to paired limed and non-limed treatments of the three field trials (Konnongorring, Kellerberrin, Wongan Hills) to determine the effects of location, lime, and residue on C mineralisation at 1 week, 1 month and 3 months; soil pH, MBC, MBN, EOC, EON, NO_3^- and NH_4^+ concentrations at 1 month and 3 months; and $CO_{2\ SOC}$, $CO_{2\ residue}$, primed C and log-transformed gene abundances at 3 months. Differences between means were determined using least significant difference (LSD) test at P=0.05. Analyses were performed with GenStat 16th edition (VSN International, Hemel Hempstead, England).

Multivariate regression approaches (Primer-E PERMANOVA, Anderson et al., 2008) were used to explore the correlative relationships between soil parameters and cumulative CO_2 derived from residue ($CO_{2\ residue}$), SOC ($CO_{2\ SOC}$) and priming ($CO_{2\ primed}$). Firstly, the BEST routine was used to identify which combinations of soil chemical and biological parameters correlated most strongly with the amount of CO_2 derived from the individual sources ($CO_{2\ residue}$, $CO_{2\ SOC}$, $CO_{2\ primed}$). Data were divided into residue-amended and non-amended datasets, parameters normalised, and the BEST routine conducted against a Euclidean-based response matrix. Secondly, a distance-based linear model (DistLM: 9999 permutations) was developed to describe the relationship between measured soil parameters and cumulative C mineralisation (the sum of $CO_{2\ residue}$, $CO_{2\ SOC}$, $CO_{2\ primed}$) in residue-amended treatments. The model was based on a Euclidean-distance matrix for cumulative CO_2 and a step-wise selection procedure with Akaike Information Criterion (AIC). Data variables were normalised before use and a redundancy analysis (dbRDA) was used to visualise the model.

3. Results

3.1. Carbon fluxes

Primed C ranged from 4.9 to 34.3 mg C g^{-1} soil C (Table 3). There was a significant and positive effect of lime addition on primed C (P< 0.01). Cumulative CO_2 released over the 3-month incubation ranged from 51.1 to 407.0 mg C g^{-1} soil C for soils with crop residue added, and from 24.2 to 61.2 mg C g^{-1} soil C for soils without additional crop residue (Table 3). The no crop residue treatment was included in order to calculate primed C. As anticipated, crop residue addition significantly increased net CO_2 fluxes (Table 3). This effect is well established. Further, the no crop residue scenario is not relevant to soil management; as such, this treatment is not discussed further. The amount of CO_2 originating from SOC ranged from 29.0 to 78.5 mg C g^{-1} soil C. There was a significant effect of lime, location and an interaction between the two (Table 3); a site effect reflecting the different initial soil properties (Table 1). The amount of CO_2 originating from residue ranged from 22.1 to 328.5 mg C g^{-1} soil C and ANOVA demonstrated a significant effect of lime, location and an interaction between the two (Table 3); again a site effect reflecting the different initial soil properties is apparent (Table 1).

3.2. Soil microbiology

Abundance of RNA genes indicated that bacteria dominated the soil microbiota (1-22 × 10^9) (Table 4). Fungi and archaea were less abundant (0.5-13 × 10^8 and 0.1-13 × 10^7, respectively). ANOVA demonstrated that archaeal abundance was significantly affected by lime, location, and an interaction between the two (Table 4). Addition of crop residue significantly increased the abundance of the entire soil microbiota studied, with the exception of bacteria (Table 4). The C-cycling functional gene glucoside hydrolase (GH48) was most abundant (1-13 × 10^6), followed by the cellobiohydrolases (*cbh*I; 2-42 × 10^5) and then the laccase-like multicopper oxidases (LMCO; 3-497 × 10^4). However, it was the LMCO gene abundances that were significantly affected by lime, as well as location (Table 4). Both archaea and LMCO were more abundant in soils where lime had been applied.

3.3. Soil chemistry

3.3.1. Nitrogen

Inorganic nitrogen was dominated by nitrate, which ranged from 4.0 to 135.7 μg N g^{-1} soil at 3 months (Table 5). ANOVA showed that inorganic N was significantly affected by lime, location and an interaction between the two (Table 5); the site effect of initial soil properties (Table 1) as is also observed in the carbon fluxes (Table 3). More N was present in organic form than inorganic form, with extractable organic N ranging from 26.4 to 196.0 μg N g^{-1} soil and microbial biomass N

Table 4
Abundance of archaeal, bacterial and fungal rRNA genes and C-cycle functional genes cbh1, GH48 and LMCO at 3 months in residue-amended (residue) and non-amended (nil) Hamilton, Wongan Hills, Kellerberrin and Konnongorring topsoils (0-10 cm) and Wongan Hills subsoil (WH subsoil) (10-20 cm) with different lime histories. For each gene, means with the same letter are not significantly different at $P \leq 0.05$. Not significant (ns), *, ** and *** indicate $P > 0.05$, $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ for three-way analyses of variance (location × lime × depth) of paired lime trial soils.

Location	pH	rRNA gene abundance						Functional gene abundance					
	($CaCl_2$)	(gene copies g^{-1} soil)						(gene copies g^{-1} soil)					
		Archaeal (× 10^7)		Bacterial (× 10^9)		Fungal (× 10^8)		cbh1 (× 10^5)		GH48 (× 10^6)		LMCO (× 10^4)	
		nil	residue	nil	residue	nil	Residue	Nil	residue	nil	residue	nil	residue
Hamilton	4.6	6.28_{abcd}	12.63_{abcd}	5.93_{abc}	11.06_{abc}	1.06_{ab}	2.31_{abc}	4.26_{a}	7.65_{abcd}	2.99_{b}	5.27_{bc}	496.89_{ab}	686.78_{ab}
WH subsoil	4.2	0.12_{a}	0.77_{a}	1.42_{a}	3.38_{abc}	0.46_{a}	7.10_{cdef}	2.33_{a}	17.68_{bcd}	0.96_{a}	3.14_{bc}	3.36_{a}	15.07_{ab}
Wongan Hills	5.0	1.62_{bcde}	4.34_{de}	4.50_{abc}	5.66_{abc}	3.17_{cdef}	11.96_{ef}	8.54_{abcd}	27.29_{d}	3.86_{bc}	4.77_{bcd}	6.61_{ab}	24.81_{ab}
Wongan Hills	5.9	3.76_{de}	9.92_{e}	5.24_{abc}	11.07_{bc}	4.11_{cdef}	13.04_{f}	14.40_{abcd}	22.59_{cd}	4.42_{bcd}	6.42_{cde}	19.62_{ab}	70.60_{b}
Kellerberrin	5.0	2.18_{cde}	4.85_{de}	10.97_{bc}	15.21_{bc}	3.76_{cdef}	12.38_{ef}	10.28_{a}	41.68_{d}	3.85_{bc}	15.23_{e}	5.05_{a}	11.74_{ab}
Kellerberrin	5.8	4.17_{de}	7.73_{e}	16.68_{bc}	22.42_{c}	5.73_{def}	11.07_{f}	10.32_{a}	41.15_{d}	5.62_{bcde}	11.48_{de}	8.84_{ab}	23.55_{ab}
Konnongorring	4.7	0.19_{ab}	0.46_{abc}	2.50_{ab}	4.84_{abc}	3.87_{cdef}	7.96_{def}	15.89_{bcd}	32.65_{d}	4.88_{bcd}	2.81_{bc}	2.95_{a}	4.70_{a}
Konnongorring	5.3	1.46_{bcde}	3.45_{cde}	3.18_{abc}	11.73_{bc}	3.29_{cde}	2.39_{cde}	3.06_{abcd}	11.06_{abcd}	2.69_{bc}	5.62_{bcde}	7.41_{ab}	12.81_{ab}
Significance level													
location		***		*		ns		ns		***		***	
lime		***		ns		ns		ns		ns		***	
residue		***		ns		*		**		***		***	
location × lime		***		ns		ns		ns		ns		Ns	
location × residue		ns		ns		ns		ns		*		Ns	
lime × residue		ns		ns		ns		ns		ns		Ns	
location × lime × residue		ns		ns		ns		ns		*		ns	

Table 5
Microbial biomass N, extractable organic N, nitrate and ammonium concentrations at 1 month and 3 months in residue-amended (residue) and non-amended (nil) Hamilton, Wongan Hills, Kellerberrin and Konnongorring topsoils (0-10 cm) and Wongan Hills subsoil (WH subsoil) (10-20 cm) with different lime histories. Least significant difference (LSD) values (P=0.05) for any two means. Not significant (ns), *, ** and *** indicate $P > 0.05$, $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ for three-way analyses of variance (location × lime × depth) of paired lime trial soils.

Location	pH	Microbial biomass N				Extractable organic N				Nitrate				Ammonium			
	($CaCl_2$)	(μg N g^{-1} soil)				(μg N g^{-1} soil)				(μg N g^{-1} soil)				(μg N g^{-1} soil)			
		1 month		3 months		1 month		3 months		1 month		3 months		1 month		3 months	
		nil	residue	nil	residue	nil	residue	nil	residue	nil	residue	nil	residue	nil	residue	nil	residue
Hamilton	4.6	69.4	78.5	50.6	57.0	156.7	113.3	196.0	184.2	101.8	73.2	135.7	122.6	2.0	1.6	2.7	1.8
WH subsoil	4.2	6.6	13.4	5.5	14.5	24.5	20.7	33.2	26.4	8.6	3.6	7.4	4.0	0.2	0.3	6.3	3.8
Wongan Hills	5.0	21.2	39.2	11.2	24.2	63.3	29.9	93.5	64.1	38.1	9.9	57.2	36.3	0.3	0.7	0.7	0.6
Wongan Hills	5.9	28.2	44.0	14.3	33.1	60.6	22.0	87.5	61.1	33.6	2.7	53.6	31.9	0.2	0.9	0.1	0.1
Kellerberrin	5.0	41.8	44.0	22.6	27.2	95.9	70.1	119.0	89.0	75.2	42.9	81.3	56.6	0.2	0.4	0.6	0.2
Kellerberrin	5.8	40.7	40.4	3.9	13.7	123.8	89.0	146.5	123.6	90.8	58.2	96.7	74.0	0.3	0.9	0.3	0.6
Konnongorring	4.7	23.9	31.8	17.1	20.6	58.0	25.2	93.0	67.6	31.4	6.7	46.3	33.5	2.9	0.8	12.7	7.1
Konnongorring	5.3	25.6	28.9	3.8	14.9	94.2	59.9	109.5	86.2	66.6	34.7	79.2	54.0	0.3	1.2	1.2	0.2
LSD (any two means)		8.6		10.9		7.0		6.4		5.9		6.5		1.2		1.5	
Significance level																	
location		***		ns		***		***		***		***		**		***	
lime		ns		**		***		***		***		***		ns		***	
residue		***		***		***		***		***		***		ns		***	
location × lime		ns		***		***		***		***		***		*		***	
location × residue		**		ns		ns		ns		ns		ns		ns		***	
lime × residue		ns		ns		ns		ns		ns		ns		**		**	
location × lime × residue		ns		ns				ns		ns		ns		ns		**	

ranging from 3.8 to 57.0 μg N g^{-1} soil (Table 5). Organic N was also significantly affected by lime, location and an interaction between the two. The exception was microbial biomass N, where ANOVA did not find a significant effect of location. Lime addition generally increased inorganic N and extractable organic N, while decreasing microbial biomass N, with the exception of the Wongan Hills location (Table 5).

3.3.2. Carbon

Microbial biomass C ranged from 25 to 320 μg C g^{-1} soil at the conclusion of the experiment (Table 6). Extractable organic C ranged from 35 to 90 μg C g^{-1} soil. ANOVA results suggest that MBC was not affected by lime addition (Table 6). The effects of location (site effects) and crop residue addition on soil carbon are well established and as such these results are not discussed further.

3.4. Correlation analyses

The BEST multivariate regression analyses show significant correlations between the amounts of CO_2 originating from the three processes (Table 7). The amount of primed C correlated significantly and positively with the abundance of archaeal rRNA genes and SOC functional gene laccase (Rho 0.46, P<0.01). Decomposition of extant SOC was positively correlated with pH and N (Rho 0.65, P<0.001). The addition of crop residue both increased CO_2-C, as has been widely reported elsewhere. Decomposition of the added crop residue correlated with pH and inorganic N, albeit less strongly than for SOC (Rho 0.43, P< 0.05).

The distance-based linear model visualised in Fig. 1 highlights the soil biological and chemical properties that correlate with the total CO_2 from the three processes of priming, SOC decomposition and

Table 6
Microbial biomass carbon and extractable organic carbon at 1 month and 3 months of incubation. Values are the average of three replicates. Results from ANOVAs are in italics below. ns, *,** and *** represent $P>0.05$, $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Location	pH	Microbial biomass carbon (μg C g^{-1} soil)				Extractable organic carbon (μg C g^{-1} soil)			
		1 month		3 months		1 month		3 months	
		- residue	+ residue	- residue	+ residue	- residue	+ residue	- residue	+ residue
Hamilton	4.6	847	501	274	320	312	184	60	63
WH subsoil	4.2	346	405	25	77	128	149	35	49
Wongan Hills	5.0	400	361	85	144	136	133	54	53
Wongan Hills	5.9	372	387	78	169	137	142	46	90
Kellerberrin	5.0	319	336	76	157	118	124	43	48
Kellerberrin	5.8	277	399	79	176	102	147	46	54
Konnongorring	4.7	321	334	66	127	118	123	44	48
Konnongorring	5.3	334	370	31	141	123	136	42	53
Paired lime trial soils LSD P=0.05				*24*				7	
Significance level									
location			*ns*		***		*ns*		**
lime			*ns*		*ns*		*ns*		*ns*
residue			*ns*		***		*ns*		***
location x lime			*ns*		*ns*		*ns*		*ns*
location x residue		*ns*		*ns*		*ns*		*ns*	
lime x residue			*ns*		**		*ns*		*
location x lime x residue		*ns*		*ns*		*ns*		*ns*	

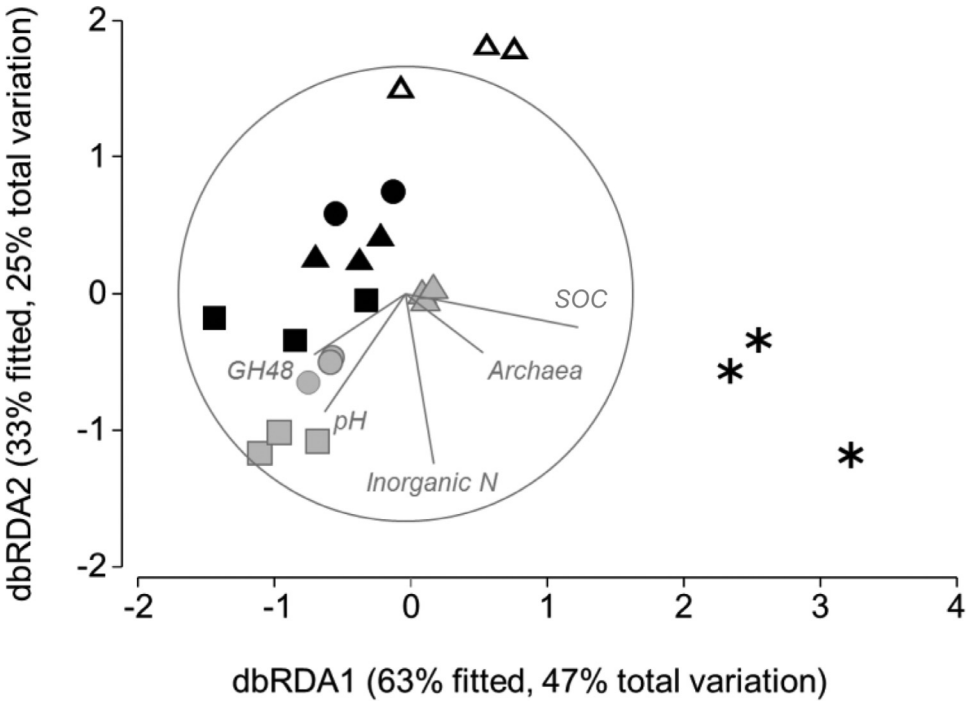

Fig. 1. Distance based redundancy analysis (dbRDA) derived from a Euclidean-distance linear model (step-wise selection with Akaike Information Criterion) describing the relationship between measured soil parameters [inorganic N, soil pH, soil organic C (SOC) and the abundance of archaea and GH48] and the overall C mineralisation response [total CO_2 derived from SOC ($CO_{2\ SOC}$) + residue ($CO_{2\ residue}$) + primed C] in residue-amended Hamilton (✱), Wongan Hills (⬧), Kellerberrin (■), Konnongorring (●) topsoils (0-10 cm) and Wongan Hills subsoil (10-20 cm) (△) with either limed (grey) or non-limed (black) histories.

crop residue decomposition combined. Archaea and glucoside hydrolase (GH48) gene abundances appear as important properties, along with pH and inorganic N (Fig. 1). In combination with the initial SOC content, these two biological and two chemical properties explained 72% of the variation in total CO_2 (Fig. 1). Limed (grey) and unlimed (black) soils clearly separate in this analysis and the 6 topsoils from the neighbouring long-term farm field trials cluster distinctly.

4. Discussion

The central purpose of this study was to explore the effect of lime on carbon cycling in agricultural soils, by quantifying CO_2 emissions from three processes; mineralisation of extant SOC, mineralisation of added crop residue and SOC priming. Measurement of the abundance of the genes involved in lignin decomposition (LMCO: laccase-like copper oxidase) and cellulose decomposition (*cbh*I: cellobiohydrolase, GH48: glycoside hydrolase) is novel in this application and provide valuable insights into these SOC decomposition processes.

4.1. SOC priming and the influence of soil microbiology

We hypothesised that priming would be greater in limed than non-limed soil, due to a more favourable pH environment for microbial activity. The first part of this hypothesis is strongly supported by the results in Table 3. Lime addition had a significant positive effect on priming. The results in Table 4 lend support to the second part of the hypothesis, that this increase in priming is related to a more favourable environment for microbes. Archaea and laccase-like multi copper oxidases were significantly more abundant in limed soils. Multivariate analyses indicated that priming correlated most strongly with the abundance of archaeal

Table 7
Multivariate correlation analysis (BEST) demonstrating the combination of soil chemical and biological parameters that correlate most strongly with the cumulative soil CO_2-C, residue-derived CO_2-C and primed CO_2-C within the residue-amended (n=24) and unamended (n=24) soils over the 3 month incubation, where EON = extractable organic N, LMCO = laccase functional gene abundance, SOC = soil organic C

Treatment	Rho	*P*	Parameters
Residue-amended soils			
soil	0.65	<0.001	Inorganic N, pH, EON, SOC
residue	0.43	<0.05	Inorganic N, pH
primed	0.46	<0.01	Archaea, LMCO, SOC
Non-amended			
soil	0.71	<0.001	SOC, inorganic N

genes, lignin decomposing (LMCO) genes, and total SOC (Table 7). Together, these results suggest that biological indicators may be more useful in understanding carbon cycling in soils than chemical indicators alone.

Soil organic carbon exists as a complex mixture of biomolecules, with a substantial component of lignin and alkyl structures (Baldock and Skjemstad, 2000). An increased abundance of lignin-decomposing genes thus suggests a greater capacity to mineralise SOC. Although the ability to decompose lignin is carried by fungi, who can generally be categorised as K-strategists which utilise C from more recalcitrant sources (Otten et al., 2001), bacterial laccases may also play an important role in the decomposition of recalcitrant compounds such as lignin (Ausec et al., 2011). Feng et al. (2019) reported that in upland red soils of China (Ferrosols derived from quaternary red clay earth) there was no significant effect of pH on LMCO gene abundances across their field sites. They do report a significant increase in LMCO gene abundance where straw was incorporated into the soil, indicating a relationship between incorporation of lignin-containing plant residues and the abundance of laccase-like genes. They report that nitrogen availability influenced lignin accumulation and that total N had a strong (69%) correlation with variation in laccase enzyme activity. This interaction between SOC-decomposing genes and N may help to explain the key role of archaea.

Archaea are generally reported to be present in smaller numbers in soil than bacteria; however, the overall effect of pH on archaeal abundance remains unclear. For example, Tripathi et al. (2013) have reported a slight negative correlation between pH and archaeal abundance whereas Bengtson et al. (2012) report an inconsistent trend. In line with the current work, it has been shown previously that in West Australian soils archaea tend to be less numerous than their bacterial counterparts (Gleeson et al., 2016). Although lower in abundance, archaea are reported to play a significant role in soil ammonia oxidation, a crucial step in the N cycle (Leininger et al., 2006), which makes N more available, enhancing conditions for microbial mineralisation. The increase in archaeal abundance in limed soils observed in the current work could be functionally linked to the higher inorganic N concentrations in these soils. Higher soil N favours the growth of r-strategists when the available crop residue has a relatively high C:N ratio (Chen et al., 2014), as was the case in the wheat residue applied in this study (C:N = 42). In this study, limed soils appear to have provided more favourable conditions for SOC priming, with more lignin-decomposing organisms (K-strategists) and better conditions for the growth of r-strategists (fresh residue, greater soil N) than their non-limed counterparts. The results of this study support the idea that priming results from the combined activity of K- and r-strategists, whose contribution to SOC mineralisation alters with the changing availability of C and N (Chen et al., 2014; Fontaine et al., 2004). Together, these results suggest that biological indicators may be more useful in understanding carbon cycling in soils than chemical indicators alone. Although not measured in the current study, consideration should also be given to the dynamics of both the ammonia oxidisers (bacterial and archaeal) and nitrite oxidisers as well as the recently discovered comammox bacteria (i.e. specific species of *Nitrospira*) that are capable of complete ammonia oxidation to nitrate (Pjevac et al., 2017) and their relationship to soil pH. In soils similar to those of the current study it has been reported that AOB are more numerous than AOA from a gene abundance perspective (Jenkins et al., 2016); however, how this manifests in an activity measure in terms of nitrification rates remains unclear.

4.2. Liming and the influence of pH on soil microbiology

We hypothesised that the increase in pH due to liming would enhance mineralisation of both extant SOC and crop residue. The results in Tables 3 and 7 lend conditional support to this hypothesis; lime and soil pH were influential but not exclusive factors impacting mineralisation. There was a significant interaction between lime and location. Mineralisation of added residue was greater at all limed sites, while mineralisation of extant SOC was greater at 2 of the 3 paired lime field trials (Table 3). The process of mineralisation of extant SOC was strongly and significantly correlated with the combination of pH, organic and inorganic N and initial SOC (Rho 0.65 $P<0.001$ Table 7). The process of mineralisation of crop residue was less strongly, but still significantly, correlated with pH and inorganic N (Rho 0.43 $P<0.05$ Table 7).

Our third hypothesis, that mineralisation would be positively correlated with the abundance of key SOM functional genes, is partially supported by the distance-based linear model visualised by the redundancy analysis in Fig. 1. SOC functional gene GH48 is positively related to total CO_2, the combined emissions from the three processes of priming, SOC mineralisation and crop residue mineralisation (Fig. 1). However, the relationship between GH48 and mineralisation is only significant in combination with archaeal abundance, pH, inorganic N and SOC. On balance, these results suggest that liming to increase soil pH may create a more favourable environment for microbes to mineralise new and existing organic material. However, these results also strongly underline the importance of considering soil biological properties as well as soil chemical properties in any attempts to manipulate soil carbon dynamics.

These findings add more nuanced understanding to the effect of lime on soil carbon dynamics. A comprehensive review of the net effect of lime on soil carbon stocks (Paradelo et al., 2015) found that in most cases, lime increased carbon stocks, likely due to increased plant growth and thus carbon inputs. In cases where carbon stocks decreased with liming, this was attributed to increased mineralisation (Paradelo et al., 2015). Studies focusing on long-term lime field trials have found mixed results. For example, Bertrand et al. (2007) found that soil carbon mineralisation was not significantly affected by lime application, while Aye et al. (2016) measured higher rates of mineralisation in limed soils. Soil pH has been found to correlate positively with microbial biomass C (Pietri and Brookes, 2008) and microbial respiration (Aye et al., 2017). Carbon stocks in soils from the current study were found to have decreased or remained unchanged with lime application (Wang et al., 2015). This study found that microbial biomass C was consistently higher in the limed than unlimed soils (Table 6), however, an increase in soil pH did not always result in an increase in mineralization of SOC. Possibly, the C remaining in these historically (>10 years) limed soils was more chemically recalcitrant, or physically protected via formation of Ca-organic complexes (Bronick and Lal, 2005). Studies wherein lime is applied at the beginning of the incubation and isotope labelling enable partitioning of mineralisation have also reached mixed conclusions about the impact of lime on soil carbon dynamics. Wachendorf (2015) found that lime addition decreased the mineralisation of both SOC and poplar residue in a 51-d study in a silty sand Podzol (Δ pH 0.1 units, short rotation coppice system). Lime addition increased mineralisation of SOC in a Chromic Luvisol (20% clay, Δ pH 1.8 units) (Ahmad et al., 2014) and two light clay soils (Andisol 11% C, Δ pH 1.3 units, Ultisol 0.25% C, Δ pH 2.3 units) (Dumale et al., 2011). The results of the current study provide direct evidence that priming can be increased by lime application, and conditional support for the theory

that microbial mineralisation of SOC and crop residue is enhanced by lime creating a more favourable environment for microbes.

4.3. Nitrogen as a moderating chemical factor

Nitrogen appears to be a key factor in understanding the effect of lime on soil carbon dynamics. The increase in pH caused by lime addition increases the availability of N (Weil and Brady, 2017). Our results indicated that inorganic N correlated positively with the total CO_2 emission from the combination of priming, SOC mineralisation and crop residue mineralisation (Fig. 1). Furthermore, inorganic N was positively correlated with mineralisation of SOC and crop residue, in combination with pH (and initial SOC content and organic N, for SOC mineralisation) (Table 7). Nitrogen is limiting to microbial growth in most of the world's soils and soil microorganisms appear to be extremely efficient at capturing any available N (Kuzyakov and Xu, 2013). Where an increase in pH has increased N availability in this study, it would then appear that microbes are in a soil environment more conducive to their ability to mineralise both extant SOC and new crop residues. This finding aligns with 'stoichiometric decomposition theory', which suggests that nutrient availability will enhance mineralisation (Chen et al., 2014). Stoichiometry of C, N and P has been highlighted as the critical factor affecting microbial functional resistance to environmental stressors in a recent study exploring abundance of cbh1 and 15 other genes associated with C, N and P cycling across 54 alfalfa-growing soils in China (Luo et al., 2010).

However, N availability did not appear to impact SOC priming (Table 7) and, in the absence of fresh organic matter to stimulate priming, more N was associated with less SOC mineralisation. Indeed, mineralisation of SOC was inversely correlated with inorganic N where crop residue was not added; the strongest correlation found (Rho 0.71 $p<0.001$ Table 7). Without an additional carbon source, it would appear that a low-N soil environment promoted increased residue mineralisation, as microbes attempt to source N, as proposed in the 'microbial nitrogen mining theory' (Craine et al., 2007). Where N inhibits laccase activity, less lignin in SOC can be mineralised. Feng et al. (2019) suggest that it is important to consider N as a moderating chemical factor in efforts to increase soil carbon sequestration, as N explained 69% of the variation in laccase enzymes in their study of soil carbon dynamics in 3 long-term straw incorporation field trials. These results emphasise the importance of considering the different processes occurring within the soil carbon cycle. SOC is widely accepted to exist as a complex continuum of progressively decomposing organic compounds (Lehmann and Kleber, 2015). Thus, it logically follows that mineralisation of this complex mixture will enlist a broad range of microbes, including both r-strategists and K strategists, with differing pH and nutrient optima.

4.4. Limitations, implications of this work and remaining unknowns

This study utilised soils from three long-term liming field trials, providing a useful opportunity to capture the cumulative effects of changes to soil function resulting from >10 years post amelioration management. While this work covered a pH range from 4.2 – 5.9, it would be valuable to validate these findings across a wider range of soil types and identify if pH thresholds exist in tipping the functional response. To gain greater knowledge of long-term cumulative effects on functional responses, greater collaboration with agricultural producers could help overcome limitations in the existence of long-term research trial sites. Where detailed records of lime application and other land management practices have been kept, this approach could enhance our understanding of soil carbon dynamics and pH across the diversity of soils that exist.

In addition to well established plant growth benefits (Gazey et al., 2014; Tang et al., 2003), this research suggests that lime addition enhances conditions for microbial mineralisation of SOC and crop residue. Furthermore, global supplies of lime are finite and transport costs already make lime application prohibitive for farmers in many parts of the world. Therefore, while lime will continue to play a role in ensuring food security, other land management options to enhance the capability of acid soils need to be explored. Some crop residues have been shown to increase soil pH (Wang et al., 2017). However, carbon inputs from crop residue alone will not be sufficient under current agricultural practices to reverse the acidification and carbon depletion of agricultural soils, where cations continue to be removed as food and fibre. The role of organic materials in increasing soil pH requires further research attention.

5. Conclusions

Soil acidity remains an ongoing threat to the security of our agricultural production systems, with important implications for nutrient-use efficiency and carbon cycling. The long-term trials used in this study demonstrate that amelioration of soil acidity through lime does not necessarily lead to increased SOC stocks, despite improvements in cropping yields and biomass production. Our research shows that pH impacted upon three distinct processes within the soil carbon cycle; mineralisation of existing soil organic carbon, mineralisation of crop residue and SOC priming. The largest influence is indirect, via the effect of pH on the soil microbiota. Archaea and SOC-decomposing gene LMCO positively correlated most strongly with priming. The net CO_2 response was positively correlated with archaea, glucoside hydrolase, pH, N and initial SOC. While the use of lime to increase food security will continue, new whole system approaches will be required to concurrently sequester carbon in acid soils. The more nuanced understanding of soil carbon dynamics resultant from this study, combining long-term field trial soils with isotopically-labelled crop residues, may be applied to inform this grand challenge.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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