FINAL REPORT

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Part 1 - Summary Details

CRDC ID: UNE1601

Project Title: Soil System Research – physical, chemical and biological processes for plant growth and nutrient cycling down the whole soil profile

Project Start Date: 01/07/2015 **Project Completion Date:** 31/07/2019

Research Program: 1 Farmers

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Date submitted: 1/10/19

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report)

Background

1. Outline the background to the project.

The soils of cotton farming systems have received considerable attention and we know much about their function and management. Most of this work to date has focused on the near-surface (0-30cm) soil layers, but there is growing evidence that the processes and mechanisms operating in deeper soil layers are of considerable significance. The work proposed will address a series of key knowledge gaps relating to soils under cotton farming systems, capitalizing on existing research and collaborations between UNE/CRDC/DPI/ACRI. UNE proposed to contribute PhD studentships to the project. In combination with these, our proposal sought funding to support a post-doctoral position, analytical and operating costs to carry out three discrete but interlinked investigations of soil systems within the whole soil profile accessed by cotton.

These three areas of research were identified as;

i) *Mechanisms of whole-profile C and N cycling*

Research in the cotton industry has demonstrated the potential of some systems (e.g. cotton-maize) to increase C and N contents in the deep soil profile. This has considerable significance for C and N cycling and nutrient supply for cotton production. This component of the project will provide a comprehensive understanding of the quantities and mechanisms of C and N placement, mineralisation and turnover through the whole soil profile under cotton/maize and comparable cotton rotations, to enable these cycles to be more fully predicted and managed. Understanding the mechanisms of C and N turnover through the soil profile has the additional benefit of quantifying the potential contribution to (N_2O), and mitigation of (C capture), greenhouse gas emissions, which are now key national and international priorities offering the potential for participation in emerging carbon markets.

ii) *Microbial processes in the soil profile*

Soil microbes are a key component of soil organic matter formation, stabilisation and destabilisation but we currently know little regarding their function and influence in the deeper soil layers. Microbial community characteristics can be traced through the soil system using a series of analytical techniques. For example, the nature and function of microbial populations can be examined using their characteristic array of phospholipid fatty acids (PLFA). Studying the PLFA signatures under cotton rotations will identify the microbes involved in SOM processes. More specific information relating to activity and functional attributes of soil microbial populations can be explored using enzyme analysis. Together these analyses will allow for a comprehensive yet detailed examination of the role of microbes in the below-ground C and N cycles under cotton.

iii) *Below ground agronomy and constraints to plant growth*

Poor root development and limited rooting depth impact cotton plant viability and productivity. Although rooting depths of less than 45 cm have been observed in some cotton soils, the causes of this poor development remain unknown. Potential limitations to subsurface plant activity can be physical (soil structure and compaction, water-availability), chemical (sodicity, salinity, subsurface nutrient limitation) and biological (pathogens). Studying the physical, chemical and biological nature of the sub-surface soils, as well as potential interactions between these characteristics, will enable development of management strategies to overcome constraints to below-ground plant development.

The original team proposed to deliver this proposal underwent several changes in both the early and later life of the project. The first was the departure from UNE of Dr Nellie Hobley and Mr Eckhard Ferber. This left the project short of its Postdoctoral appointment and one PhD candidate, investigating the below ground agronomy, respectively. The Postdoctoral position was filled with the appointment of Dr Yui Osanai, who has excelled in her delivery to this project, but did require a negotiation of milestones against time and an extension to the original project timeframe. UNE were unable to recruit and replace Eckhard's PhD candidature within a realistic time frame

and so aspects of the milestones associated with this section of the project were taken on by Dr Oliver Knox in conjunction with work he was doing no UE1403 and CRDC/CRC SI UNE1603.
Katherine Polain completed the PhD appointments to this project and she has proven herself to be a highly competent and diligent soil microbiologist over the course of the project. Her PhD is nearing completion as we write this report. Katherine was affected more than most of us with the sudden and horrendous circumstances around the death of her supervisor and our friend and colleague, Prof Lily Pereg.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

General overview

UNE 1601 commenced in July of 2015 and started sampling the ACRI long term rotation trial, which is now being run by Dr Guna Nachimuthu in October 2015. Sampling pre-sowing, in crop and post-harvest was been undertaken in every season and phase of the rotations since commencement and was completed in late 2017 providing three seasons and one complete rotational cycle to analyse.
Initial analysis of the soil biological, physical and chemical soil states at each point of sampling and to a depth of 1 m was completed and interpretation and reporting is now well advanced with only a few milestones still awaiting analysis and reporting.
Extension activities have been undertaken and a couple of peer reviewed publications printed with others currently under review. Members of the team have presented at field days and made contributions to a several Spotlight and Cottongrower articles. The work has also been disseminated at conferences with Dr Osanai presenting an overview of the Carbon work at the AASCS meeting in Canberra in 2017 and microbiological work, being undertaken by Katherine Polain, presented at the ASSS meeting in Canberra in November of 2018. Both are presenting work from this project at the Soil Organic Matter Conference in October of 2019. This will be after the Association of Australian Cotton Scientists meeting in Armidale, which Oliver Katherine and Yui are all heavily involved in the development and delivery of.

Activity against specific milestones

1. Mechanisms of whole-profile carbon capture under cotton systems

1.1. Milestone completed. Dr Yui Osanai appointed.

1.2. Milestone completed. Field sampling and soil analyses completed.

1.3. C and N characterisation within the soil profile has been completed. We found that soil organic C fluctuated substantially during the two year sampling period, reflecting field operations. Despite this, inclusion of maize in crop rotation increased soil organic C in the deeper profile overtime, but only under minimum tillage with wheat rotation. No substantial changes have been observed in systems without maize or under maximum tillage.

1.4. Associated changes in $\delta 13C$ signature seem to suggest that the increase in soil organic C in the deeper profile is indeed maize-derived C. Soil fractionation analyses were conducted to assess the potential mechanism of SOC change under maize. If the change in SOC is associated with change in particulate organic C fraction, root biomass increase is the most likely mechanism. We found that the increase in subsoil SOC was strongly associated with changes in mineral-associated organic C, rather than particulate organic C, suggesting that the increase was not simply due to the increased root biomass.

1.5-1.6. Further in-depth analyses suggest that changes in subsoil SOC was most likely driven by the movement of C from the topsoil in the form of dissolved organic C. Because of the dependence of subsoil SOC dynamics on topsoil SOC dynamics, any management or environmental changes that affect topsoil SOC will ultimately impact subsoil SOC. This explains the inconsistent patterns observed in this field trial over the last few years, and therefore the duration of this study is too short to conclusively address the potential of maize rotation to offer longer term improvements in soil C. We are currently preparing two manuscripts on SOC dynamics. Soil nutrient analyses were also completed, showing strong temporal fluctuations, reflecting fertiliser application, plant uptake and mineralisation. We are hoping to prepare an additional manuscript focusing on N dynamics.

2. Microbial processes in the soil profile

2.1. Katherine returned from maternity in February of 2018 and completed her field work and the majority of associated experiments (respiration, nitrogen mineralisation and molecular). Her initial ^{18}O phosphate work has been published as has a paper on the decision not to use lubricants in our soil coring. Further papers on soil microbial activity and population diversity are well advanced in their status of preparation.

2.2. Microbial activity signatures have been capture by examining respiration and substrate responses of the soil. These have shown a divergence between the cotton-maize and cotton-cotton soils, however, the changes

are often limited to in-crop analysis, implying that the crops ability to drive long term changes to the microbiology of the soil, even under these divergent systems, is limited. Metagenonmic analysis of the bacteria, archaea and fungi responsible for these respiratory processes is complete and the work is being prepared for publication and further dissemination.

2.3. Katherine's work on microbial nitrogen cycling experiment used a quantitative gene analysis technique (QPCR) to analyse the potential abundance of various genes involved in the nitrogen cycle. Lab incubation assays have also been completed. It is envisaged that this data will add weight to the functional assays into mineralisation of N and add to the growing body of work on N management in cotton.

2.4 Thesis completion is expected in late 2019 early 2020. Katherine has moved to an indefinite period of suspension of her studies, but a plan for completion has been put in place and we will continue to assist Katherine in any way we can.

3. Below ground agronomy and constraints to plant growth identified

3.1. Oliver took on most of the responsibilities of the original appointment for this part of the project from 2016.

3.2. The project has added to the evidence of the link between traffic and compaction, which has remained evident throughout the sampling periods. Initial analysis showed a good relationship between bulk density and penetrometry resistance, which was independent of soil moisture that would normally affect rheological properties of the soil. A breakdown in this relationship occurred in some of the later analysis, but was associated blockages and breakages in coring tubes during sampling.

3.3. In collaboration with Brendan Griffiths, Jamie Barwick and Tom Dowling and John Burrows of Goanna Telemetry and in conjunction with work undertaken in UNe1403 and CRDC/CRC SI UNE1703 we analysed capacitance probe data from across the cotton industry for 2015/16, 2016/17 and 2017/18 seasons. Analysis of the data have identified that ~25% of all fields showed subsoil constraint (i.e. no root activity at peak vegetative growth below 60 cm). The data did not give a correlation between constrained and unconstrained fields and NDVI satellite imagery due to cotton's compensatory measures in leaf area and canopy development A more detailed report against this milestone has been made for UNE1403.

3.4. Management suggestions, beyond wide scale adoption of CTF and reduced traffic in general, are being considered as part of support to the USQ led work on the alleviation of sub soil constraints with CRDC and soil constraint for GRDC. UNE is delivering trials across NSW to evaluate deep ripping in combination with a number of chemical and organic amendments. Much of the work has been delayed due to the drought and mechanical issues, which have now been rectified.

4. Annual reporting

4.1. Completed as required

4.2 This report constitutes the projects final reporting requirements.

5. Paper production, industry engagement and conference attendance

Conferences

O. Knox (2015) The potential for water to cause compaction, oral presentation at the 2015 Cotton Collective.

K. Polain et al. (2016) Sub-soil microbial activity under rotational cotton crops in Australia, a poster presentation at the European Geologsciences Union 2016 conference, Vienna (presented by L. Pereg).

K. Polain et al. (2016) $\delta^{18}O_P$ as a marker for depth resolved microbial turnover of phosphorus in native and cropped Australian vertisols, a poster presentation at the International Phosphorus Workshop, Germany (presented by C. Guppy).

Y. Osanai et al. (2017) Mechanisms of whole-profile carbon cycling in cotton-based cropping system, oral presentation at 3rd Cotton Research Conference, Canberra.

O. Knox et al. (2018) Understanding soil systems research, oral presentation at Australian Cotton Conference, Gold Coast.

K. Polain (2018) Who's lurking in your soil, oral presentation (3MT) at Australian Cotton Conference, Gold Coast.

K. Polain et al. (2018) Digging deeper – microbial potential in sub-soil nutrient cycling, oral presentation presented at National Soils Conference, Canberra.

Y. Osanai et al. (2018) Mechanisms of whole-profile carbon cycling in cotton-based cropping system, a poster presented at National Soils Conference, Canberra.

O. Knox et al. (2019) Spacial cotton roots. Joint meeting between DOPIE (formerly OEH) and UNE. June.

Extension events

O. Knox (2015) Soil pit demonstration, at the 2016 Monsanto Cotton Grower of the Year field day, Mungindi.
O. Knox (2016) Fostering collaboration in the cotton industry. Presentation to the Agricultural Industry Advisory Council, February.
O. Knox (2016) Cotton production, issues and challenges. Quadrant Brazilian visitors, Gunnedah.
O. Knox (2018) Beliefs come first. Presentation to the Rockhampton beef group over concerns about GM.
O. Knox (2019) How healthy is your soil. Dalby consultants and growers meeting, February.
O. Knox (2019) Soil Health and the VSA. Soil pit demonstrations for consultants in Jondaryan and Moree, August.

Industry publications

Y. Osanai and O. Knox (2018) Reconsider the importance of soil organic carbon, Spotlight, Winter 2018.
K.Polain, D. Jones, G. Joice and O. Knox (2018) Do you lube before you probe. April May edition, Australian Cotton Grower.
K. Polain and O. Knox (2018) Who's lurking in your soil. December edition, Australian Cottongrower

Papers

Polain K, Guppy C, Knox O, Lisle L, Wilson B, Osanai Y, Siebers N (2018) Determination of Agricultural Impact on Soil Microbial Activity Using δ18OP HCl and Respiration Experiments. ACS Earth and Space Chemistry, 2, 683-691.
Knox OGG, Osanai Y, Polain K, Pereg L, Nachimuthu G, Wilson B, Waters W (2019) Lessons from extension activity related to cotton rotation impacts on soil—A scientist's perspective. Soil Use and Management, 35, 141-149.
Polain K, Joice G, Jones D, Pereg L, Nachimuthu G, Knox OGG (2019) Coring lubricants can increase soil microbial activity in Vertisols. Journal of Microbiological Methods, 165, 105695.As the projects analysis is completed more papers and industry outputs will be proposed.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Investigation 1: Mechanisms of whole-profile C and N dynamics

Recent studies in the Australian cotton industry have demonstrated the potential of some systems (e.g. cotton-maize) to increase carbon (C) in the deep soil profile (Hulugalle *et al.*, 2014), yet the processes and mechanisms that determine the rate of C cycling in deeper soil layers are relatively unknown.

Therefore, Investigation 1 aimed to elucidate mechanisms for whole-profile C and N dynamics by examining changes in soil organic C (SOC), soil organic fractions, C isotope signatures, soil N and inorganic N, microbial decomposition processes in topsoil vs. subsoil, and microbial responses to physical constraints (disturbance and compaction) in cotton systems under three historical cropping systems (Maximum tillage continuous cotton, minimum tillage continuous cotton and minimum tillage cotton-wheat) with or without maize rotation.

Site description

The field experiment was conducted at the Australia Cotton Research Institute in Narrabri (30°10'S, 149°40'E), New South Wales in Australia, where a crop rotation trial has been established for 30 years. The study site has a semi-arid climate with a hot summer and a mild winter. The mean daily maximum and minimum temperature of the hottest month (January) is 34°C and 20°C, respectively, and that of the coldest month (July) is 18°C and 4°C, respectively. The mean annual rainfall is 568 mm and is primarily summer dominant, falling on an average of 64 days per year. The soil is a self-mulching cracking clay, classified as grey vertosol (Isbell, 1996, World Reference Base: Vertisol), with mean particle size distribution of 53% clay, 21% silt and 26% sand in the 0–1.2 m depth (Nachimuthu *et al.*, 2018).

Experimental design

The study was conducted between October 2015 and October 2017, using the long-term (over 30 years) field trial consisted of three historical cropping systems (HCS) of maximum tillage continuous cotton (Max Till/CC), minimum tillage continuous cotton (Min Till/CC) and minimum tillage cotton-wheat (Min Till/CW) that were replicated in four blocks. In 2011, the experimental plots were re-designed by splitting each HCS plot to introduce maize into the rotation as an alternate summer crop in half of the plots (Fig. S1). The study duration and the

timing of crop rotation meant that each of the six systems had the following cropping sequence during the study: cotton-fallow-cotton-fallow (Max Till/CC and Min Till/CC), maize-fallow-cotton-fallow (Max Till/CM and Min Till/CM), fallow-fallow-cotton-wheat (Min Till/CW) and maize-fallow-cotton-wheat (Min Till/CWM). For a detailed description of the field layout, dimension and number of rows within each plot, see Nachimuthu *et al.* (2018).

Tillage practices

Experimental plots were cultivated in May/June after the harvest of the summer crops (cotton or maize). Maximum tillage consisted of slashing of non-harvestable biomass, followed by disc-ploughing and incorporation of slashed biomass to 20 cm, chisel ploughing to 30 cm, followed by 1 m spaced bed construction. Minimum tillage consisted of slashing of non-harvestable biomass, followed by root cutting, pupae busting and incorporation of slashed biomass to 10 cm below the surface of the bed, followed by bed renovation with a disc-hiller.

Crop management

Cotton seeds were sown at the rate of 16–18 kg ha^{-1} in spring (October/November) according to the treatment cycle. The varieties sown were: Sicot 71BRF® in 2015 and Sicot 746B3F in 2016. Fertiliser was applied at the rate of 180 kg N ha^{-1} after sowing and 80 kg N ha^{-1} during peak flowering, which occurred in January in both cropping seasons. Cotton was furrow-irrigated seven times in the 2015/16 season and nine times in the 2016/17 season at the rate described in Nachimuthu *et al.* (2018) when rainfall and soil water storage could not meet evaporative demand. Cotton plants were harvested in April/May with a mechanical four-row cotton picker, and the remaining non-harvestable biomass was slashed to a height of 0.1 m, followed by root cutting or chisel ploughing depending on the tillage treatment.

Maize seeds (Var P1467) were sown at the rate of 20 kg ha^{-1} in November 2015 in systems that included maize in the rotation. Fertiliser was applied at the rate of 260 kg N ha^{-1} one month after sowing in 2015. Maize was furrow-irrigated six times in 2015/16 season at the rate of 100 mm per irrigation. Maize plants were harvested in April, and the remaining non-harvestable biomass was slashed and root-cut or chisel ploughed depending on the tillage treatment.

Wheat seeds (*cv.* Crusader) were sown at the rate of 60 kg ha^{-1} in winter (May/June) in 2015 and 2017 in systems that included wheat in the rotation. Wheat received a total of 80 kg N ha^{-1} during the cropping season in 2015 and 2017. Wheat plants were furrow-irrigated at the rate of 100 mm per irrigation once in 2015 and twice in 2017. Wheat was harvested with a combine harvester, and the remaining stubble was either left as a stubble fallow until the following spring or directly planted with maize (into standing stubble).

Field sampling and initial processing

Soil samples were collected from each plot before (October) and after (May/June) the summer crop season during the two-year treatment cycle. A portable coring rig (Gibson Engineering, Brisbane) was used to extract cores to 1 m depth. Each core was divided into 5 depth intervals (0–15, 15–30, 30–50, 50–70 and 70–100 cm) in the field. Soil samples were oven-dried at 40°C and sieved to < 2 mm to remove gravel and plant materials before further processing. Bulk density was determined from air-dried < 2 mm soil mass (corrected for 105 °C oven-dried mass) and sample volume (corrected for gravel content). Gravimetric soil water content was also assessed by drying subsamples of air-dried samples at 105°C.

Soil fractionation

Soil samples were partitioned into particulate organic fraction and mineral-associated organic fraction using a particle size fractionation method described in Sanderman *et al.* (2012). Briefly, 10 g of dried soil sample (< 2 mm) was dispersed in 40 mL of sodium hexametaphosphate solution (5 g L^{-1}) overnight (16 h) on an orbital shaker at 180 rpm. Using a wet-sieving system (Fritsch Analysette 3 PRO; Fritsch, Idar-Oberstein, Germany), the dispersed soil solution was fractionated into a particulate organic fraction (> 50 μm in size) and a mineral-associated fraction (≤ 50 μm in size). All fractions were oven-dried and weighed. Dissolved organic fraction was obtained as water-extractable C and N. Briefly, 10 g of dried soil samples (< 2mm) were dispersed in 50 mL of de-ionised water for 1 h on an orbital shaker at 180 rpm. The mixtures were centrifuged for 30 min

at 3000 rpm and filtered through Millipore Express® 0.22 µm polyethersulfone membrane filters. Extraction was repeated up to five times to gain enough material for elemental and isotope analyses. Filtrates were frozen until the determination of C, N and $\delta^{13}C$ analysis as follows.

Carbon, nitrogen and $\delta^{13}C$ analyses

To measure the organic C, N and $\delta^{13}C$ signature of the bulk soil (i.e. SOC), particulate and mineral-associated fractions (POM and MAOM, respectively), sub-samples were ball-milled to powder (< 100 micron) and analysed by a Sercon GEO 20/20 IRMS equipped with ANCA-GSL module (Sercon Ltd., UK). For dissolved organic matter (DOM) samples, aqueous filtrates were analysed for the total organic C concentrations by a TOC analyser (Sievers InnovOx Laboratory TOC Analyzer; GE, US). The remaining filtrates were lyophilized and analysed for C, N and $\delta^{13}C$ signature by a Sercon GEO 20/20 IRMS. All samples were checked for the presence of inorganic C and treated with 2% phosphoric acid to remove inorganic C prior to the analysis. The C isotope data were expressed in parts per mille (‰) relative to the international standard of Vienna Pee Dee Belemnite (V-PDB) and calculated as:

$$\delta^{13}C\ (‰) = \left[(R_{sample} - R_{standard})/R_{standard}\right] \times 10^3 \quad (1)$$

where R_{sample} is the ratio of ^{13}C:^{12}C in the sample and $R_{standard}$ is that of V-PDB.

Soil organic carbon stocks and natural ^{13}C abundance

The calculation of SOC stocks for the 0–100 cm profile was performed on the basis of equivalent soil mass based on the method of Wendt and Hauser (2013). Briefly, the SOC stocks were calculated considering the SOC concentrations and the measured soil masses in the 0–15, 15–30, 30–50, 50–70 and 70–100 cm soil layers for each core, with the cumulative reference soil masses of 16200 Mg ha^{-1} for 0–100 cm soil profile. A cubic spline function, which consisted of a piecewise series of cubic polynomial curves was used to calculate the cumulative SOC stocks in the cumulative reference soil masses.

The relative contribution of C_3 (cotton and wheat) and C_4 (maize) sources for SOC stocks were calculated for those systems with maize rotation by using a two end-member mixing model according to Balesdent *et al*. (1987):

$$f_{C4} = \frac{\delta^{13}C_{SOC} - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}} \quad (2)$$

$$f_{C3} = \frac{\delta^{13}C_{SOC} - \delta^{13}C_{C4}}{\delta^{13}C_{C3} - \delta^{13}C_{C4}} \quad (3)$$

where $\delta^{13}C_{SOC}$, $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ were $\delta^{13}C$ signature of SOC, C_3 plant biomass (i.e. cotton and wheat) and C_4 plant biomass (i.e. maize), respectively. We adopted this approach, as the systems with maize rotation received both C_3 and C_4-derived C during the experimental period (as maize was planted as a summer crop in alternate years). The $\delta^{13}C$ signature of whole plant biomass (both aboveground and belowground) for cotton, wheat and maize collected from the experiment were -27.8 ± 0.2, -25.1 ± 0.2 and -12.3 ± 0.1‰, respectively. For the systems with wheat rotation (i.e. Min Till/CWM), the average of cotton and wheat $\delta^{13}C$ signature was used for the calculation. The C_3-derived and C_4-derived SOC stocks (Mg ha^{-1}) were calculated by multiplying SOC stocks by f_{C3} or f_{C4} for each depth and treatment.

Soil inorganic N

Soil inorganic N was determined by measuring ammonium and nitrate concentrations using the 2M KCl extraction method (Rayment & Lyons, 2010). Briefly, 4 g of dried soil samples dispersed in 40 mL of 2M KCl for 1 h on an orbital shaker at 180 rpm. The mixtures were then filtered through Whatman 42 filter papers. The concentrations of ammonium and nitrate in the filtered extracts were determined colourimetrically using a spectrophotometer (SpectraMax M2, Molecular Devices Corporation, California).

Data analyses and statistics

All data were analysed by analysis of variance (ANOVA) using the 'stats' package in R statistical software (ver. 3.6.1, R Core Team, 2018). All data were checked for normality and heteroscedasticity prior to the analyses, and log-transformed where necessary. Tukey's HSD test was used for *post hoc* multiple comparison. The effects were considered significant at $P < 0.05$,

however, marginally significant effects ($0.05 < P < 0.1$) were also reported. Non-significant effects at $P > 0.1$ were reported as n.s. Correlation analyses (Pearson's) and partial correlation analyses were performed using the 'ppcor' package (Kim, 2015) to account for the effect of depth on measured variables while maintaining a large number of data points for the analysis.

Structural equation modelling

We used structural equation modelling (SEM) to identify the relative importance and effects of the agricultural management (i.e. HCS and maize rotation) and soil properties (i.e. soil inorganic N, SOM fractions) on net changes in SOC stock. SEM offers the ability to separate multiple pathways of influence and view them as a system, and to partition direct and indirect effects that one variable may have on another and estimate the strengths of these multiple effects (Grace, 2006). Firstly, we developed a priori model which articulates the proposed causal influences based on the known effects and relationships among the managements, soil properties and the response variable (i.e. SOC). Our model (Fig. 1) proposed the general hypothesis that the agricultural managements (HCS and maize rotation) impact soil inorganic N and SOM fractions, and the overall SOC stock. HCS was ranked as Max Till/CC < Min Till/CC < Min Till/CW, based on their reported benefit for increasing SOC stock (Hulugalle & Scott, 2008). We used C:N ratios of soil fractions as an indication of decomposition processes, after confirming the significant relationship between the C:N ratios of SOM and microbial respiration (Fig. 2). As we were interested in the changes in SOC stock during the full cropping cycle (two years), we used net changes in SOC, SOM fractions and inorganic N availability. To eliminate any differences caused by depths within the profiles (due to the grouping of the top two profiles and the bottom three profiles into the topsoil and the subsoil, respectively), the SEM analysis was done on the residuals (i.e. controlling the effect of depths), and therefore variation in the data caused by depths within each profile cannot influence the outcome of the model.

We used the multi-group modelling approach where soil profile (i.e. topsoil and subsoil) served as a grouping variable in order to compare changes in the strength of the pathways in the model, depending on which profile of soil are examined. A multi-group model starts with the hypothesis that the same model structure fits all groups and that the pathways are of equal sign and magnitude among groups. We used the Chi-square test ($\chi 2$) to evaluate the overall goodness of fit. Since the purpose of multi-group analyses is to highlight the differences among groups by imposing a common causal structure for all groups, the overall model fit is expected to be poorer than fitting a model for each group separately (De Vries et al., 2012). Therefore, we further confirmed adequate model fit by using the root mean square error of approximation (RMSEA) and the Bollen-Stine bootstrap test. Finally, we calculated the standardised total effects of all predictor variables. SEM was conducted in Amos 25 (IBM SPSS).

Investigation 2: Microbial processes in the soil profile

Impact of coring lubricants on soil biota

It is essential that sampling procedures for biological measurements, are done in a way that reflects the soil processes and limits sampling artefacts. To determine if a range of common soil coring lubricants could alter microbial activity of soils, we measured their effect on microbial activity in substrate induced respiration experiments.

Experimental soil sampling

Five soil cores were collected from the 'Trevenna' farm at the University of New England, New South Wales (Australia) (Latitude 30°29'12.21"S, Longitude 151°38'0.90"E). Each core was recovered from a depth of 100 cm using a powered soil corer, 42 mm in diameter. These cores were recovered directly into a 40 mm polyvinyl chloride (PVC) half-pipe insert, to store and support the cores, which were sealed at the end with plastic wrap and transported back to the laboratory in a chilled box for processing within 24 hours of sampling.

Soil Properties

The soils were classified as black-grey vertosol (Isbell, 2016) or vertisol (international classification), comprised of 74 % clay, 16% silt and 9% sand at a pH_{CaCl} of 5.6 (McPhee *et al.*, 2010). The gravimetric water content (GWC) at the time of sampling in the 10-30, 30-50 and 50-70 cm regions of the core were 20, 36 and 36 % respectively, but averaged 30 % in the 70 – 100 cm depths of the profile, but these differences were not significant (P=0.22).

Core partitioning and respiration set up

Each 100 cm core was partitioned into 4 depths (10-30, 30-50, 50-70 and 70-100 cm) for analysis. From each depth partition, four sub-cores measuring 42 mm (diameter) by 30 mm (length) were cut and weighed in preparation for respiration incubations. The first experiment was conducted by exposing the circumferential surface of these intact soil cores to common coring lubricants and measuring carbon dioxide (CO_2) evolution using a Respicond respirometer (Nordgren, 1988b). The remaining soil at each depth were sampled using a sterile cork borer to produce a further four mini sub-cores measuring 4 mm (diameter) by 20 mm (length), which were transferred to a 1.2 ml 96 deep well plate for a second experiment involving increased soil exposure to the lubricants with CO_2 evolution determined by MicroResp (Campbell *et al.*, 2003).

The recovered 30 mm by 42 mm cores were designated as either a control (water) or exposed to one of either silicone spray, mould stripper or WD-40. The exposure to the oil was conducted by spraying the oil onto the surface of a 100 x 200 mm plastic sheet, which was then weighed. An intact core was placed onto the plastic sheet and rolled, using the sheet to apply the lubricant to the core outer surface and mimic normal field coring conditions. The core was then inserted into a respirometer pot (Nordgren, 1988b) and the amount of lubricant applied was determined by reweighing the plastic sheet and calculated from the difference in the weight of the lubricant on the plastics prior to and after rolling of the soil core. The pots were loaded into a 20 °C water bath and incubated for 48 hours. Carbon dioxide evolution was determined by the decrease in conductivity, which was determined automatically and converted to either hourly CO_2 or cumulative CO_2 by the Respicond software (Nordgren, 1988b).

The 4 mm by 20 mm cores were placed into a 96 well plate, with 20µL of either water (control), glucose solution (100 g L^{-1}), silicone oil, WD-40, mould stripper or olive oil. These cores were incubated in a MicroResp system (Campbell *et al.*, 2003) for 24 hours. The respired CO_2 was captured in a detection plate and the colour change was measured on a SpectraMax M2e multi-mode plate reader (Molecular Devices Corporation) at 570 nm, with all data handling performed according to the MicroResp user guide (Campbell *et al.*, 2003).

Statistical analysis

All data was tabulated in Microsoft Excel and imported into the GenStat program (VSN International Limited, U.K.) for statistical analyses. Where sample size was considered small, paired T-test were used to assess significance between measures. For all other analysis, Analysis of Variance (ANOVA) was used to assess significant differences ($P \leq 0.05$) between the experimental factors of depth, lubricant and lubricant by depth.

Determination of the longer term impact or rotation and agricultural practices on soil biological activity

Improved understanding of microbial activity and associated nutrient cycling in agricultural systems is required to maximise production whilst maintaining and improving soil fertility. We compared past and current microbial activity using the stable $\delta^{18}O_{P\ HCl}$ pool and respiration incubations, under crop and native systems to a depth of 1 m.

Sampling of the crop cores took place during January 2016 (mid-season), while native vegetation samples were collected in May 2016. The native vegetation site was a further 5 km west of ACRI (Latitude 30o12’10.08”S, Longitude 149o32’44.88”E) and comprised of river red gum (Eucalyptus camaldulensis)/coolabah (Eucalyptus coolabah) open woodland with a redgrass (Bothriochloa macra) and prickly acacia (Vachellia nilotica) understorey. All samples were extracted using a powered soil corer 42 mm in diameter, collecting 4 soil cores in each system to a depth of 100 cm.

Intact cores were recovered into polyvinyl chloride (PVC) half-pipes to store and support the core, sealed with plastic wrap and transported back to the laboratory in cooled, insulated containers for processing. In the laboratory, soil cores were sub-divided into five depths (0-15, 15-30, 30-50, 50-70 and 70-100 cm).

Basalt rock samples were collected from Sawn Rocks (Latitude -30°8'42.12", Longitude 150°3'12.41" and elevation 460 m), located 40 km away from the sample sites. These rocks were formed during the same period of volcanic activity and under similar conditions as the events under which the soil parent material was derived.

Sequential extraction of P

Soils were sequentially extracted using a modified Tiessen and Moir (1993) method. Resin and sodium bicarbonate soluble P are extremely small fractions in our soils, so they were omitted in favour of NaOH extraction, which incorporates these two (Tiessen & Moir, 1993). Briefly, 0.5 g of the air-dried and sieved soil sample was mixed with 30 mL of 0.1 M NaOH and shaken for 16 hours on an orbital shaker at 220 rpm. Samples were centrifuged at 900 x g at room temperature for 30 minutes and the supernatant was collected for analysis. The soil pellet was resuspended in 30 mL of 1 M HCl and once again shaken for 16 hours and centrifuged to collect the supernatant for analysis. The resultant soil pellet post HCl extractions was digested using 10 mL of a 1 L mixture of 0.3 % potassium dichromate, 43.4% perchloric acid, 42% nitric acid and 1% bromine in deionised water. The NaOH, HCl and digest extracts were run on a Model 725 radial viewed Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) with mass flow controller (Agilent, Australia) against known standards of ammonium dihydrogen phosphate (99.99%) to determine P concentration at a wavelength of 213.618 nm.

Oxygen isotopic composition of phosphate ($\delta^{18}O_{P\ HCl}$)

Before purification and extraction, soil samples for $\delta^{18}O_{P\ HCl}$ analysis were oven-dried at 40 °C until a constant mass was obtained. Samples were then ground to pass a <2 mm sieve (Tamburini *et al.*, 2010) We determined the oxygen isotopic composition of phosphate in HCl extracts ($\delta^{18}O_{P\ HCl}$) as we found from sequential extraction that this is the largest P-pool in the studied soils and thus is likely to reflect microbial P turnover the strongest, even though it is not directly involved in microbial P cycling. However, at high P cycling some of the cycled P is also precipitated in the more stable P pools (e.g., the HCl-pool) and thus cycling effects are visible in HCl-P (Angert *et al.*, 2011, Joshi *et al.*, 2016). The applied procedure for $\delta^{18}O_{P\ HCl}$ extraction and purification conformed to the procedures described by Tamburini et al. (2010) and Amelung et al. (2015). About 10 g of the air-dried and sieved soil was mixed with 100 mL of 0.1 M sodium hydroxide (NaOH) in a 1:10 w/v ratio, and shaken for 16 hours on an orbital shaker at 220 rpm. Samples were centrifuged at 900 x g at room temperature for 30 minutes and the supernatant was removed. The soil was then extracted with 100 mL 1 M hydrochloric acid (HCl) for a further 16 hours, centrifuged and the supernatant filtered through Whatman 42 filter paper, which was collected for purification. Purification of the extracted phosphate was done according to Tamburini et al. (2010), where the filtrate underwent ammonium phospho-molybdate (APM) precipitation and dissolution followed by magnesium ammonium phosphate (MAP) precipitation and dissolution, anion removal and finally, silver phosphate precipitation (Tamburini et al., 2010). The original method specified cation removal with AG50 X8 cation resin, however, our extraction procedure varied from that of Tamburini et al. (2010), by using a DOWEX® 50 WX8 (200 – 400 mesh) resin for Mg^{2+} cation removal, which was cleaned and used following the original method (Tamburini *et al.*, 2010). About 2.5 mg (equivalent to 500 µg oxygen) of purified Ag_3PO_4 precipitate sample was weighed in triplicate into a 6mm x 4mm silver foil cups and analysed using a Sercon 20-22 continuous flow isotope ratio mass spectrometer (IRMS) connected to a high temperature (1400 °C) pyrolysis furnace (tuned for carbon monoxide analyses) using a 66-place solid sample auto sampler. Reference materials used were internationally certified IAEA-602 benzoic acid (+ 71.4‰ VSMOW), commercially available IA-R022 calcium carbonate (+ 7.52‰ VSMOW) and Sercon verified cuttlefish (-3.28‰ VSMOW), which were used as standards in the analysis of $\delta 18O_{P\ HCl}$ in

the extracted soils. Use of IAEA-601 benzoic acid (+23.3‰ VSMOW) is a preferred standard however, both the calcium carbonate and cuttlefish standards used have been inter-laboratory verified and thus can be used for this work. The analytical precision of IRMS measurements was approximately 0.4 delta units, with replications in the same samples indicating an average precision of 0.37 delta units for the whole procedure.

Due to the wide range in the available $\delta^{18}O‰_{\text{V-SMOW}}$ values in the standards, a regression line was constructed to correct for signal compression in the measured values by substituting into the equation y = 1.70x + 15.9, where 'x' was the $\delta^{18}O‰_{\text{V-PDB}}$ value obtained from the instrument. Once corrected for compression, the data was converted to $\delta^{18}O‰_{\text{V-SMOW}}$ (de Groot 2008), where $\delta^{18}O‰_{\text{V-SMOW}} = 1.03 \times \delta^{18}O‰_{\text{V-PDB}} + 30.91‰$.

The thermodynamic equilibrium O-isotope fractionation between dissolved phosphates and water were calculated based on a simplified equation proposed by Chang and Blake (2015):

$$\delta^{18}O_P = e^{\left(\frac{14.43}{T} - \frac{26.54}{1000}\right)} \times (\delta^{18}O_W + 1000) - 1000 \quad (1)$$

Here, $\delta^{18}O_P$ is the stable oxygen isotope ratio of PO_4 at equilibrium [‰], T is the ambient soil temperature [K] and $\delta^{18}O_W$ is the stable oxygen isotope ratio of water [‰]. As the extraction of pore water from Australian vertisol soils is inherently difficult and likely to produce fractionated values, the mean value of -5.8‰ for $\delta^{18}O_w$ published by Hughes *et al.* (2012) on rainwater samples collected over 30 years, has been used as it would closer reflect the values of soil water influencing the HCl-P extractable pool. The mean ambient field temperature used was 25.3 °C, which was measured with soil thermistors at 10 cm depth. This information was accessed on "The FastStart™ Fund Soil Temperature Network". The minimum (20.2 ºC) and maximum (35.5 ºC) temperatures and the value of -1.7 ‰ for $\delta^{18}O_w$ were used to calculate the estimated equilibrium range. The -1.7 ‰ uncertainty was determined by interpolation of $\delta^{18}O$ precipitation values for Australia, available from the Global Network of Isotopes in Precipitation (GNIP) and determining the variance (IAEA/WMO, 2018, Tadros *et al.*, 2014).

Oxygen isotopic composition of Sawn Rocks and fertilisers

The Sawn Rock samples, a selection of Australian derived fertilisers (Mono-Ammonium Phosphate (MAP), Di-Ammonium Phosphate (DAP) and 2 Triple Super Phosphates) and apatite rock samples from the mine from which the phosphate fertiliser were produced (Duchess mine at Phosphate Hill in Western Queensland) were ground and sieved to <250 μm. About 18.0 mg of sample was weighed and analysed on the IRMS using the same standards and parameters as the extracted phosphate samples.

Basal respiration and microbial biomass

At each of the analysed depths, two portions of the core measuring 30 mm (length) by 42 mm (diameter), each weighing approximately 60 g, was transferred into specimen jars to pre-incubate in the dark at 25 ºC for three days (Anderson & Domsch, 1978a). The moisture content of the pre-incubated samples was adjusted to an approximate gravimetric water content (GWC) of 30% with deionised water for basal samples and glucose solution for substrate induced respiration samples (to determine microbial biomass), to achieve a final concentration of 50 mg glucose g^{-1} soil. These samples were placed in a respirometer pot, which was loaded into a 20 ºC water bath. This apparatus works by the absorption of CO_2 transpired by the soil into a hydroxide (OH^-) trap, forming carbonate ions (CO_3^{2-}), where conductivity decreases. The change in conductivity is calibrated against CO_2 absorption providing a measure of respiration (Nordgren, 1988a). Removal of organic matter (i.e. roots) and any macrofauna in the soil, ensures the CO_2 respired is due to microbial activity (Nordgren, 1988a). Evolved CO_2 was measured using an electronic respirometer system for both cumulative basal and substrate induced respiration (mg CO_2) over a 24 hour period, which was subsequently converted to cumulative mg CO_2 g^{-1} of dry weight soil (Nordgren, 1988a). Microbial biomass was calculated according to the equation derived by Anderson and Domsch (Anderson & Domsch, 1978a) x = 40.4y = 0.37 where y is the maximum initial rate of respiration (mg CO_2 g^{-1} h^{-1}) in response to the addition of glucose and x is the mg of microbial C g^{-1} of soil.

Statistics

All data was tabulated in Microsoft Excel and imported into the GenStat program (VSN International Limited, UK) for statistical analyses. Data and residual normality was tested with the Anderson-Darling test with Analysis of Variance (ANOVA) used to assess significant differences ($P \leq 0.05$) between the experimental factors, these being depth, system and system by depth. Where a loss of sample or technical replicate occurred, unbalanced ANOVA was used.

The impact of rotation and depth on microbial activity and biomass

Australian cotton crops are traditionally grown in Vertosol (Australian classification) or Vertisol (International classification) soils, which are highly dynamic in terms of vertical (self-mulching) and lateral (swelling and cracking) movements to at least 1m in depth, encouraging the movement of C down the soil profile and way from the crop rhizosphere (Hullugalle *et al.*, 2001, Isbell, 2016, Michéli *et al.*, 2006). Therefore, there is the potential for significant microbiological activity and biomass to be occurring down the soil profile. The primary aim of our work was to the measure the distribution of soil microbial activity (SMA) using basal respiration measurements and biomass (SMB) using substrate induced respiration (SIR) measurements, down the Vertisol profile to the depth of 1m, over two growing seasons.

Sample collection

Sampling took place over two growing seasons in October, January and June, which corresponded to pre, in and post crop, respectively. One core sample was collected per replicate plot, of which there were four each for continuous cotton (CC) and cotton-maize (CM) rotations, resulting in 8 cores collected each sampling event and a total of 48 separate cores collected over the two seasons. Samples were extracted using a powered soil corer with a 42 mm inner diameter, to the depth of 100 cm. Coring lubricants were not used in the extraction process, as they have been found to stimulate microbial activity, therefore inflating SMA measurements in this soil type (Polain *et al.*, 2019). Intact cores were sampled into polyvinyl chloride (PVC) half-pipes to store and support the core, sealed with plastic wrap and transported back to the laboratory in cooled, insulated containers for processing. In the laboratory, soil cores were sub-divided into five depths (0-15, 15-30, 30-50, 50-70 and 70-100 cm), which were again sub-sampled for microbial respiration, biomass and $\delta^{18}O$ analyses. Soil samples were assessed both individually for each depth and as an aggregated mean for top (0-30 cm) and sub- (30-100 cm) soil samples from each core.

Microbial respiration and biomass

Microbial respiration was determined by using an electronic respirometer system (Respicond, Nordgren Innovations AB, Sweden) according the method outlined by Anderson and Domsch (1978b). Briefly, at each identified depth, two portions of the cores measuring 30 mm (length) by 42 mm (diameter), each weighing approximately 60 g, was transferred into specimen jars to pre-incubate in the dark at 25°C for three days. The GWC for each sample was adjusted to approximately 30% with either deionised water (basal samples) or 50 mg/mL glucose solution (substrate induced respiration). Samples were placed in respirometer pots, which were loaded into a 20°C water bath. Carbon dioxide evolution was measured over a 24 hour period, which was subsequently converted to cumulative mg CO_2 cm^{-3} of dry weight soil (Nordgren, 1988b). Microbial biomass was calculated from the substrate induced respiration according to the equation derived by Anderson and Domsch (1978b) $x = 40.4y \times 0.37$. Where y is the maximum initial rate of respiration (mg CO_2 h^{-1} cm^{-3}) in response to the addition of glucose and x is the mg of microbial C cm^{-3} soil. The metabolic quotient (qCO_2) was determined by the ratio of SMA (µg CO_2 hr^{-1} cm^{-3}) to SMB (mg $C_{microbial}$ cm^{-3}) (Anderson & Domsch, 1990, Spohn, 2015).

Oxygen isotopic composition of phosphate ($\delta^{18}O_{P\ HCl}$)

Soil samples for $\delta^{18}O_{P\ HCl}$ analysis were oven-dried at 40°C until a constant mass was obtained and then ground to pass a <2 mm sieve (Tamburini *et al.*, 2010). The procedures for $\delta^{18}O_{P\ HCl}$ extraction, purification and analysis are described explicitly in Polain *et al.* (2018). As an overview,

approximately 10 g of the air-dried and sieved soil underwent two extractions to remove organic matter, followed by a series of precipitation and dissolution steps to purify the samples. Purified samples were then analysed using a Sercon 20-22 continuous flow isotope ratio mass spectrometer (IRMS), with reference materials and data handling conforming to the methods detailed in Polain *et al.* (2018). The thermodynamic equilibrium range for O-isotope fractionation between dissolved phosphates and water was previously determined to be 12.8 ‰ and 17.2 ‰, with a mean equilibrium of 15.0 ‰ (Polain *et al.*, 2018)

Statistical analysis

All data was tabulated in Microsoft Excel and imported into the GenStat program (VSN International Limited, UK) for statistical analyses. Data and residual normality was tested with the Anderson-Darling test with Analysis of Variance (ANOVA) used to assess significant differences ($P \leq 0.05$) between the experimental factors, these being depth, system and system by depth. Where a loss of sample or technical replicate occurred, unbalanced ANOVA was used.

Sub-soil microbial diversity under rotational cotton and cotton-maize systems

The development of high through put sequencing platforms and universal primers for both prokaryotic and eukaryotic microbiota, allows for assessment and monitoring of soil microbial diversity in response to changing conditions (i.e. management practice or climate change) (Lehman *et al.*, 2015). However, there has been limited research into the diversity of sub-soil microbiota in Vertosols and the effect of crop management on these communities.

Sample collection

Samples were collected over two seasons, prior to pupae busting and planting (October 2015 and 2016) and at flowering stage in-crop (January 2016 and 2017). There were four replicate plots each for continuous cotton (CC) and cotton-maize (CM), with one core extracted per replicate plot, at each time of sampling, resulting in total of 32 cores extracted over the two seasons. These soil cores were extracted using a powered soil corer, with a 42 mm inner diameter, to the depth of 100 cm and placed into polyvinyl chloride (PVC) half-pipes, wrapped with plastic wrap to store and support the core. Cores were transported to the laboratory in cooled, insulated containers, where sub-samples were removed in triplicate from three depths (0-15, 30-50 and 70-100 cm) and stored at -20°C until required.

DNA extraction

DNA was extracted from each sub-sample using PowerSoil® DNA Isolation Kits (MO BIO Laboratories, Inc) according to manufacturer's instructions. Briefly, 0.25g of soil was weighed and subjected to a series of steps to promote cell lysis, removal of inhibitors (i.e. non-DNA material), washing of DNA, before being eluted in 65 µL elution buffer. The triplicate samples (see 2.2 Sample collection) were pooled, with DNA quantity (1 ng/µL to 50 ng/µL required) and purity (A260/A280 ratio range 1.6 to 1.9) assessed using a NanoDrop™ 8000 Spectrophotometer (ThermoFisher Scientific, USA).

Sequencing

Three technical replicates of the pooled soil DNA soils were sent to the Australian Genome Research Facility (AGRF) in Brisbane (Queensland) for Diversity Profiling using the MiSeq Illumina Platform. This service identifies the relative proportion of organisms within mixed microbial communities (AGRF, 2017). AGRF validated primers 341F and 806R were used to target 16S variable regions V3-V4 for bacteria and archaea, whilst primers 1F and 2R were used to target ITS regions for fungi.

Diversity analyses

Reports containing Operational Taxonomic Units (OTUs) tables and taxonomy summary files were received from AGRF upon successful sequencing. OTUs were used in the construction of mathematical functions (indices) to measure soil microbial diversity (Daly *et al.*, 2018). Gini-Simpson Diversity Index (D_{GS}) was used to compare microbial diversity between samples, with values ranging 0 (low diversity) to 1 (high diversity) (Kim *et al.*, 2017) and Gini-Simpson Evenness

Index (E_{GS}) was used to determine the equatorial dispersion of organisms amongst species (Tuomisto, 2012), with values ranging from 0 (uneven distribution of species) to 1 (even distribution of species) (Maurer & McGill, 2011). Sørensen's Index of Similarity (I_S) was used to compare similarities between samples (Benizri & Amiaud, 2005)

Statistical and analyses

All data was tabulated in Microsoft Excel and imported into the IBM SPSS Statistics 25 program for statistical interrogation. Analysis of Variance (ANOVA) was used to assess significant differences ($P \leq 0.05$) between the experimental factors of depth, depth by system, depth by sample time (crop stage and season).

Investigation 3: Below ground agronomy and constraints to plant growth identified

Cone penetrometry resistance.

As part of the work conducted in this project to assess the implications of field management and the resultant changes in soil physical condition, we monitored soil penetrative resistance using a CPII Cone Penetrometer (Rimik, Toowoomba) in all field sampling the work period. Part of the motivation for this is that there is a single reference that cotton roots are impeded at a soil restrictive force of 2500 KPa (Taylor et al., 1966), although this has been standardised by Fritton (1990), the combination of visual and measured soil parameters would imply that this value is significantly higher, probably nearer to 4000 KPa or higher.

The cone penetrometer was fitted with a RTK GPS device, a 75 kg load cell and was used with a 75 cm shaft tipped with a 130 mm^2 displacement tip. Data was recovered and analysed using the Rimik Penetrometer File Reader. Bulk density was obtained from the cores taken to service the carbon and microbial aspects of the project as well as the soil GWC. Mechanical impact on the soil was not directly measurable, but the number of passes in each row of the trial was possible to predict from the NSW DPI farm records and machinery used. As such, by taking penetrometry readings in 3rd and 7th hill and adjacent furrow (either side of the hill) we were able to make general assessments of cultivation and traffic on the overall resistance posed by the soil to crop roots over the sampling periods.

Gathered insertion data was mapped using GIS Visualiser (Figure 25) and the penetrative data assessed in 5 cm increments from 0 to 75 cm deep by crop, time, system and location (furrow/hill) with ANOVA using Genstat. Where the load on the cell became excessive (>6600 kpa) data was treated as being missing.

Physical treatments on microbial respiration

Microbial basal respiration and respiration response to added DOC were assessed on soil samples collected at the end of the study using MicroResp™ (The James Hutton Institute, UK; Campbell et al., 2003). In order to overcome the difficulty in soil handling associated with the heavy clay content of the soil, 0.6 g of air-dried soil was individually weighed and added to each well of the deep-well microtiter plate without the use of the delivery device. Soil samples were then brought to ~50% water holding capacity and pre-incubated in a dark at 25 °C for 3 days. Following the pre-incubation, soil samples were re-adjusted to ~70% water holding capacity, either with water or DOC extracted from plant biomass. To recover plant DOC dried cotton and maize plant biomass (the whole plants including roots) was cut into small pieces (~ 2mm), mixed with de-ionised water at 1:20 ratio and shaken for 2 h on an orbital shaker at 180 rpm. The mixtures were filtered through Millipore Express® 0.22 µm polyethersulfone membrane filters, and the filtrates were analysed for the total organic C concentrations by a TOC analyser. DOC was added to the samples at the rate of 7.2 mg C mL soil water^{-1}. CO2 production rate was measured over 6 h at 25 °C as previously described (Campbell et al., 2003), and was expressed as mg CO_2-C g soil^{-1} h^{-1} or µg CO2-C g soil C –1 h^{-1} (i.e. normalized for soil C), depending on the analysis.

The effect of physical treatments on microbial respiration and respiration response to added DOC were also assessed on soil samples collected at the end of the study using MicroResp™ method

described above with some modification. Soil samples were prepared as described above, and after the pre-incubation, physical treatments were applied prior to water and DOC addition. Compaction treatments were applied by using a custom-made compression device to compact individual soil samples in deep-well microtiter plates to achieve the bulk density of 1.0 and 1.5 g soil cm^{-3}. Non-adjusted control samples had a bulk density of 0.86, while a 'disturbance treatment' was achieved by individually poking and breaking up the pre-incubated soil samples with a needle. Bulk density of the disturbed samples were not determined, due to the uneven nature of the broken soil surface. Water and DOC were added as previously described, and CO_2 production rate was measured as described.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Investigation 1: Mechanisms of whole-profile C and N dynamics

Soil organic carbon

Temporal changes in soil organic carbon

The concentrations of SOC fluctuated significantly amongst the five sampling points during the two-year treatment cycle (Fig. 1), however, there were no consistent patterns associated with the timing of the field management (i.e. pre-planting and post-harvest operations). Sampling time had a significant impact on SOC ($P < 0.0001$), and interacted with the effects of HCS, maize and depth.

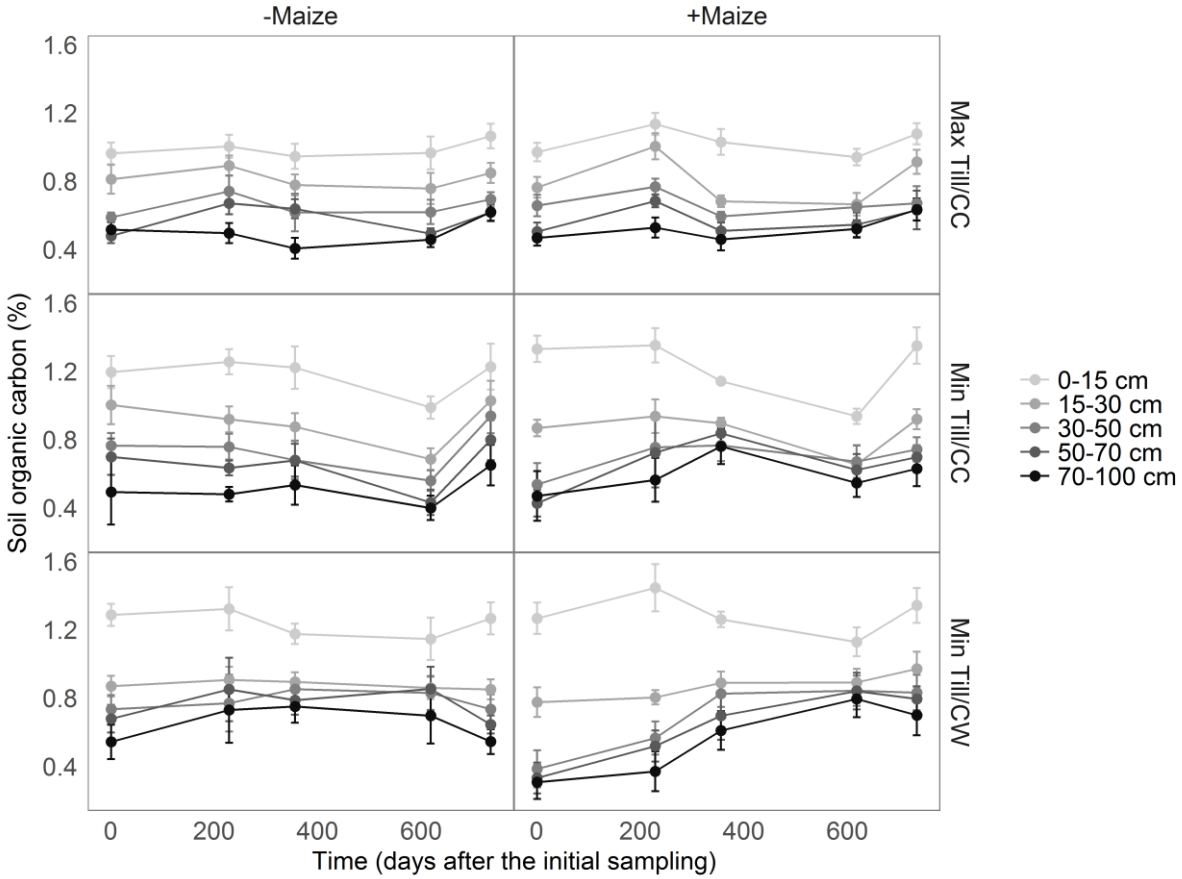

Fig. 1 Temporal changes in soil organic carbon concentrations under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) during the full treatment cycle (from Oct 2015 to Oct 2017). The horizontal bars are the mean's standard errors (n=4). The days of the samplings are Day 0 = October 2015 (pre-planting), Day 229 = May 2016 (post-harvest), Day 356 = October 2016 (pre-planting), Day 618 = June 2017 (post-harvest), and Day 734 = October 2017 (pre-planting).

Vertical patterns in soil organic carbon

Despite the strong temporal effect, there was a clear vertical trend in SOC concentrations where the concentrations decreased with increasing depth across all treatments ($P < 0.0001$, Fig. 2). However, the magnitude of this decline was smaller in maximum tillage treatment, which was evident in a marginally significant depth × HCS interaction effect on SOC ($P = 0.05$).

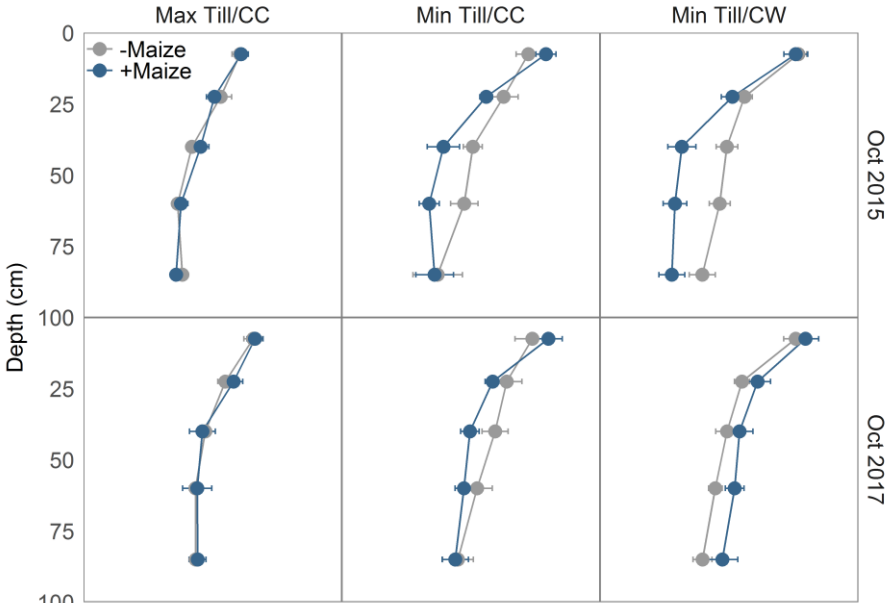

Fig. 2 Soil organic carbon (%) of soils under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

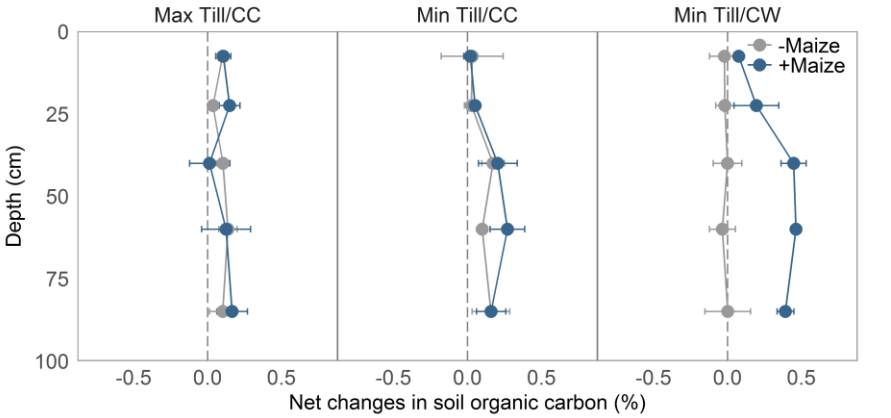

Fig. 3 Net changes in soil organic carbon (SOC) under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

Soil organic carbon stocks

The SOC stocks at the start of the experiment ranged from 88 to 122 Mg ha^{-1} for 0–100 cm depth across the treatments (Fig. 4a). While HCS and maize treatments also influenced SOC, there was a significant interaction effect of HCS × maize × treatment cycle on SOC concentrations ($P = 0.005$). This was evident when examining the net changes in SOC between the start and the end of the two-year treatment cycle (Fig. 3), where a significant increase in SOC occurred in Min Till/CWM at the end of the two-year treatment cycle compared to the beginning of the cycle.

At the end of the 2-year treatment cycle, SOC stocks increased in most systems compared to the start, however, the increase was significantly higher in the systems with maize than the systems without maize by 23% (equivalent to 21 Mg ha^{-1}, $P = 0.04$, Fig. 4b). This increase was marginally more pronounced in the minimum tillage systems than the maximum tillage systems (maize × HCS interaction, $P = 0.06$).

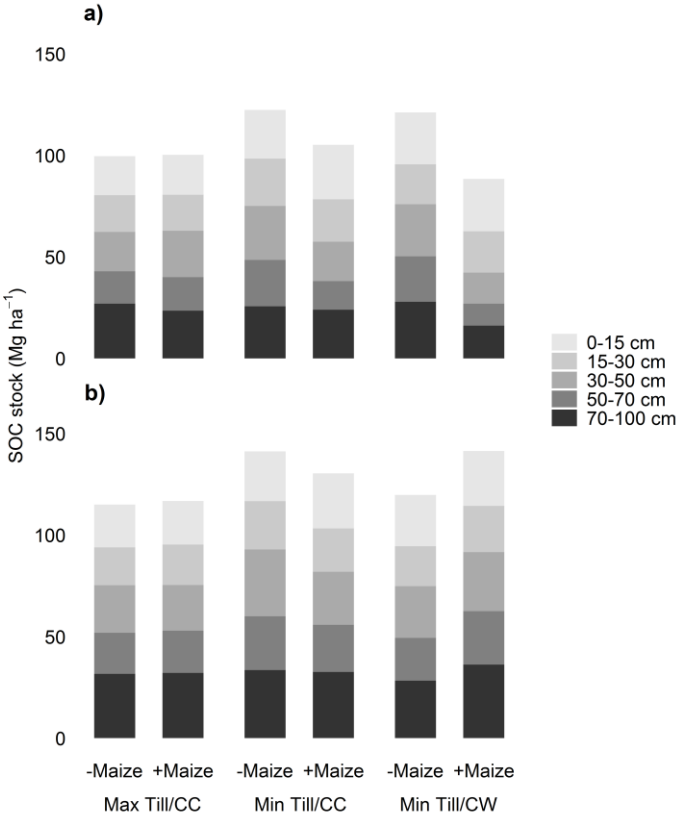

Fig. 4 The whole-profile soil organic carbon (SOC) stock at the start of the experiment (a) and at the end of the full treatment cycle (b under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/- Maize).

Soil fractions

Soil organic C contains a mixture of organic materials that are in various stages of decomposition processes. Some are more recently incorporated into the soil, while others are old, highly decomposed materials that are recalcitrant and protected against further decomposition. Various methods exist to characterise and categorise organic matter into various pools or fractions. One of those method is based on particle size. Larger particle size fraction (>50 µm, referred to as particulate organic carbon) represents organic matter that are at early stages of decomposition, while finer particle size fraction (<50 µm, referred to as mineral-associated organic C) represents organic matter that are highly decomposed. Dissolved organic fraction represents water-soluble organic C that are highly mobile and labile in nature.

Proportion of DOC, POC and MOC in SOC pool

Across the treatment, mineral-associated organic C (MOC) fraction comprised 92% of the total SOC pool on average across the depths, while particulate organic C (POC) fraction comprised only 7% of SOC pool on average. DOC fraction contributed less than 1% to the total SOC pool. There was a clear vertical trend in the proportion of POC fraction in SOC pool, with the largest proportion (up to 30%) of POC observed at the 0–15 cm depth, which sharply declined with depth ($P < 0.0001$), was influenced by HCS (HCS × depth interaction, $P = 0.01$). The proportion of POC at 0–15 cm was significantly influenced by tillage, and it was nearly doubled under minimum tillage compared to that of maximum tillage (Tukey's HSD, $P < 0.0001$). No differences were observed in the proportion of POC below 15 cm depth. The opposite pattern was observed for the proportion of MOC, where maximum tillage reduced the proportion of MOC at 0–15 cm (Tukey's HSD, $P < 0.0001$), reflecting that the contribution of DOC fraction to the total SOC pool was too small to influence the overall pattern. The proportions of POC and MOC were not significantly affected by the maize treatment nor treatment cycle.

Vertical patterns in DOC, POC and MOC

Vertical patterns in the C concentrations of soil fractions (DOC, POC and MOC) generally followed the same pattern as SOC (Fig. 5, 6, and 7). POC fraction was not influenced by maize treatment and treatment cycle, and was influenced significantly by depth ($P < 0.0001$) and marginally by HCS ($P = 0.06$) only. DOC and MOC fractions were more responsive to HCS, maize and treatment cycle that POC fraction, and were generally similar to the effects observed in SOC.

Additionally to the significant effects observed in SOC, the treatment cycle had a significant effect on DOC fraction (P = 0.04), and interacted marginally with HCS (P = 0.07), depth (P = 0.07) and maize and depth (P = 0.098). As for MOC fraction, there was also a significant HCS × maize × depth × treatment cycle interaction (P = 0.04), where the increase in MOC in Min Till/CWM at the end of the two-year treatment cycle was more pronounced in the lower soil depths than the upper soil depths.

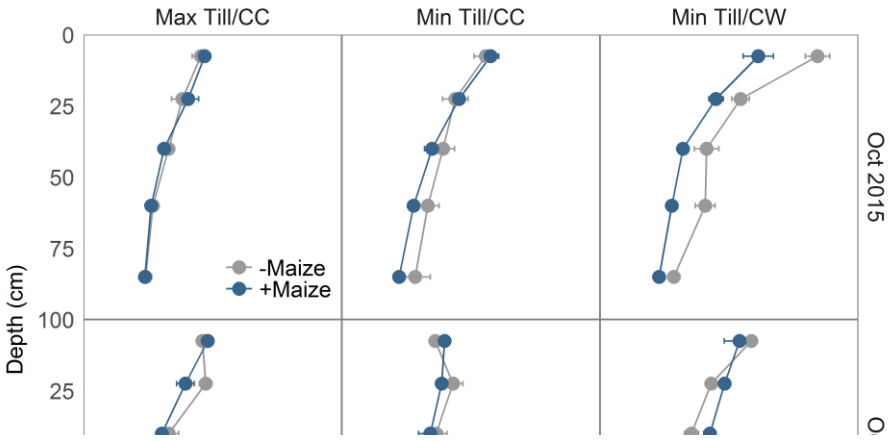

Fig. 5 Dissolved organic carbon (%) of soils under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

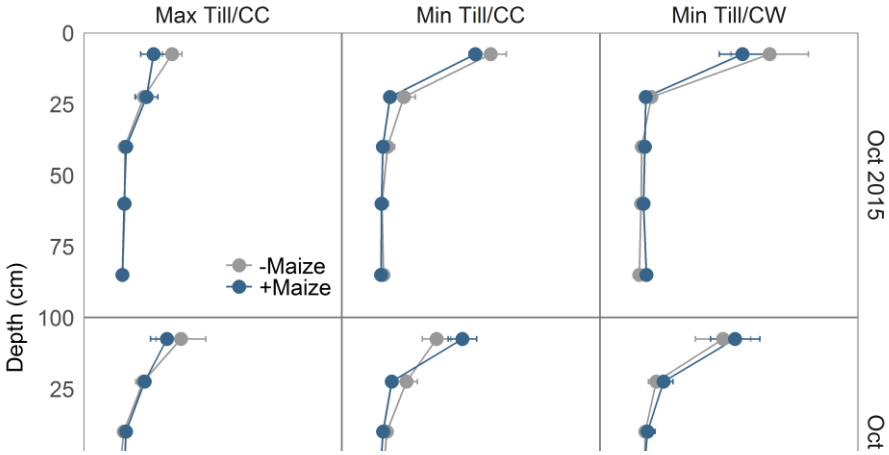

Fig. 6 Particulate organic carbon (%) of soils under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

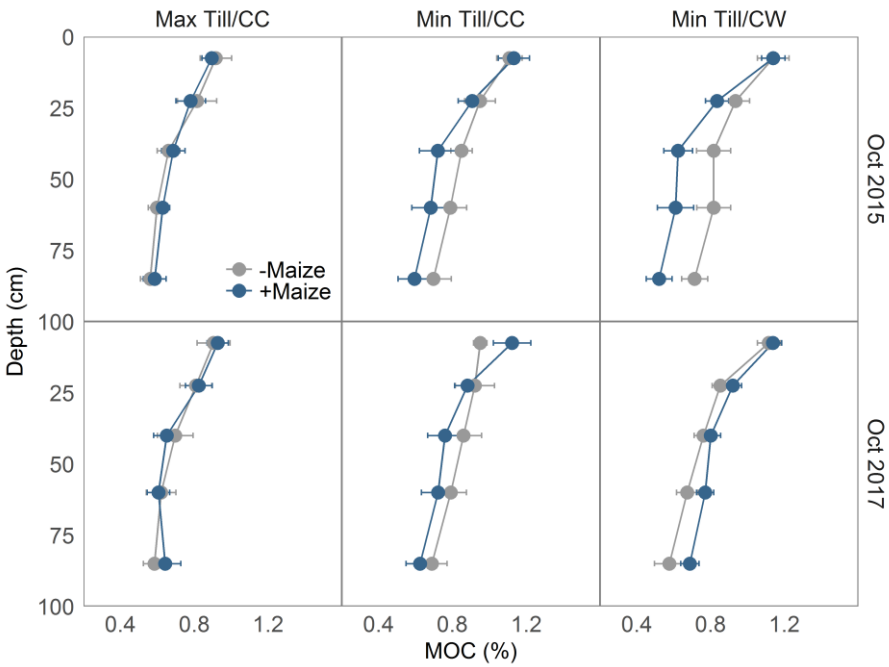

Fig. 7 Mineral-assocaited organic carbon (%) of soils under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

Carbon isotope analyses

Carbon isotope analysis is a useful tool to quantify C_4-derived C (i.e. maize) in C_3-cultivated soils (i.e. cotton soil), utilising the difference in $\delta^{13}C$ signatures between C_3 and C_4 plants. Soil C stock is largely determined by the balance between C input by plants and C losses through microbial

respiration, and the $\delta^{13}C$ signature of soil organic C (SOC) often reflects the $\delta^{13}C$ signature of the plant input. Thus, examining the $\delta^{13}C$ of SOC may give some insights into the mechanisms that control C cycling of the whole soil profile under different systems.

Vertical patterns in $\delta^{13}C$ value of SOC, DOC, POC and MOC

We found that the $\delta^{13}C$ value of SOC and MOC showed a strong vertical pattern of gradual increase with increasing depth (P<0.0001, Fig. 8 and 11). The $\delta^{13}C$ value of DOC and POC showed a similar vertical patterns where a significant maize effect was evident in the upper soil profile (Fig. 9 and 10), indicating that these fractions were predominantly composed of relatively younger SOC than MOC fraction.

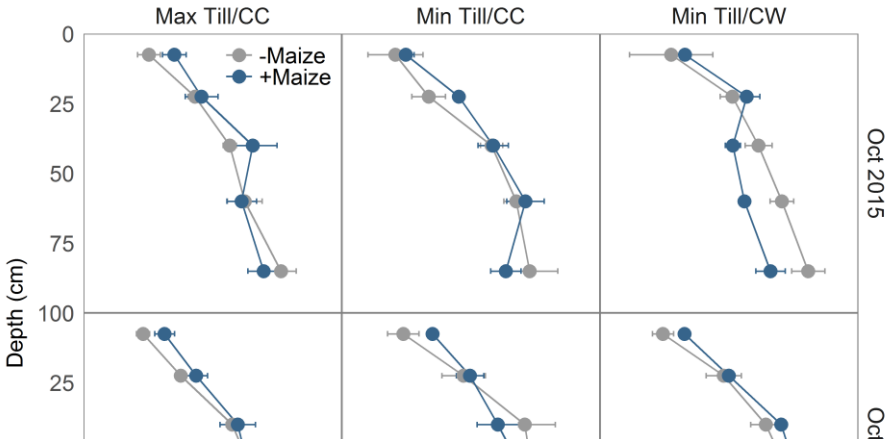

Fig. 8 $\delta^{13}C$ values of soil organic carbon collected under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

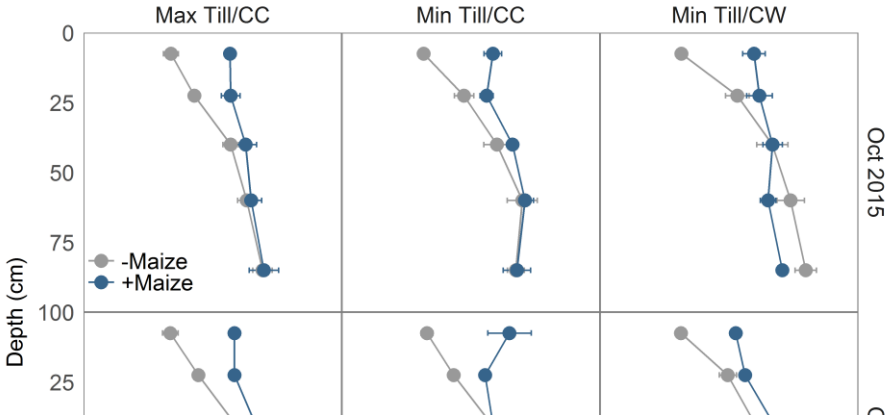

Fig. 9 $\delta^{13}C$ values of dissolved organic carbon collected under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

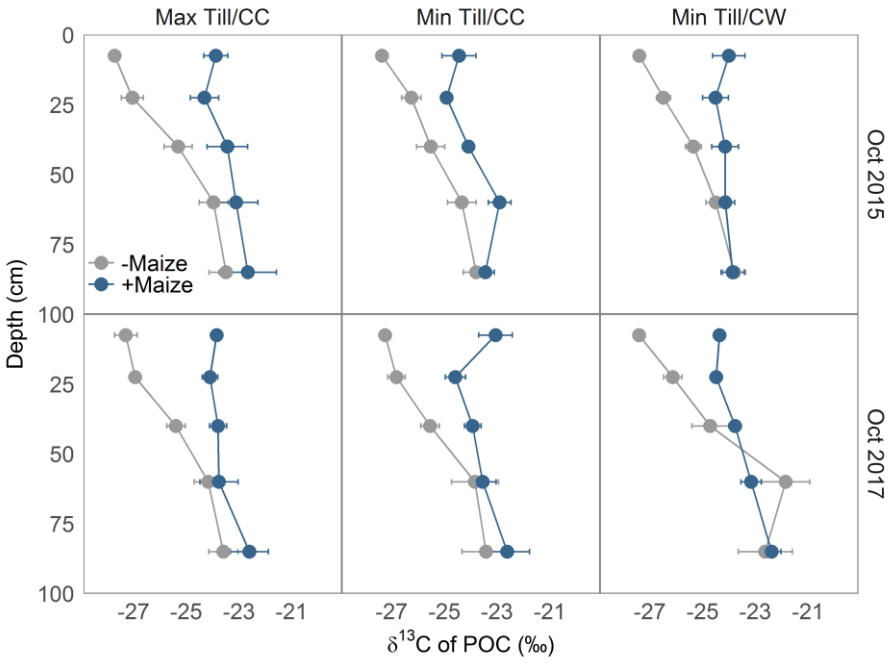

Fig. 10 $\delta^{13}C$ values of particulate organic carbon collected under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

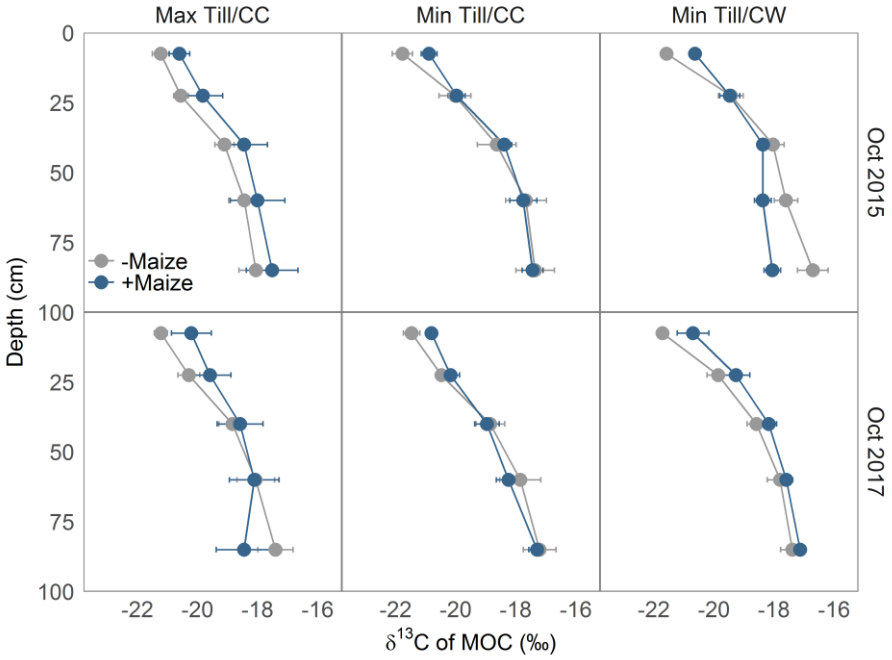

Fig. 11 $\delta^{13}C$ values of mineral-associated organic carbon collected under Max Till/CC, Min Till/CC and Min Till/CW with and without maize rotation (+/-Maize) at the beginning (Oct 2015) and end (Oct 2017) of the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

Cotton vs Maize contribution to SOC

To quantify the source of new C input into the soil, the relative contributions of C_3 and C_4-derived C to SOC stocks were assessed in the systems with maize rotation. Generally, there were net increases in the contribution of both C_3 and C_4-derived C at the end of the full treatment cycle at all depths in all systems (Fig. 12). However, the magnitude of the increases differed between HCS (P = 0.0004, Table 3), and were greater in Min Till/CWM than in both Min Till/CM (Tukey's HSD, P = 0.02) and Max Till/CM (Tukey's HSD, P = 0.002). This HCS effect was greater in the lower soil profile than the upper soil profile (HCS × depth interaction, P = 0.03, Table 3). The amount of C added to SOC stock by C_4-derived C was marginally greater than that of C_3-derived C (P = 0.07, Table 3).

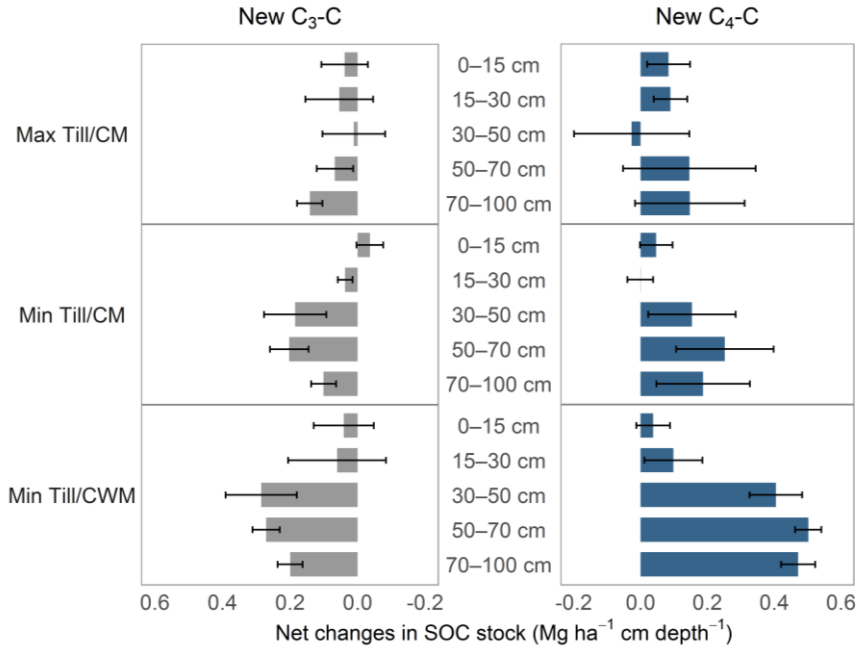

Fig. 12 The contribution of C_3-derived and C_4-derived C under Max Till/CM, Min Till/CM and Min Till/CWM during the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

Soil total N and inorganic N

There was a strong and significant correlation between SOC and total N (Fig. 13), indicating that both temporal and vertical patterns in soil total N mirrored those of SOC. Temporal changes in soil inorganic N were variables and not appropriate to compare between HCS or maize treatments due to the differences in the timing and the rate of N fertiliser application. However, across the two-year treatment period, the average amount of soil inorganic N (combined nitrate and

ammonium) was higher in Min Till/CC than the other systems, and was the highest in Min Till/CC without maize rotation (Fig. 14).

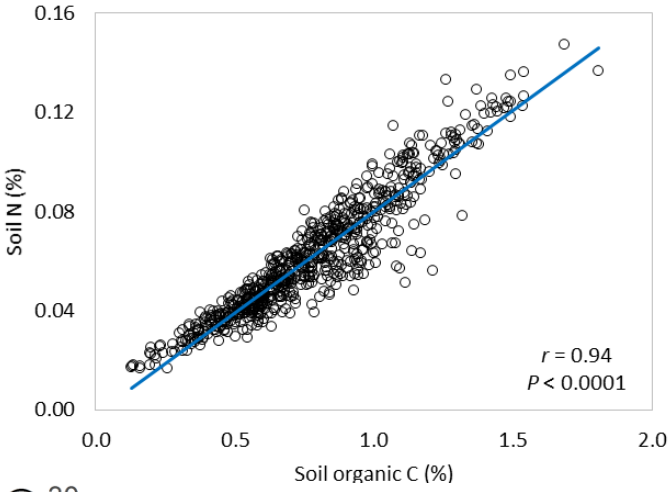

Fig. 13 Correlation between SOC soil total N for the entire dataset. A correlation coefficient (Pearson's *r*) is presented with *P*-value.

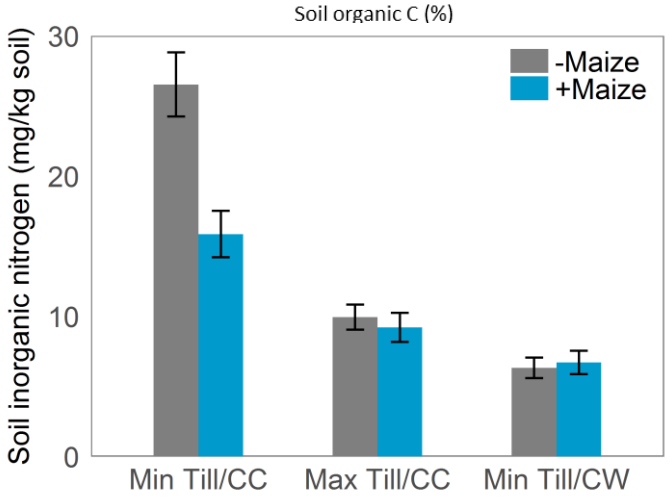

Fig. 14 The average soil inorganic N concentrations in the soil under Max Till/CM, Min Till/CM and Min Till/CWM during the full treatment cycle. The horizontal bars are the mean's standard errors (n=4).

Topsoil vs. subsoil SOC dynamics

The results above showed clear vertical patterns where soil properties and the effects of agricultural management differed between the topsoil (0–30 cm) and the subsoil (30–100 cm). Here, we explored how HCS and maize rotation may affect SOC dynamics by changes in decomposition processes in these soil profiles.

C:N ratios of soil organic matter and microbial decomposition

To assess how HCS and maize rotation affected SOC stocks during the 2-year treatment cycle, we first explored the relationship between microbial respiration and C:N ratios of SOM. There was a significant negative relationship (Fig. 15), suggesting that changes in C:N ratios could be used as a proxy for microbial decomposition.

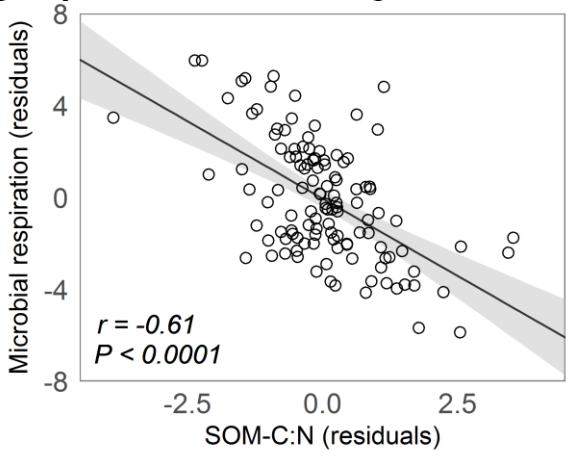

Fig. 15 Partial correlations between microbial respiration and C:N ratios of SOM. A correlation coefficient (Pearson's *r*) is presented with *P*-value, and the shaded band represents 95% CI.

Structural equation modelling

We developed *a priori* model and fitted the same model for topsoil and subsoil dataset. The overall fit of the model was adequate (χ^2=4.8, *df*=10, *P*=0.90; RMSEA=0.000, *P*=0.96; Bootstrap *P*=0.92), and the impact of HCS and maize rotation on SOC differed between the soil profiles (Fig, 16). In the topsoil, maize rotation directly increased SOC, while it also caused a decrease in SOC via increasing inorganic N, which decreased POM-C:N affecting SOC (Fig. 16a). HCS did not directly impact SOC, but indirectly decreased it via inorganic N and POM-C:N. In the subsoil, changes in SOC was strongly influenced by changes in MAOM fraction (Fig. 16b). Both maize rotation and HCS had a positive direct effect on SOC, while they also had indirect effects through different pathways. The standardised total effect on SOC was the greatest for MAOM-C:N, followed by maize rotation in the subsoil, indicating their dominance in affecting SOC changes in this cropping system during the study.

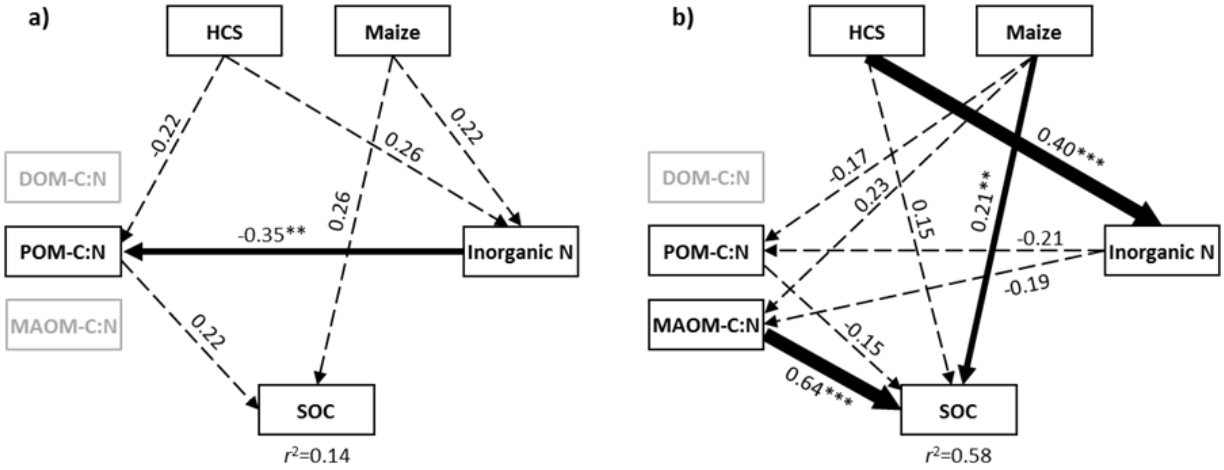

Fig. 16 The direct and indirect effects of HCS and maize rotation on SOC changes in the topsoil (a) and subsoil (b) through changes in C:N ratios of soil fractions and inorganic N. Numbers adjacent to arrows are standardised path coefficients. Arrow width is proportional to the strength of path coefficients. Only significant (*P*<0.05) or marginally significant (*P*<0.10) relationships are presented. **P*<0.05, ***P*<0.01, ****P*<0.001.

Agricultural management influenced SOC dynamics differently between the topsoil and subsoil. In the topsoil, changes in POM fraction influenced SOC dynamics. Thus, any management change that affects POM fraction is likely to impact SOC stock in the topsoil. In contrast, changes in the subsoil SOC was dominated by MAOM fraction, which were influenced by both HCS and maize rotation directly and indirectly. The lack of DOM involvement is likely due to the small percentage of this fraction in SOC pool and its labile nature. However, the direct effect of maize rotation on SOC storage in the subsoil suggests that the direct transfer of C into MAOM fraction, such as a vertical movement of C, may dominate C dynamics in the subsoil in these soils. Therefore, agricultural management that affects physical properties of the soil could have a significant consequence for subsoil C storage.

Investigation 2: Microbial processes in the soil profile

Microbial activity and biomass

Impact of coring lubricants on soil biota

Core circumference oil application and respiration

Exposure of the circumferential surface of intact soil cores to common coring lubricants found the adherence of silicone oil (0.36g lubricant 100g^{-1} soil) to the soil cores to be significant ($P < 0.001$) and three times higher than either WD-40 or mould stripper (0.12 and 0.11 g lubricant 100^{-1} soil, respectively). There was no significant difference in the cumulative CO_2 after 48 hours respiration between any of the experimental variants (depth, lubricant or lubricant by depth). However, the average hourly CO_2 showed a significant difference ($P = 0.031$) in substrate by depth. This difference was driven by the increased CO_2 evolution by microbial communities in the top soil

(10-30 cm) of the control samples. When we compared the activity of microbial communities at depth (Table 1), there was a trend of inflated microbial activity measurements with increasing depth in response to mould stripper and silicone oil, with no discernible pattern for the response to WD-40.

Table 1. The effect of applied lubricants on microbial activity down the soil profile (n = 5). *Activity calculated as a percentage of the activity of the untreated control.

Lubricant	Depth (cm)	% activity*	Effect
Mould stripper	10-30	3	Inhibited
Silicone oil	10-30	3	Inhibited
WD-40	10-30	4	Inhibited
Mould stripper	30-50	89	Inhibited
Silicone oil	30-50	67	Inhibited
WD-40	30-50	144	Inflated
Mould stripper	50-70	131	Inflated
Silicone oil	50-70	100	None
WD-40	50-70	92	Inhibited
Mould stripper	70-100	133	Inflated
Silicone oil	70-100	125	Inflated
WD-40	70-100	133	Inflated

Lubricant effect on mini sub-cores

The 4 mm by 20 mm cores were exposed to approximately 5mL lubricant 100 g^{-1} soil, where there was a significant difference in microbial activity by lubricant ($P = 0.001$, Figure 17) and by depth ($P = 0.004$), but there was no apparent substrate by depth interaction ($P = 0.98$). Overall microbial activity measurements were inflated by 6 % in response to the application of silicone oil and mould stripper (Figure 17), whilst olive oil and WD-40 had no discernible effect on the respiration activity measured. Whilst there was no interaction between depth and substrate, respiration was significantly higher in the 30-50 and 50-70 cm profile soils than in the 10-30 and 70-100 cm profile samples, which were statistically similar.

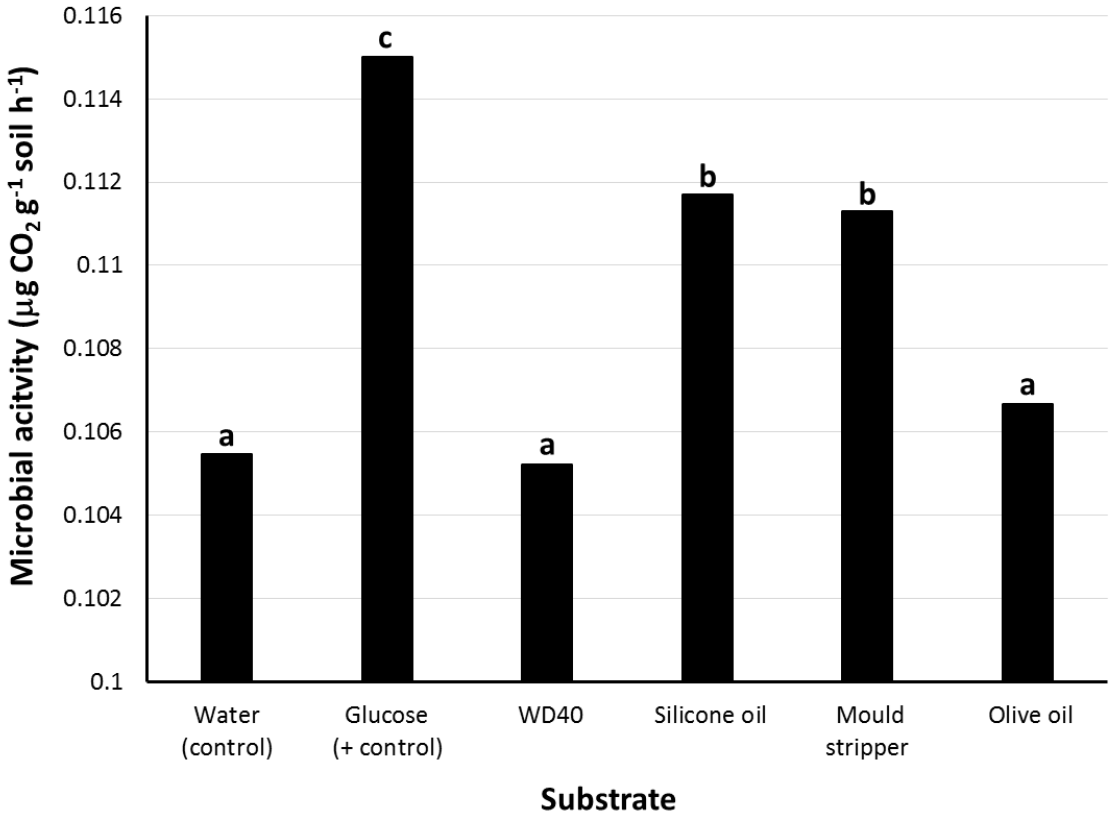

Figure 17. Mean average respiration rates for sixteen 0.54 g soil samples taken between 10 and 100 cm depth and exposed to 20 µl of one the substrates (glucose, WD40, Silicone oil, Mould stripper oil, Olive oil) or water as a control. Similar alphabetical numbers represent similar means based on ANOVA and $P<0.05$.

Application of lubricants to the surface of field fresh soil cores did not significantly influence the activity of microbial communities (0.08 to 0.11 g lubricant to 100 g^{-1} soil). However, when the ratio of lubricant to soil was increased to 5mL lubricant 100 g^{-1} soil, there was a significant ($P = 0.001$) effect on microbial activity. Silicone oil and mould stripper increased measurements by at least 5 %, whilst olive oil and WD-40 had no difference. The results imply that when using coring rigs to recover soil for microbial functional analysis, the use of coring lubricants is best avoided.

Determination of the longer term impact or rotation and agricultural practices on soil biological activity

Soil P pools

Sequential extraction of P in the NaOH, HCl and digest (residual P) extractable pools showed the HCl pool to be significantly ($P < 0.001$) larger, in both crop and native systems, with total P (sum of all extracts) being overall higher in the native system (Table 2). There was a significant difference ($P < 0.001$) in system by depth in the HCl pool. The native system had decreasing HCl extractable P with depth, where the crop system had increased HCl extractable P in the top (7.5 cm) and subsoil (85 cm).

		µg P g^{-1} soil						
System	Depth (cm)	NaOH Mean	SE	HCl Mean	SE	Digest Mean	SE	Total P
Crop	7.5	30.3	± 1.4	467.8	± 4.3	176.4	± 0.8	674.5
Crop	40	14.1	± 0.3	383.7	± 14.0	180.0	± 6.2	577.8
Crop	85	9.4	± 0.6	546.1	± 67.2	170.7	± 1.3	726.2
Native	7.5	137.8	± 17.4	609.3	± 88.6	127.2	± 5.9	874.3
Native	40	29.0	± 2.8	452.0	± 26.1	103.9	± 13.2	584.8

Native	85	16.0	± 2.8	407.0	± 0.5	99.5	± 6.8	522.5

Table 2. Mean P values in sequential extracts down the soil profiles under crop and native system. Standard error (SE) values are based on n = 4.

Oxygen isotopic composition of phosphate ($\delta^{18}O_{P\ HCl}$)

In order to evaluate whether the $\delta^{18}O_{P\ HCl}$ of the native and cropped system were influenced by microbial activity, we analyzed parent material from Sawn Rocks for its $\delta^{18}O_{\ VSMOW}$ composition. The value was measured as 7.8‰ (Table 3), which was depleted by 5.9‰ in the crop and 3.13 ‰ in the native systems. In addition, we also determined the $\delta^{18}O_{\ VSMOW}$ composition of P fertilisers in order to assess all influencing factors to the $\delta^{18}O_{P\ HCl}$ values of the cropped soil samples. The average value for the fertilisers was 20.5‰ and close to the value of the apatite rock (20.4‰) from which they were derived.

	$\delta^{18}O_{\ VSMOW}$ (‰)	
Sample	Mean	SE
Sawn Rocks	7.8	± 0.04
Mono-Ammonium Phosphate (MAP)	20.2	± 0.2
Di-Ammonium Phosphate (DAP)	19.0	± 0.1
Triple Super Phosphate 1 (TSP 1)	22.0	± 0.1
Triple Super Phosphate 2 (TSP 2)	20.7	± 0.1
Australian apatite (Duchess)	20.4	± 0.0

Table 3. Mean $\delta^{18}O$ values (‰) of the Sawn Rock samples, fertilisers derived from Australian apatite (mined from Duchess) and rock sourced from Duchess Mine. Standard error (SE) is based on n = 11 for the Sawn Rock and n = 3 for the fertilizers and Duchess rock.

The $\delta^{18}O_{P\ HCl}$ values of the extracted soils were significantly ($P < 0.001$) enriched down the whole profile under the crop, in comparison to the native system (Figure 18). The $\delta^{18}O_{P\ HCl}$ values down the soil profile significantly ($P < 0.001$) depleted in the native system 12.3‰ to 9.4‰. In comparison, the $\delta^{18}O_{P\ HCl}$ value of the crop system showed an overall depletion from 14.2‰ in the top 0 – 15 cm to 13.3‰ in the 70 – 100 cm of the soil, with all values being within the estimated equilibrium range of 12.7‰ to 17.1‰, but falling short of the mean equilibrium of 14.9‰.

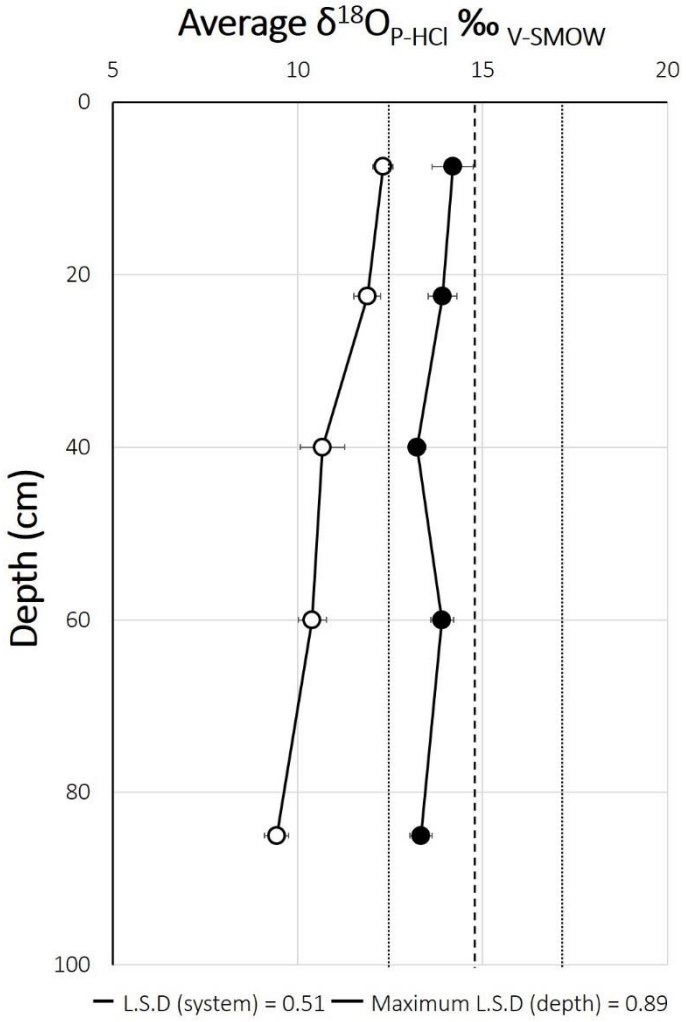

Figure 18. Average $\delta^{18}O_{P\ HCl}$ values down the soil profile under crop (●) and native (O) systems, with error bars indicating the standard error of the mean based on n=4 (independently replicated samples). The calculated theoretical equilibrium $\delta^{18}O_{P\ HCl}$ range are indicated by the dotted vertical lines and the mean value by the vertical dashed line. Least significant differences (L.S.D) in the systems and depths are indicated by the bars at the base of the graph.

Microbial activity and biomass

There was a significant (P = 0.05) difference in CO_2 evolution under basal conditions between systems (Figure 19), which was most increased in the 15 – 50 cm of the crop profile. There was no significant difference between the systems in response to the addition of glucose (SIR), although the evolved CO_2 concentration was consistently higher in soils from under the crop system.

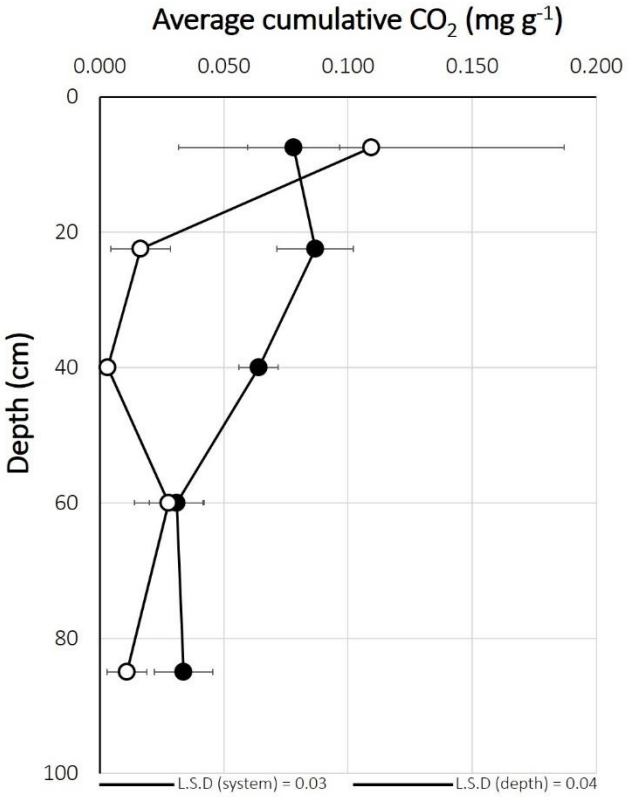

Figure 19. Average cumulative carbon dioxide (CO_2) evolved (mg g^{-1} dry soil) after 24 hours of incubation in basal conditions down the soil profile under crop (●) and native (O) systems. Error bars are the standard error of the mean based on n=4, with each value based on an average of the values for the indicated mid sample depth. Least significant differences (L.S.D) in the systems and depths are indicated by the bars at the base of the graph.

There was a significant difference in CO_2 evolution at depth under basal conditions (P = 0.02) and in response to glucose (P = 0.005), which was driven by a higher level of microbial activity in the top 0 – 15 cm of the soil profiles, in comparison to the subsoils. The response to glucose was lower in the native system however, the decline in activity with depth was not as pronounced as the cropping system. Overall, microbial communities under the crop system were more active under basal conditions and in response to glucose. However, whilst microbial activity in the 0-30 and 30-100 cm proportions of the profile was 56 to 44%, respectively, for the cropping soil, the 0-30 cm section of the native system accounted for 82% of the respiratory activity.
There was a significant difference (P < 0.001) in microbial biomass between systems (Figure 20), with the average overall microbial biomass in the crop system being five times the amount of the biomass in the native system. This was in addition to an inverse relationship of biomass trends between the systems, where microbial biomass increased with depth in the native system and decreased with depth in the crop system. A strong (R = 0.80) and significant (P = 0.005) correlation between microbial biomass and $\delta^{18}O_{P\ HCl}$ values was observed.

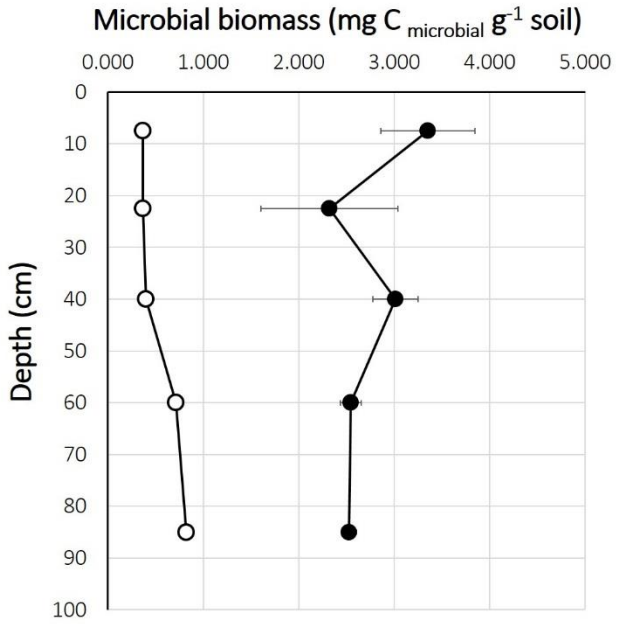

Figure 20. Average microbial biomass (mg C $_{microbial}$ g^{-1} soil) down the soil profile under crop (●) and native (O) systems. Error bars are the standard error of the mean based on n=2, with each value based on an average of the values for the indicated mid sample depth.

Contrary to current beliefs, agricultural practices have not decreased microbial activity in our crop system. Differences in average $\delta^{18}O_{P\ HCl}$ signatures indicated higher past microbial activity down the entire soil profile under the crop system (13.7 ‰) compared to the native system soil profile (11.0 ‰), whilst current microbial activity was double under the crop system, especially between 15 and 100 cm. The data also highlighted the importance of investigating microbial activity and biomass at depth. In these relatively dry, carbon and nutrient poor cropping soils, the influence of water is perhaps the key to explaining our results.

The apparent influence of water on the microbial activity in these soils contrasts with observations made elsewhere in wetter environments, where higher C and fertility status of the soils appears to be more important, thus additional research will need to be undertaken to explore this further. However, if our interpretations are correct, the requirement for water to drive microbial activity in Australian vertosols raises additional challenges in the face of growing global populations due to the additional pressures this will place on water resources to assist farmers in producing more from the available land.

The impact of rotation and depth on microbial activity and biomass

The distribution of average SMA ($P = 0.024$) and SMB ($P < 0.001$) significantly differed down the soil profile. The SMA was higher at both 0-15 cm and 30-50 cm (4.43 ± 0.48 µg CO_2 h^{-1} cm^{-3}) compared to the SMA at 50-70 cm (3.39 ± 0.37 µg CO_2 h^{-1} cm^{-3}), however, the soil below 30 cm produced 47% of the soil profiles SMA. Soil microbial biomass was five times greater (8.79 ± 2.35 mg C $_{microbial}$ cm^{-3}) at 0-15 cm, compared to the 70-100 cm sub-soils (1.62 ± 0.22 mg C $_{microbial}$ cm^{-3}), which contributed only 26% to the profiles overall SMB.

Crop system did not significantly influence the distribution of average SMA ($P = 0.223$) down the soil profile, although CM trended in increased SMA at 0-15 cm, 30-50 cm and 50-70 cm. Crop system did significantly influence the distribution of average SMB ($P < 0.001$), with an increased SMB at 0-15 (11.82 ± 4.42 mg C $_{microbial}$ cm^{-3}) and 15-30 cm (8.13 ± 3.56 mg C $_{microbial}$ cm^{-3}) in the CC system, whilst CM had a higher SMB at 70-100 cm (1.92 ± 0.37 mg C $_{microbial}$ cm^{-3}).

Seasonality did not significantly affect the distribution of average SMA ($P = 0.547$) down the soil profile. However, SMA was over three times greater ($P < 0.001$) down the entire soil profile in Season 1 (5.96 ± 0.27 µg CO_2 h^{-1} cm^{-3}), compared to Season 2 (1.84 ± 0.11 µg CO_2 h^{-1} cm^{-3}). Seasonality did significantly ($P < 0.001$) influence the distribution of SMB down the soil profile,

with Season 1 (7.60 ± 2.07 mg C $_{microbial}$ cm^{-3}) having a higher average SMB down the entire soil profile, compared to Season 2 (1.15 ± 0.24 mg C $_{microbial}$ cm^{-3}), which also had a decreasing SMB to depth.

Cropping status significantly affected the distribution of both average SMA ($P = 0.024$) and average SMB ($P < 0.001$) down the soil profile. Soil microbial activity was highest in-crop at 15-30 cm (5.72 ± 1.28 µg CO_2 h^{-1} cm^{-3}), followed by post crop at 15-30 cm and pre crop 30-50 cm (3.61 ± 0.59 µg CO_2 h^{-1} cm^{-3} and 3.82 ± 0.47 µg CO_2 h^{-1} cm^{-3}, respectively), with pre crop 15-30 cm recording the lowest average SMA (2.62 ± 0.30 µg CO_2 h^{-1} cm^{-3}). SMB was significantly higher pre and in crop at 0-15 cm (9.86 ± 2.23 mg C $_{microbial}$ cm^{-3} and 15.57 ± 6.61 mg C $_{microbial}$ cm^{-3}, respectively) in comparison to other crop status and depths. Post crop SMB was the lowest of the crop status, regardless of depth, with an average of 1.34 ± 0.15 mg C $_{microbial}$ cm^{-3}.

The qCO_2 differed significantly with depth ($P < 0.001$), with an increase below 30 cm, especially at 70-100 cm (2.46 ± 0.23 µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) (Figure 21). Crop system did not significantly influence the qCO_2 down the soil profile when $\alpha \leq 0.05$ ($P = 0.072$). However, when $\alpha \leq 0.10$, the influence of system on qCO_2 with depth, does highlight significant differences in values in the top 0-15 cm, with CM (2.55 ± 0.47 µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) having a qCO_2 value over 1.5 time great than CC (1.51 ± 0.29 µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) (Figure 2B). Seasonality effected the qCO_2 values with depth ($P = 0.009$), particularly at 30-50 cm, with Season 2 having an increased qCO_2 value (2.72 ± 0.40 µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) compared with Season 1 (1.90 ± 0.30 µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) at the same depth (Figure 2C). Overall, Season 2 trended in higher qCO_2 values until 50 cm, where deeper soils had similar qCO_2 values, regardless of season. Crop status also had a significant influence on the qCO_2 values to depth ($P = 0.001$), with increased values evident pre (70-100 cm) and post (0-15, 30-50, 50-70 and 70-100 cm), whilst in crop values were significantly lower (0-15, 30-50, 50-70 and 70-100 cm) (Figure 21). Interestingly, there was no significant difference between qCO_2 values at 15-30 cm, regardless of crop status.

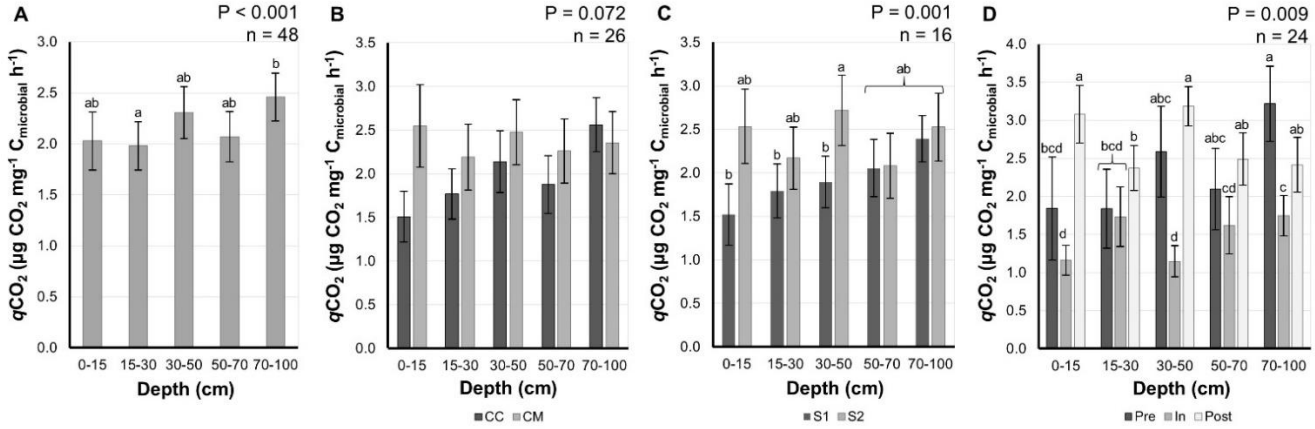

Figure 21. The average microbial metabolic quotient (µg CO_2 mg^{-1} $C_{microbial}$ h^{-1}) by depth (A), crop system by depth (B), season by depth (C) and crop status by depth (D), down the down profile. Significant differences between values are indicated by lower case letters ($\alpha \leq 0.05$) and the average number of samples indicated by 'n'.

All average $\delta^{18}O_{P\ HCl\ V\text{-}SMOW}$ signatures fell within the previously determined equilibrium range of 12.8 ‰ to 17.2 ‰ (Polain *et al.*, 2018) (Figure 22). When $\alpha \leq 0.10$, there was a significant difference ($P = 0.098$) in $\delta^{18}O_{P\ HCl\ V\text{-}SMOW}$ signatures by depth (Figure 22), with a difference of 0.90 ‰ (± 0.2 ‰) from top (0-15 cm) to sub (70-100 cm) soil. These signatures were also significantly influenced ($P = 0.06$, $\alpha \leq 0.10$) by crop system at 0-15 cm, with CC having an enriched $\delta^{18}O_{P\ HCl\ V\text{-}SMOW}$ signature of 15.62 ‰ (± 0.40 ‰), compared to 12.77 ‰ (± 0.60 ‰) in the CM system (Figure 22).

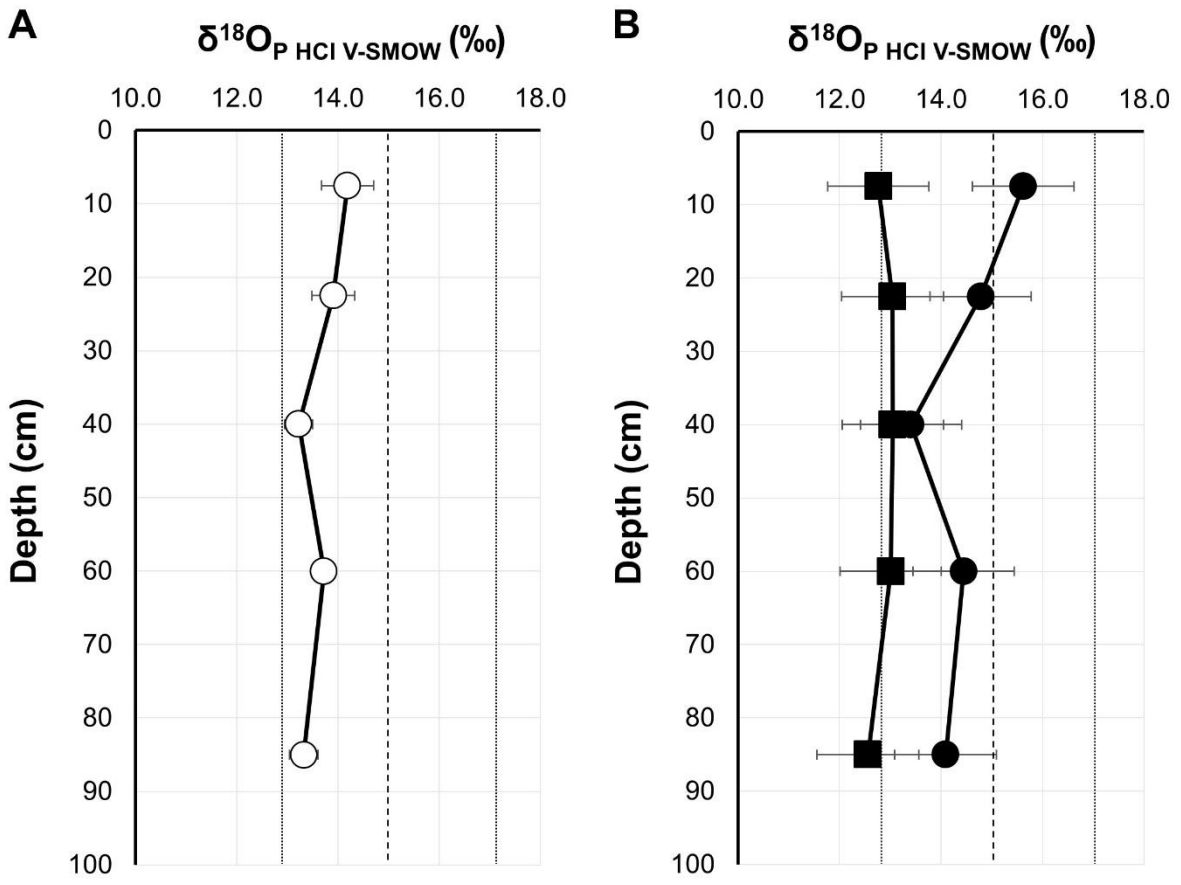

Figure 22. The average $\delta^{18}O_{P\ HCl\ V\text{-}SMOW}$ signatures down the soil profile (0-100 cm) (A) and under continuous cotton (CC ●) and cotton-maize (CM ■) systems (B), errors bars are the standard deviation of the mean. The dotted vertical lines indicated the previously determined equilibrium range of 12.8 ‰ and 17.2 ‰, whilst the vertical dashed lines indicate the previously determined mean equilibrium value of 15.0 ‰ (Polain *et al.*, 2018).

Our work highlights the need to investigate microbial properties beyond the top 30 cm of the soil profile in dynamic soils, such as Vertosols. Whilst our hypothesis of declining microbial activity and biomass with increasing soil depth was upheld, the activity measurements were greater than expected to 50 cm. Our hypothesis that microbial activity and biomass would be enhanced down the soil profile under the CM system was refuted, with no difference in microbial activity between the systems down the soil profile and an increased biomass in the CC system top soils. The overall fluctuations in crop status and seasonality do not appear to affect overall microbial activity, with long-term microbial activity prevalent in sub-soils. The most likely explanations for our observations are intricately tied to the dynamic properties and management of our soils, with their vertical and lateral movements a result of drying and rewetting from irrigations practice allowing, both availability and mobility of soil C down the soil profile, to be utilised by microorganisms.

Sub-soil microbial diversity under rotational cotton and cotton-maize systems

Diversity of microbial species

Depth significantly ($P < 0.001$) influenced the diversity of bacterial (16S) communities, whilst there was no apparent influence by depth on fungal (ITS) community diversity ($P = 0.524$). The average bacterial Gini-Simpson Diversity Index (D_{GS}) decreased from $1.00 \pm 1.90 \times 10^{-04}$ (0-15 cm) to 0.99 ±

1.02 x 10^{-03} (70-100 cm) (Figure 23), whilst average fungal D_{GS} differed by 0.01 from 0-15 to 70-100 cm, with an increase of 0.04 at 30-50 cm (Figure 23).
Crop system significantly (P = 0.025) influenced bacterial community diversity at depth (70-100 cm), with a decrease in dominant species in the continuous cotton (average D_{GS} = 0.99 ± 1.64 x 10^{-03}), compared to cotton-maize (average D_{GS} = 0.99 ± 9.49x 10^{-04}) (Figure 1B). Fungal community diversity down the soil profile was not significantly (P = 0.914) influenced by crop system however, the average DGS trended in being 0.03 ± 0.02 lower under the CM system (Figure 1E). There was no significant difference on the effect of season, crop stage and system on subsoil bacterial (P = 0.830) or fungal (P = 0.443) diversity (Figure 23), with D_{GS} values averaging 0.99 ± 5.08 x 10^{-04} (bacteria) and 0.90 ± 1.37 x 10^{-02} (fungi). However, bacterial community diversity decreased with depth at each crop stage over the two seasons, irrelevant of crop system, except for the notable decreases in diversity in CC at 70-100 cm, pre-crop for both seasons 1 and 2 (Figure 23). Fungal community diversity did not demonstrate a clear depth differences over the two growing seasons however, there was a notable difference between crop system diversity at 0-15 cm pre-crop, season 1, with a decrease in diversity in the CM system (Figure 23).

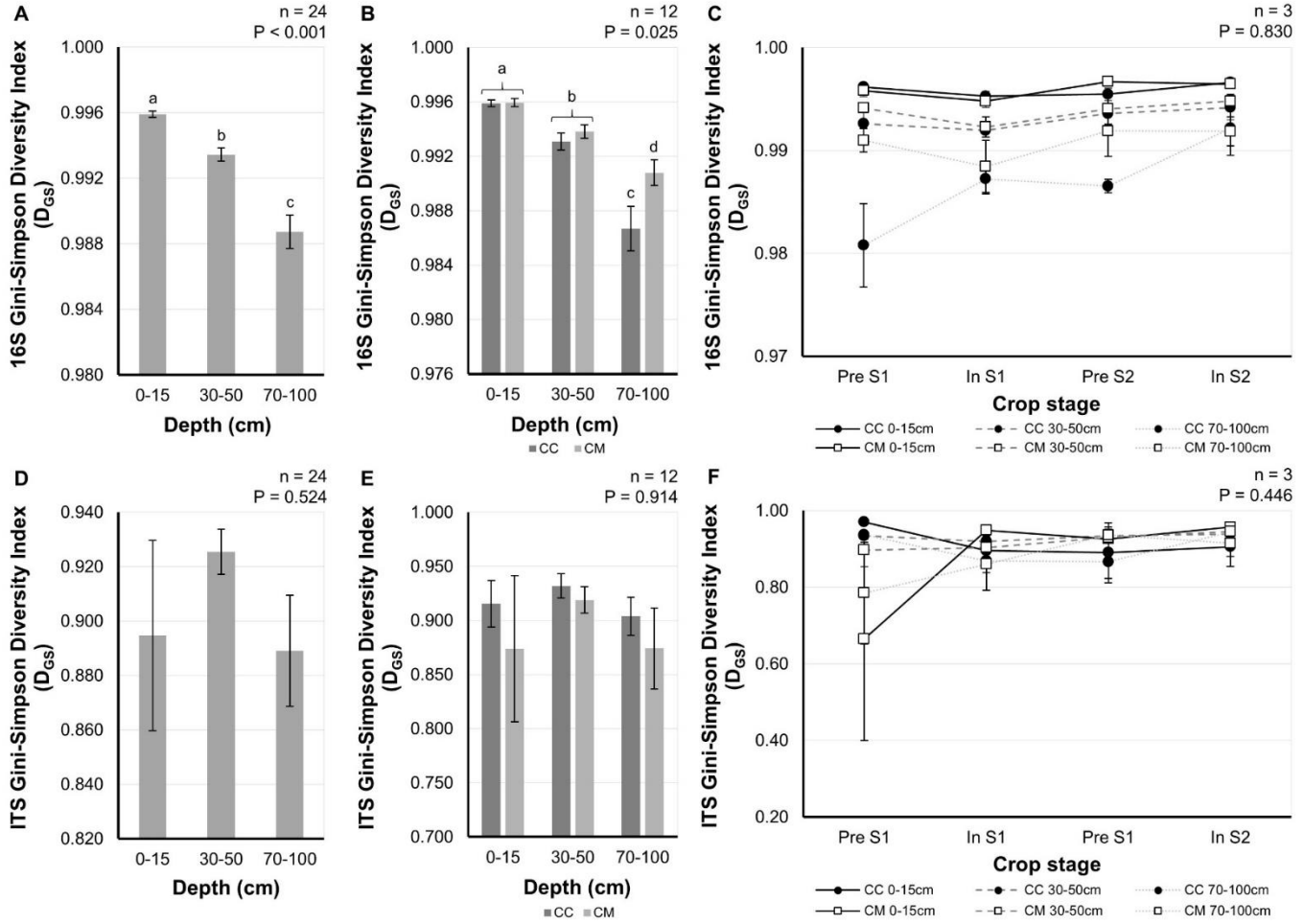

Figure 23. Diversity of bacteria (16S) and fungi (ITS) communities, as affected by depth (**A**, **D**), depth and crop system (**B**, **E**) and depth, crop system and crop stage (**C**, **F**). Lower case letters indicate significant differences (P) when α = 0.05 and average number of replicate samples (n).
Distribution of microbial species

Depth significantly influenced the distribution of bacterial and fungal species (both P < 0.001) down the soil profile. Bacterial species distribution equality increased down the soil profile, with the average Gini-Simpson Evenness (E_{GS}) value for bacterial species increased between depths 30-50 cm (3.93 x 10^{-04} ± 1.51 x 10^{-05}) and 70-100 cm (5.14 x 10^{-04} ± 2.33 x 10^{-05}). The distribution of fungal species increased in equality with increasing depth, with average EGS increasing from 0-15 cm (3.20 x 10^{-03} ± 7.22 x 10^{-04}) down the profile to 70-100 cm (8.25 x 10^{-03} ± 7.25 x 10^{-04}).

Crop system did not significantly influence the distribution of either bacterial (P = 0.188) or fungal (P = 0.296) species down the soil profile. The average bacterial E_{GS} value for both CC and CM was $10^{-04} \pm 5.84 \times 10^{-05}$, whilst the average fungal EGS valued differed by less than 0.001 between each system, at each depth. The CM system trended in having a higher level of distribution at 0-15 and 30-50 cm for both bacterial and fungal species, whilst there was a higher level of distribution in the CC system at 70-100 cm.

Season, crop stage and system did not significantly influence the distribution of bacterial (P = 0.297) or fungal (P = 0.521) species down the soil profile, with average E_{GS} of $4.42 \times 10^{-04} \pm 4.05 \times 10^{-05}$ (bacteria) and $5.56 \times 10^{-03} \pm 4.31 \times 10^{-04}$ (fungi). The distribution of bacterial species was relatively consistent across system, crop stage and season, with a slight separation by depth, where the distribution had a higher E_{GS} in the sub-soils (Figure 2C). The distribution of fungal species displayed higher variability across all factors, with some separation by depth evident, due to an overall increase in sub-soil E_{GS} values.

Microbial community composition

The average number of 16S OTUs (3614 ± 736) was significantly higher (P < 0.001) than the average number of ITS OTUs (229 ± 127) for the duration of the experiment, with an average 16S to ITS ratio of 16:1. This ratio significantly varied (P = 0.001) by depth, with the number of 16S OTUs increasing with increasing depth (9:1 at 0-15 cm, 13:1 at 30-50cm and 15:1 at 70-100 cm) however, these ratios were not affected by rotation. Similarities between bacteria and fungi community profiles significantly decreased (P < 0.001) between the depths of 0-15 cm and 70-100 cm, with Sørensen's Index of Similarity (I_S) values of 0.77 and 0.53 for bacterial and fungal communities, respectively. At each depth, Acidobacteria (14-19%), Actinobacteria (29-49%) and Proteobacteria (14-27%) were the prevalent bacterial phyla (Figure 24), with Ascomycota (47-61%) and Basidiomycota (19-35%) being the prevalent phyla for fungi (Figure 24). Crop system did not significantly influence bacteria and fungi profiles with depth, however, I_S values between CC and CM systems decreased by 0.08 (bacteria) and 0.16 (fungi) from 0-15 to 70-100 cm.

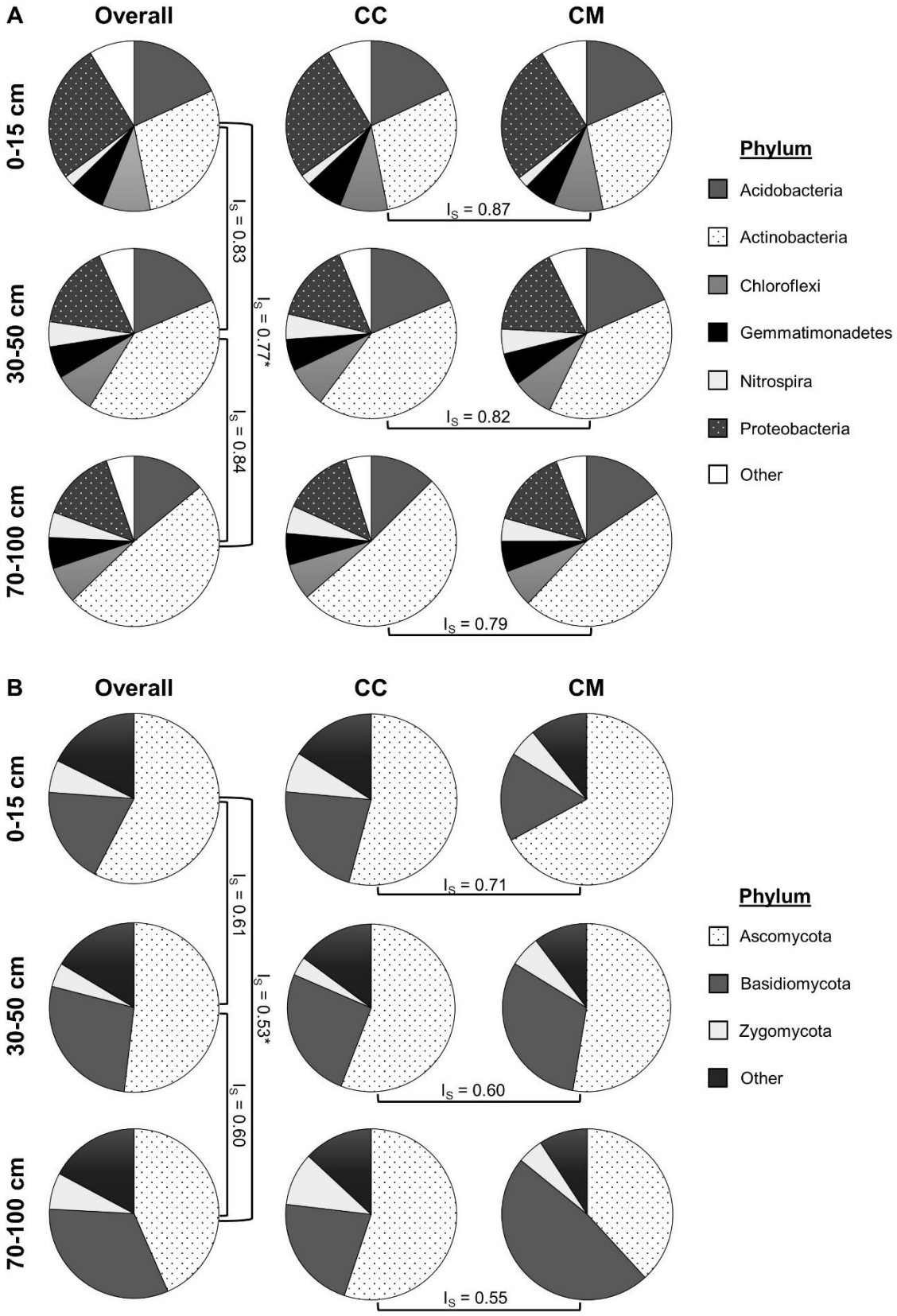

Figure 24. Microbial diversity profiles for bacteria (**A**) and fungi (**B**) down the soil profile under continuous cotton (CC) and cotton-maize (CM) crop systems. Similarities between microbial communities at each depth (overall and by system), are denoted by the Sørensen Index of Similarity (I_S) values, with an asterisk (*) indicating a significant difference of $P < 0.001$ when $\alpha = 0.05$.

Figure 25 An overlay of the RTK GPS positions of the cone penetrometry sites take in May 2017. Similar results were plotted with each sampling. The step in the samping (toward the top of the image) was due to the lysimeter, which was located in plot 16. There are actually three pins at each point (two furrow and one hill), but the field boundaries are lost with further magnification.

Soil resistance across the whole dataset

The model of the entire data-set indicated significant increases in soil resistance in plots incorporating a wheat rotation compared with continuous-cotton. The furrows had significantly higher resistance than the rows, but there was no significant difference between the two furrows (one with high traffic, one without traffic). The model was excellent, explaining 97 % of variance in the data. However, given the assumption that the resistance under wheat rotation was >6600 kPa at all depths in 2016 following wheat (the soil was too hard for insertion), this result should be treated with caution.

After limiting the data to the minimum tillage plots only, the effect of rotation was enhanced. It was the only significant factor in the soil resistance for this data set and accounted for 98 % of variance. Once again, soil resistance was significantly higher under cotton-wheat rotations than under continuous cotton rotations.

The investigations limited to the continuous cotton rotations revealed that the only significant effect was a lower resistance in the rows than in the furrows. In contrast to the effect of wheat in rotation, this model was poor, explaining only 14 % of variance.

Soil resistance in the top 6-15 cm

Across all plots, the models confirmed the effects of wheat rotation and location in row or furrow obtained for the entire data set. Additionally, minimum tillage plots had a significantly lower resistance than maximum tillage plots. A possible effect of

maize incorporation reducing resistance was indicated ($p<0.1$). Once again, the model was very good, accounting for 94 % of variance in the data.
After limiting the data to the minimum tillage plots only, the model confirmed the importance of wheat incorporation on increasing soil resistance. Location in row or furrow was also important, but the difference was in traffic load: there was a significantly higher resistance in the furrow with high traffic than in the furrow with low traffic, but the difference between the coefficient of the low traffic furrow and the row was not significant. The model was very good, accounting for 94 % of variance in the data.
The investigations limited to the continuous cotton rotations revealed that both tillage and location were important, with maize being potentially important ($p<0.1$). Minimum tillage plots had significantly lower resistance at the surface than maximum tillage plots, and the rows had significantly lower resistance than the furrows. The model explained 50 % of variance.

Soil resistance in the bottom 70-75 cm

In contrast to the surface soils, the model of the greatest depth across all sites (70-75 cm, all plots) confirmed only the effects of wheat rotation, which resulted in higher resistance than continuous cotton. The incorporation of maize also had a significant effect, reducing the resistance slightly compared with plots without maize. There was no effect of location (row vs. furrow) or tillage on the soil resistance at this depth. Once again, the model was very good, accounting for 92 % of variance in the data.
After limiting the data to the minimum tillage plots only, the model confirmed the importance of wheat incorporation on increasing soil resistance at depth. Location in row or furrow was not important, but maize incorporation resulted in a significant reduction in resistance in the subsoil compared with plots without maize incorporation. The model was excellent, accounting for 95 % of variance in the data.
The investigations limited to the continuous cotton rotations indicated that only maize incorporation was important, with maize in rotation significantly reducing soil penetration. However, the model explained only 7 % of variance, indicating a small effect.

Traffic load and proximity to irrigation ditch

The models of the position in field (proximity to irrigation ditch) and the traffic load showed no impact of irrigation ditch proximity on soil resistance. There was, however, a significant effect of traffic load on soil resistance, with furrows receiving higher traffic loads having greater resistance than furrows receiving less or no traffic. This effect was apparent in both tillage treatments. The models explained between 16 and 28% of variance.
Tillage appears to lower the soil resistance, but only at the surface. In contrast, maize incorporation appeared to result in a small, but significant, reduction on subsurface resistance. The effect of traffic was only apparent in the minimum tillage plots, with the maximum tillage plots not resulting in any difference between furrows of different tillage loadings. This is likely a result of the complete reformation of the beds in the maximum tillage plots. The effects of tillage are only discernible near the surface.

Physical constraints to microbial activity

Both results from stable isotope analysis and SEM analysis indicate a potentially important role of DOC in SOC storage and stabilisation in the subsoil in cotton-based cropping systems. Here, we assessed how microbial respiration and microbial

response to added DOC were affected by physical constraints, in particular, by differences in bulk density. The reason for this was to link the physical monitoring back to the microbial observations and bring these aspects of the project together.

Bulk density (BD)

Bulk density differed between HCS, and differed between the sampling, most likely due to the field operations, as observed with pentrometry. Nevertheless, BD increased with depth, and was consistently lower in Max Till/CC than Min Till/CC and Min Till/CW in the topsoil (Fig. 26).

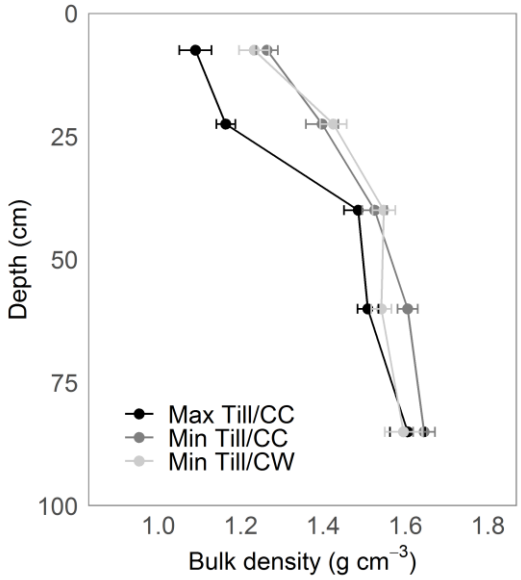

Fig. 26 Bulk density of soil under Max Till/CM, Min Till/CM and Min Till/CWM measured at the end of the full treatment cycle (Oct 2017). The horizontal bars are the mean's standard errors (n=4).

Physical treatment effects on microbial respiration

Basal respiration decreased along a BD gradient (i.e. disturbed, no adjustment, slightly compacted and compacted, $P < 0.0001$) and depth ($P < 0.0001$), and BD treatment and depth also interacted with each other ($P < 0.0001$, Fig. 27a). Basal respiration was generally the highest in the disturbed samples, followed by non-adjusted control, slightly compacted and compacted samples at all depths. While the effect of BD treatment was generally greater at 0–15 cm depth, each BD treatment differed slightly in their effects on basal respiration along the soil profile. There were no differences between the depths in basal respiration among compacted soil samples, while the disturbance fluctuated with depth. When basal respiration was normalised by SOC content, the respiration was higher at the lower than the upper soil depths (Fig. 27b), and the effect of depth interacted with BD treatment ($P < 0.0001$) where the magnitude of BD treatment was the greatest at 0–15 cm than the other depths.

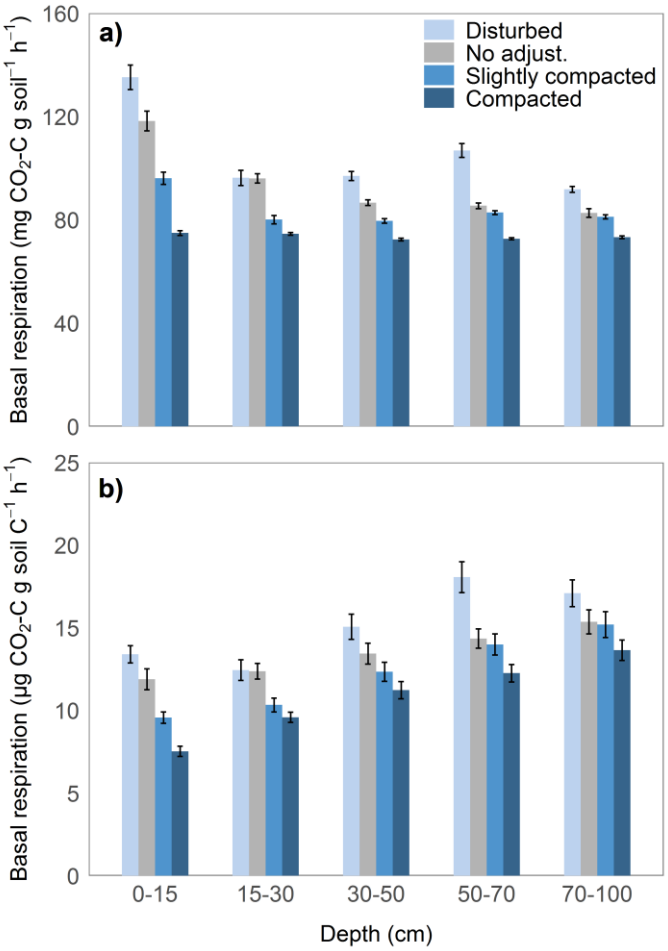

Fig. 27 The effect of bulk density treatments on basal respiration (a) and basal respiration per unit of soil C (b) of soil samples collected from the experimental field based on the average values and standard errors across the field treatments.

Physical treatment effects on respiration response to added DOC

While the study was conducted with cotton-derived DOC and maize-derived DOC separately, there were no differences in microbial respiration response between them. Therefore, the results were pooled across the DOC type.

Respiration response to added DOC under BD treatment showed different patterns to that of basal respiration, although the effect of depth remained the same. Disturbance reduced the respiration response by 12.5% on average compared to the non-adjusted control at all depths, while compaction strongly reduced the respiration response by 34.4% but only when compacted to the bulk density of 1.5 g cm^{-3} (Fig. 28a). Slight compaction of 1.0 g cm^{-3} had no effect on the respiration response to added DOC at any depth. Both disturbance and compaction effects were generally stronger at the upper soil depths than the lower soil depths (DB × Depth interaction, $P < 0.0001$). When normalised by SOC content, respiration response to DOC was slightly but statistically higher at the lower depths ($P < 0.0001$, Fig. 28b), which was most pronounced in samples compacted to the bulk density of 1.5 g cm^{-3} (DB × Depth interaction, $P < 0.0001$).

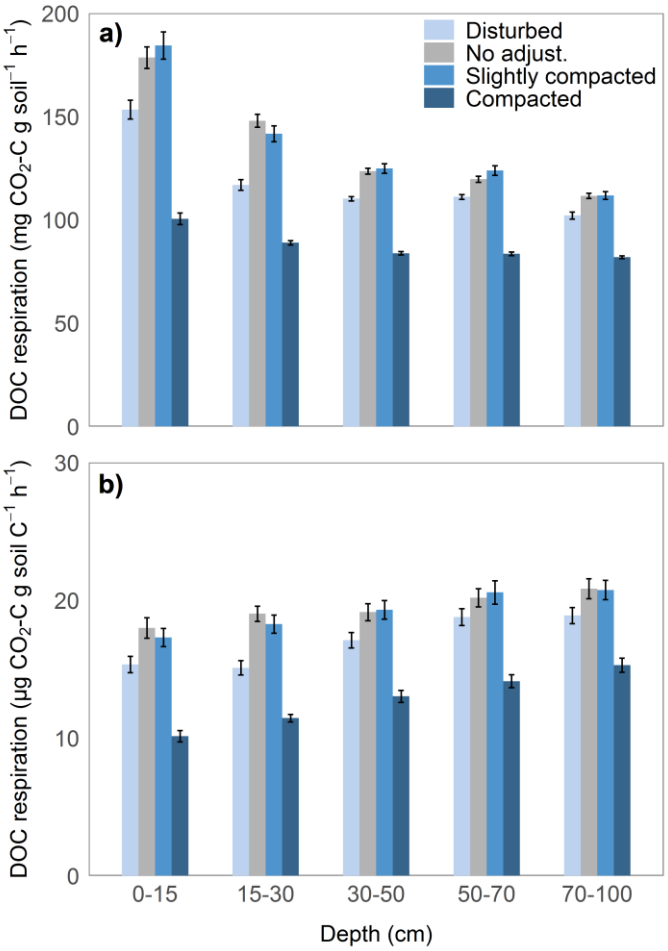

Fig. 28 The effect of bulk density treatments on DOC-induced respiration (a) and DOC-induced respiration per unit of soil C (b) of soil samples collected from the experimental field based on the average values and standard errors across the field treatments.

Potential respiration response vs. estimated field response

Estimated respiration response to added DOC differed significantly between HCS and depth ($P < 0.0001$ for both, Fig, 29a), which interacted with each other ($P = 0.001$). The effect of HCS was stronger in the upper soil profile, where Max Till/CC had the highest respiration response to DOC (mean = 165±5 mg CO_2-C g soil $^{-1}$ h $^{-1}$), followed by Min Till/CW (mean = 144±6 mg CO_2-C g soil $^{-1}$ h $^{-1}$) and then by Min Till/CC (mean = 118±5 mg CO_2-C g soil $^{-1}$ h $^{-1}$) at 0–15 cm depth. At 15–30 cm depth, the difference between Min Till/CW and Min Till/CC disappeared while Max Till/CC still maintained the highest respiration response. The effect of HCS on the respiration response at the lower depths were either small or non-significant. At 50–70 cm depth, there was a small but significant difference between HCS where the respiration response of Max Till/CC was higher than that of Min Till/CC, with that of Min Till/CW not being different from either of them.

Respiration response of non-adjusted control samples to added DOC was used as the potential respiration response to examine the effect of field treatments on respiration response. Potential respiration response was generally higher than the estimated respiration response by 41% on average, particularly in the lower soil depths (Fig. 29a, b). Potential respiration response differed significantly between HCS ($P < 0.0001$, Fig, 19b), which interacted with both maize treatment ($P < 0.0001$) and depth ($P = 0.01$). Potential respiration response to DOC were the highest in Min Till/CW and Max Till/CC (mean = 193±5 and 191±9 mg CO_2-C g soil $^{-1}$ h $^{-1}$, respectively) than Min Till/CC (mean = 153±8 mg CO_2-C g soil $^{-1}$ h $^{-1}$) at 0–15 depth, and the effect of HCS remained the same for 15–30 cm depth. No differences between HCS were observed below 30 cm depths.

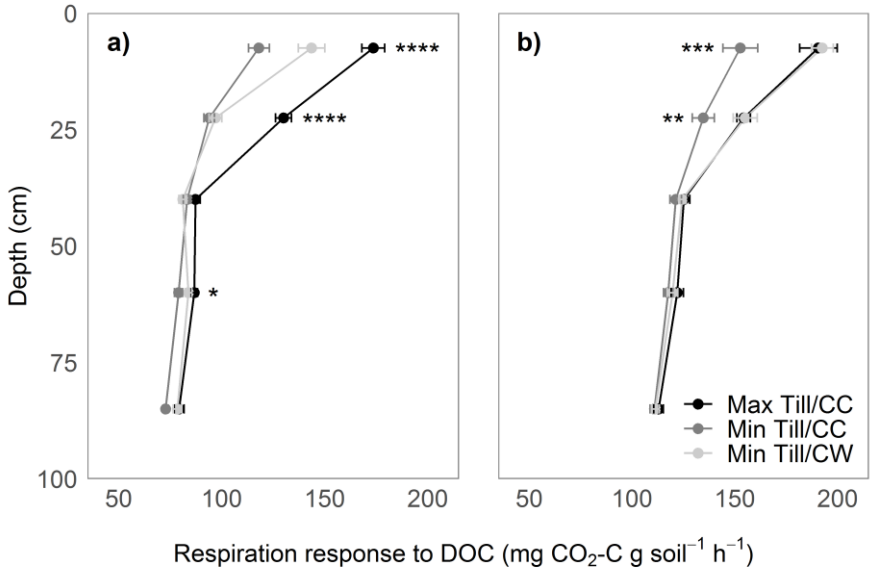

Fig. 29 Estimated respiration response at field bulk density (a) compared to potential respiration response measured in the absence of bulk density treatment (i.e. non-adjusted control samples).

These results suggest that under field conditions, respiration response to labile substrate supply will be low in the deeper soil profile due to both physical and substrate constraints. The contrasting effect of disturbance on basal and DOC-induced respiration also suggests that there is a complex interplay between physical and biological processes in regulating C fluxes. Our study demonstrates that soil physical conditions modulate microbial responses to substrate availability, and that agricultural practices that affect physical conditions can have a significant impact on C dynamics and sequestration in these soils.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

Key question(s) to be researched in project *Eg. Extension-How can we assist in extending knowledge about safe use of pesticides?*	**Expected Outputs** *Eg. A number of workshops are organised asking entomologists to present & discuss finding with growers*	**Expected Science Outcomes** *(NB: A direct science outcome might not be applicable for all extension*	**Outputs from UNE 1601** *and contribution to Outcomes*	**Outcomes achieved to date**
What are the sources and mechanisms by which soil C and N are distributed and cycling optimised in the whole-soil-profile of cotton farming systems and how can this be optimised?	2 x on farm field days, plain English factsheet, dissemination of information to farmers via social media.	Clear understanding of the mechanisms C and N cycling in the whole soil profile for more efficient storage, turnover and management.	Oliver has contributed to several field days and a nitrogen tour where he has presented work around N. Both Yui and Katherine have presented at national and international, in Katherine's case, work relating to C and also P distribution and cycling. C and N have been the subject of associated Spotlight articles and the P cycling work published. All activity has been promoted via social media (Facebook and Twitter).	Yui's latest modelling outlines some clear differences in surface to subsurface (30-100 cm) C cycling processes. The work is supported by Katherine's microbial activity determinations and respiratory quotient assessments. Diversity in our crop rotation appears to be capable of adding C to our soil systems, but the type of C and its final fate in this process will vary depending on where in the profile the soil is assessed. Several of these processes are

				also linked to the inorganic N in the soil and the C:N ratios.
What is the potential of cotton soil systems to capture additional soil carbon and reduce N losses?	2 x on farm field days, plain English factsheet, dissemination of information to farmers via social media.	More complete understanding of the practices that promote efficient C and N cycling through the whole-profile of soils under cotton management systems.	Yui has presented findings on the C work throughout the project at AACS conferences, the Cotton Conference and has submitted publications summarising the work in addition to a Spotlight article (Winter 2018). Katherine has presented aspects of the microbial work, which touches on C and N cycling, at several conferences, whilst Oliver has been involved in workshops and field days regarding N management. Oliver and Katherine have contributed to Cotton Hub social media exposure around these events.	Outputs have been generated with more in the pipeline. Extension activities have been completed and there has been industry engagement over the potential for changes, particularly to soil C, from the studied rotations.
What are the soil constraints affecting root exploration of the soil	On farm feedback to growers and mitigation discussions.	Investigation of potential to extrapolate capacitance data to developing soil	Brendan and Oliver have presented at several field days, which have	Confidence and expansion of the use of capacitance probes

and causing irrigation recharge issues on cotton farms around Goondiwindi?		constraints across soils supporting cotton.	included several soil pits on the implications for rooting constraints and soil management. They have presented conference presentations on this work.	appears to still be growing across the cotton producing valleys despite the down turn in hectares due to drought. There is awareness that this technology can inform rooting depth and more growers and consultants are now seeking identification of the constraints behind there (evidence in personal communication from PCT).
What potential exists to manipulate cotton exudates through simple management to affect pathogen interactions and beneficial soil functional processes?	Identification of exudates from current commercial cotton varieties and the potential to manage these to the advantage of cotton production	Influence of agronomic management on cotton exudates and the potential this has to manipulate pathogen interactions and soil mineralisation processes	Hamid's work supports that there is some deviation in cotton exudates toward black root rot, but only Sicot 730 appears to offer a reduced attractiveness. Oliver has been unable to produce a definitive cotton root exudate from working with chemist and NMR. Oliver has also shown that cotton exDNA is relatively low compared to pea and	There appears to be similarity in cotton exudates, even without actual chemical profiles, but the responses in spinach suggest ideas around sugar to selectively train a rhizosphere or endosphere has potential to offer plant improvements.

			maize and also unresponsive to wilts. Upama in her thesis demonstrated improved beneficial colonisation of spinach with just a simple sugar in the seed coat.	

6. **Please describe any:-**
   a) **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**
   None
   b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
   The structural equation modelling adds new insights into the processes involved in the development of more stable forms of carbon under the rotations studied. This adds potential to developing better understanding of the impact of crop rotations on halting, or possibly remediating, soil carbon loss.
   The microbial assessment indicates that in this agricultural system, and possibly others, the amount of microbial activity and the location within the profile has been underestimated. This has implications for the amount of C potentially lost form agricultural systems as well as developing a more accurate understanding of nutrient cycling and just how cotton attains the nutrients that current yields dictate for the plant.
   The non-use of certain lubricants in sampling soils for carbon and microbial work has been a major insight from this work and the reporting of this has had other researchers approach members of the group to use similar techniques to establish the fate of herbicides in different soils and also micro-plastic impacts on soil microbiology.
   The 'podger' and spacer system designed and built in this project to examine the effects of soil bulk density on microbial function using the MicroResp™ system is unique, but we plan to disseminate the design via publication rather than protect the design as the global demand for such a tool would be minimal.
   c) required changes to the Intellectual Property register.
   None

Conclusion

7. **Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?**

- Maize rotation increased SOC stock in Min Till/CW system, particularly in the subsoil.
- Vertical movement of C in the form of DOC is the likely mechanism for the increased SOC in the subsoil.
- A strong correlation between SOC and soil N indicates that C and N dynamics are interlinked.
- Soil inorganic N availability is higher in Min Till/CC system, particularly without maize rotation.
- Agricultural management impacted SOC storage differently between the topsoil and the subsoil.
- Future research should explore the movement of C in the soil (i.e. leaching, root exudates), and the role of DOC in C stabilisation via microbial interactions or mineral interactions and how the relative importance of these interactions may change along soil profile.
- Microbiology can be as active in 30-100 cm sub-soil of the cotton system profile as it is in the 0-30 cm.
- The biomass of the biology is still higher in the top 30 cm of the profile, but the impact of the impact of C inputs to this biomass may result in different metabolic processes.

- Diversity is not as influenced by rotational changes as hypothesised, implying that there may be greater resistance and resilience in the system than previously presumed.
- The extent of sub-soil compaction issues that affect cotton rooting is evident in about 25% of fields in which cotton is currently grown.
- Whilst sodicity appears to be the major constraint it is not always the most limiting chemical aspect of the soil, which highlights the need for proper investigation prior to potential remediation.
- Microbial respiration response is reduced under compaction. Soil physical conditions modulate microbial responses to substrate availability, and that agricultural practices that affect physical conditions can have a significant impact on C dynamics and sequestration in cotton-based cropping systems.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

We plan to continue to use the MicroResp technique that has been key to some of the delivery aspects of this project.

(b) for the future presentation and dissemination of the project outcomes.

Both Katherine and Yui are presenting at the Soil Organic Matter International conference in Adelaide in October of 2019. Yui is also presenting at the AACS conference in Armidale in October of 2019. These are the final official presentations from this work.

Oliver has received advice on how to attempt to attain copyright and publish 'The Very Hungry Helicoverpa', which was written after the 2018 CRDC People Forum in Brisbane.

Katherine has prepared a summation of her microbial activity and biomass work, which will hopefully be submitted in October. Her work on profile microbial diversity is also planned for submission before the end of the year. Her nitrogen cycling work is complete, but awaiting analysis and writing up, but this is planned for late 2019 early 2020 and her thesis will then be completed with a viewpoint that encompasses aspects of a literature review.

Oliver has been discussing with Brendan how we publish the capacitance data, given we cannot present actual maps of the farms we have assessed and there is also scope to develop and deliver the work on traffic and compaction, either as part of this or a standalone. This latter aspect has been marked as low priority for now.

Oliver and David Backhouse are going to pursue publication of one of Lily's last papers, which contains part of Hamid's thesis work on black root rot and cotton exudates.

(c) for future research.

Yui and Oliver will pick up aspects of this project and UNE1403 in the new project UNE2001. We hope to continue to use students to support peripheral aspects of the research and address some of the questions that the observations made during this project raise.

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

See Part 3 of this report, under objectives, number five 'Paper production, industry engagement and conference attendance'

B. Have you developed any online resources and what is the website address?

No online resources have been developed to support this project, but aspects of the work has been extended via social media and blog posts via the handles @CottonHubUNE and on https://www.une.edu.au/research/research-centres-institutes/cotton-hub

Part 4 – Final Report Executive Summary

Provide a one-page summary of your research that is not commercial in confidence, and that can be published on the internet. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

UNE1601 came about from a merger of two proposals. One proposed to develop a more detailed understanding of C under cotton, whilst the other focused on the potential for rotations to modify the soil microbial biology. To address the combined goals of the project, work was undertaken at ACRI on the long term rotational trial managed by Dr Guna Nachimuthu and broken down into three areas of investigation. These were; (i) Mechanisms of whole-profile C and N cycling, (ii) Microbial processes in the soil profile, and (iii) Below ground agronomy and constraints to plant growth.

Addressing the 'Mechanisms of whole-profile C and N cycling' became the focus for Dr Yui Osania. Her research in this area establishing that the maize rotation increased SOC stock in a Min Till/CW system. The mechanism for this was likely to be vertical movement of C in the form of DOC and that a strong correlation between SOC and soil N was evident, which indicated that C and N dynamics are interlinked. The conclusions from this were that agricultural management impacted SOC storage differently between the topsoil and the subsoil and that future research should explore the movement of C in the soil (i.e. leaching, root exudates), and the role of DOC in C stabilisation via microbial interactions or mineral interactions throughout the soil profile.

'Microbial processes in the soil profile' became the area of focus for Katherine Polain's PhD candidature. Katherine's work looked at both short and long term influence of the cotton rotaion under minimum tillage and found that microbial diversity was not influenced by rotational changes, which implied that there may be greater resistance and resilience in the system than previously presumed. Katherine's work also highlighted that whilst the microbial biomass may be higher in the top 30 cm of the profile the activity can be as high in the 30-100 cm sub-soil of the cotton system profile as it is in the top. The implications from these observations are that we may need to look deeper in our systems if we want to truly understand the nutritional cycles that feed our plants nutrient demand and that might limit the losses of C from our soils.

The final aspect of the project was the 'Below ground agronomy and constraints to plant growth', which initially had to become more aligned to other projects when the appointed candidate had to withdraw and could not be replaced. Oliver, Brendan, Katherine, Guna, Brian and Yui did their best to address this aspect of the project. The associated work showed that ~25% of cotton fields are affected by sub-soil constraint, whilst the work at ACRI clearly established that furrow traffic led to soil compaction and with this a loss in microbial activity. Field vehicular traffic remains a necessity of our production systems, but the work done here adds to the number of proponents pushing for farms not only to move to minimum tillage, but controlled traffic farming systems.

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