

More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints

Final report for research project 4.2.003

Brian Dzoma and Nigel Wilhelm

March 2024



PROJECT PARTICIPANTS

SARDI, a division of PIRSA (South Australian Research & Development Institute, Department of Primary Industries and Regions, South Australia)

NSW DPI (Department of Primary Industries, New South Wales)

AIR EP (Agricultural Innovation and Research Eyre Peninsula)

MFMG (MacKillop Farm Management Group Inc)

DISCLAIMER

Any opinions expressed in this document are those of the authors. They do not purport to reflect the opinions or views of the Soil CRC or its partners, agents or employees.

The Soil CRC gives no warranty or assurance and makes no representation as to the accuracy or reliability of any information or advice contained in this document, or that it is suitable for any intended use. The Soil CRC, its partners, agents and employees disclaim any and all liability for any errors or omissions or in respect of anything or the consequences of anything done or omitted to be done in reliance upon the whole or any part of this document.

PEER REVIEW STATEMENT

The Soil CRC recognises the value of knowledge exchange and the importance of objective peer review. It is committed to encouraging and supporting its research teams in this regard.

The author(s) confirm(s) that this document has been reviewed and approved by the project's steering committee and by its program leader. These reviewers evaluated its:

- originality
- methodology
- rigour
- compliance with ethical guidelines
- conclusions against results
- conformity with the principles of the [Australian Code for the Responsible Conduct of Research](#) (NHMRC 2018), and provided constructive feedback which was considered and addressed by the author(s).

KEYWORDS

Calcareous soils, soil constraints, phosphorus, carbon coated mineral, iron deficiency, cropping

CITATION

Dzoma, B. & Wilhelm, N. (2024). More profitable crops on highly calcareous soils by improving early vigour and overcoming constraints, *Project 4.2.003 Final Report*, Cooperative Research Centre for High Performance Soils (Soil CRC).

Contents

Executive Summary	4
Introduction	6
Background.....	6
Methodology	11
Communication	15
List of Publications	17
Results	19
Discussion.....	37
Conclusion	40
Recommendations.....	42
Acknowledgements.....	43
References.....	43
APPENDIX A	46
APPENDIX B	56
APPENDIX C	61

Executive Summary

This report summarises findings of the project 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints', from four years of activities where the first three years were initially contracted and then extended for a fourth year.

Highly calcareous soils are common in some areas of south-eastern Australia and challenge crop production with a range of constraints. The challenges limit the effectiveness of improved agronomic practices developed elsewhere. This collaborative project, which started in 2020, aimed to identify and overcome calcareous soil constraints in order to lift crop production on these difficult soils. Replicated field trials were conducted on the upper Eyre Peninsula (EP) and in the South East (SE) of South Australia (SA) from 2020 to 2023 to investigate impacts of subsoil and topsoil amelioration strategies.

Findings from the work:

- Short-term topsoil strategies resulted in better gains in crop biomass and yield when compared to longer-term subsoil strategies.
- A carbon-coated mineral (bespoke biochar) applied in the topsoil improved crop vigour, biomass and grain yield as well as provided benefits to the following crop.
- Increasing seeding rates and nutrition at sowing was effective at achieving high plant densities, crop biomass and grain yield.
- High soil strength is an issue in calcareous soils, however, positive responses to deep ripping are less likely and usually limited by the hostile subsoil.
- On very highly calcareous soils, improving the fertility of subsoils has the potential to improve grain yield, but responses only started showing in the third crop after application compared to the control.
- Correcting severe yellowing in broad beans with multiple applications of iron chelate (EDDHA) resulted in more vigorous growth during winter and large grain yield increases on a highly calcareous soil in the SE region of SA.

Our findings may have direct applicability to other similar regions, despite considerable differences in farming systems and agroclimatic conditions. Addressing multiple constraints in calcareous soils using integrated solutions will ensure an increase in crop productivity and subsequently profitable and sustainable farming systems.

OBJECTIVES

The main aim of this research initiative was to identify and overcome the impacts of topsoil and subsoil conditions of highly calcareous soils on crop production in the SE and upper EP of SA. A holistic approach to addressing these constraints to crop productivity and profitability was implemented to better understand:

- The benefits of organic matter and extra nutrient inputs on crop health and growth.
- The impacts of a range of amendments on subsoil properties typical of Calcarosols.
- The contribution of novel approaches (carbon coated minerals [CCM]) to phosphorus (P) supply, crop vigour and root health.
- Current problems with crop emergence and fertiliser toxicity under marginal moisture conditions.

- The impact of high soil strength and the contribution of a physical intervention to ameliorate compaction.
- The role of manganese (Mn) and/or iron (Fe) deficiencies in broad beans on highly calcareous soils in the SE of SA.

Outcomes of this project are focused on the development of management strategies which improve soil condition, crop early vigour, crop productivity and farm profitability.

RESULTS

- Higher seeding rates and nutrition at sowing is effective at achieving high plant densities, crop biomass and grain yield.
- CCM in the topsoil improved crop vigour, biomass and grain yield
- Short-term topsoil strategies resulted in better gains in crop biomass and yield when compared to longer-term subsoil strategies.
- High soil strength is an issue in calcareous soils but positive responses to deep ripping are not common and are usually limited by the hostile subsoil.
- Soil amendments had little impact when placed deep during ripping, so amelioration of these soils has little merit.
- Crops growing on a mildly calcareous soil were less responsive to the strategies investigated than those grown on highly calcareous soils of upper EP.
- Severe yellowing in broad beans was overcome with multiple applications of an iron chelate (Fe-EDDHA) and resulted in large grain yield increases. Applications of Mn did not improve bean production.

NEXT STEPS

A research proposal has been submitted to the CRC and will seek to build on the legacy of this project 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints' – Soil CRC code 4.2.003 and Grains Research and Development Corporation (GRDC) agreement HPS2006-001OPX. This proposal is currently being considered as an out-of-session proposal and by GRDC for co-funding. This project will add value to the overall aim of the SoilCRC Program 4 by providing integrated and sustainable soil P management strategies to farmers and advisers dealing with highly calcareous soils.

The Fe component of the work was submitted to SAGIT as a new project in their current funding call but was unsuccessful. Negotiations are under way with GRDC as an alternative funding source.

TIMING

If approved, the P management project will commence in August 2024 with glasshouse pot experiments and with field work starting in 2025.

The Fe component will commence field work in 2025.

Introduction

The main aim of this research initiative was to identify and overcome the impacts of topsoil and subsoil conditions of highly calcareous soils on crop production in the South East (SE) and upper Eyre Peninsula (EP) of SA. A holistic approach to addressing these constraints to crop productivity and profitability was implemented to better understand:

- The benefits of organic matter and extra nutrient inputs on crop health and growth.
- The impacts of a range of amendments on subsoil properties typical of Calcarosols.
- The contribution of novel approaches (carbon coated minerals [CCM]) to phosphorus (P) supply, crop vigour and root health.
- Current problems with crop emergence and fertiliser toxicity under marginal moisture conditions.
- The impact of high soil strength and the contribution of a physical intervention to ameliorate compaction.
- The role of manganese (Mn) and/or iron (Fe) deficiencies in broad beans on highly calcareous soils in the SE of SA.

Prior to conducting field experiments, a comprehensive literature review (attached) of current knowledge of constraints to crop production on highly calcareous soils was completed by the project team and lodged with the CRC at the end of January 2021. This review summarised the key characteristics of highly calcareous soils, their diagnosis, and the types and extent of constraints to crop nutrient uptake, growth, water-use efficiency and profit. Knowledge gaps in both the scientific and 'grey' literature were highlighted.

Background

Calcareous soils (Calcarosols) are those where calcium carbonate is a large part of the soil composition within at least some layers of the soil profile. Calcarosols occupy about 60% of the cropping soils in south-eastern Australia, and more than 1.1 million hectares (ha) of SA cropping areas are highly calcareous. Within SA, key sub-regions that have a high proportion of highly calcareous soils include upper and western EP and lower Yorke Peninsula (YP) while moderately calcareous soils are prevalent in the crop producing areas of central EP, upper YP, the Murray Mallee and the lower SE.

Highly calcareous soils challenge crop production with a range of constraints and this limits the effectiveness of improved agronomic practices. Early crop vigour is poor and crop production continues to be limited to very low nitrogen (N) and water use efficiencies. This collaborative project funded by the CRC for High-Performance Soils and GRDC brought together a multidisciplinary team with expertise in calcareous soils and/or soil constraints to identify constraints and develop strategies to overcome them.

Outcomes of this project were focused on the development of management strategies which improve soil condition, early crop vigour and crop productivity and profitability. Addressing these constraints and providing practical soil management solutions for farmers and advisers, including the specific soil type targets for this management, will enable an increase in crop productivity and profitability.

This project worked closely with a complementary project funded by GRDC and led by Dr Therese McBeath and Dr V Gupta of CSIRO ('Understanding constraints to crop profits on highly calcareous soils'. CSP2009-003RTX). The CSIRO project investigated water relations, nutrient cycling and microbiology of highly calcareous soils.

PREVIOUS RESEARCH AND LITERATURE

This section is a summary of previous research and literature from a comprehensive literature review conducted at the start of the project and submitted to the CRC as part of the project's reporting in December 2020.

Pathogens and disease

Soil-borne pathogens are one of the critical bottlenecks to plant growth and production in cereal crops grown in calcareous soils. Previous research demonstrated the major impact of disease and nutrient deficiencies on cereal crop yields on calcareous soils at Streaky Bay on EP. Using fumigation in combination with extra fertilisers showed that suppression of pathogens combined with optimised nutrient supply could produce crops at water limited yield potential, removing the 44% yield gap (Gupta et al. 2011). Although improved P nutrition and fumigation on alkaline soils at Minnipa and Franklin also increased yields (by 16–22%), grain yields still only reached 53–73% of water-limited yield potential, suggesting that other constraints had not been addressed (Roget et al. 2004). Research in non-calcareous soils indicated that application of N may alleviate effects of rhizoctonia disease, but such effects have not been investigated in calcareous soils (Wall et al. 1994).

Phosphorus concentration in shoot tissue has been shown to fall below critical ranges in rhizoctonia-diseased plants and since P availability is generally low in calcareous soils, rhizoctonia impacts may be exacerbated in calcareous soils without adequate P supply (Wall et al. 1994). Similarly, zinc (Zn) fertilisation was found to increase tolerance to rhizoctonia disease in *Medicago truncatula* and may play a role in alleviating rhizoctonia impacts in calcareous soils (Streeter et al. 2001).

Organic matter quantity and turnover

Soil microbial activity and biological processes are influenced by chemical (e.g., pH, carbonate associated toxicity, salinity) and physical (e.g., aggregation, pore structure, poor water-holding capacity) properties. It has been suggested that in calcareous soils edaphic factors such as high pH and salt/carbonate toxicities will influence diversity and function of organisms (Rath et al. 2019; Wakelin et al. 2008). Results from a survey of surface soils from more than 25 properties on upper EP over two years indicated that microbial biomass (MB) levels ranged between <100 and 1000 mg C g⁻¹ soil, with a trend of higher MB under pasture-wheat rotation compared to continuous crop systems or remnant vegetation. There was also a positive relationship between soil organic carbon (SOC) and MB carbon (McNeill and Day 2001). An investigation of soil biology diversity and abundance in EP soils indicated that in the alkaline calcareous soils at Port Kenny, MB accounted for only 1.4–1.52% of SOC (Masters 2018). The level of microbial activity and microbial responses to management in calcareous soils can be linked to the composition of soil microbial community, as composition in calcareous surface soils was found to be significantly different to that in non-calcareous alkaline and Mallee soils. For example, calcareous soils in general seem to harbour a higher proportion of fungi relative to bacteria compared to other agricultural soils. Calcareous soils from EP showed lower genetic diversity of bacterial community compared to non-calcareous and Mallee soils (Gupta 2015). In controlled-environment experiments using alkaline highly calcareous soils, application of P fertiliser significantly altered the

microbial catabolic activity and diversity indicating that P availability may be constraining microbial activity (Davey et al. 2021).

Nutrient availability and supply

Nitrogen (N) availability is one of the key factors influencing cereal crop production in rain-fed cropping regions of the EP, including in calcareous soils (Adcock 2005). N mineralised from soil organic matter and crop residues has been shown to make a large contribution (~50%) of cereal crop uptake in rain-fed systems in many agricultural regions of Australia, and is expected to be similar in calcareous soils (Angus and Grace 2017). In a controlled environment experiment, Hancock et al. (2011) showed that while N fertiliser recovery (measured using ^{15}N tracing) was greater in average (decile 5) compared with dry (decile 2) conditions, losses of N fertiliser were lower in these calcareous soils than might be expected, by 13–24%. In the SE, where the calcareous soils are also often rich in organic matter (3–7% organic C) and many farmers are trying to achieve hyper-yielding crops, current N calculators seem to be over-predicting N mineralisation and hence estimating low fertiliser N rates to apply. A key consideration in highly calcareous soils, especially when soil moisture is low, is the risk of fertiliser toxicity. The risk is highest for fertilisers that will release ammonia when added to soil. For example, 23 kg N ha^{-1} as urea placed with the seed-reduced plant establishment in three trials on the upper EP whether the crop was dry sown or sown on the break (Cook and Wilhelm 2019). Another study demonstrated that the minimum critical distance at which fertiliser should be separated from seed in order to avoid fertiliser toxicity was higher in calcareous soils for commonly used fertilisers including diammonium phosphate (DAP) and urea (Dockerill 2019).

Plant P nutrition is a challenge on highly calcareous soils due to strong sorption and slow dissolution processes which reduce P availability to crops (Lombi et al. 2004). Addressing P access and availability in these soils is one way of increasing crop productivity and thus reducing the yield gap in moisture-limited environments like the upper EP. A study was conducted on upper EP comparing a range of fluid fertiliser products compared with granular products in field experiments with spring wheat on highly calcareous grey sandy loams (>50% carbonate) and a calcareous red brown sandy loam (5–10% carbonate). Grain yield was increased by 23% by applying fluid compared with granular fertiliser on the grey calcareous soil and by 19% on the calcareous red brown sandy loam (Holloway et al. 2001). Liquid sources of P fertiliser were shown to be of significant benefit to fertiliser P recovery and crop yield of grey highly calcareous (90% of tests) soils in the late 1990s and early 2000s (Holloway et al. 2001a; Holloway et al. 2007). Under controlled conditions, plants grown in a grey highly calcareous soil from EP, when provided with P in different forms, took 50% of their P from fluid fertiliser but only 30% of their P from granular fertiliser, indicating the efficiency of fluid fertiliser was at least 20% better than the granular product (Bertrand et al. 2000).

Stabilisation of soil organic carbon with iron oxide minerals and biochar

Soil organic carbon (SOC) plays a vital role in maintaining soil quality and ecosystem functions, supporting agronomic productivity and resilience to induced stresses including drought and climate change. By increasing the stabilisation of rhizodeposits, it should be possible to build SOC stocks, and potentially shift the soil microbial community to a more structured community with improved carbon use efficiency (CUE) (Fang et al. 2018). While there is still uncertainty around the exact value to production from improving SOC stocks, estimates have shown that wheat yield can increase by $20\text{--}70 \text{ kg ha}^{-1}$ for every 1 t ha^{-1} increase in the SOC pool in the root zone (Lal 2006). There is, however, a paucity of knowledge on the role of improved SOC stocks in highly calcareous soils. Constraints to

production in calcareous soils that could be addressed through increasing SOC content could include:

- Reducing N volatilisation (via NH_3 and denitrification) by storing more N in the microbial and SOC pools, and through providing adequate protons in soil to enhance ammonium content.
- Increasing available P content by intercepting available P before it is locked up as calcium phosphate, concomitantly increasing the microbial cycling of P.
- Modification of soil redox potential (eH) through biochar application, thus lowering the free energy required for plant nutrient uptake (Chew et al. 2020).
- Modification of the soil microbial community through biochar application. This should directly influence soil organic matter content and improve soil-available N, P, and potassium (K).

Dispersion and alkalinity

The presence of dispersed clay particles in the soil solution can result from the abundance of sodium (Na^+) ions, and to K^+ ions to a lesser extent (Rengasamy and Marchuk 2010), that are adsorbed on the exchange surfaces of clay particles. Clay dispersion increases the severity of physical constraints in dense clay subsoils. In calcareous soils, high exchangeable sodium percentage (ESP) and high pH are always linked together and it is difficult to apportion their effects on the resulting poor soil physico-chemical conditions and consequently on crop growth. An excess of exchangeable sodium and high pH caused by the presence of sodium carbonate or bicarbonate impart poor physical conditions to these soils. In many saline sodic soils that do not contain sodium carbonate, the pH is around 8.2–8.3. Gupta and Khosla (1982) indicated that pH, with its intimate relationship with sodicity of calcareous soils, is likely to affect the whole range of physico-chemical and surface properties of soils.

Subsoil constraints

Work from the EP shows that many of the highly calcareous sandy loams (located at Cungena and Nunjikompita, SA) can contain quantities of boron (B) which present a low hazard to wheat production whilst the redder variants (Minnipa and Poochera soils) can contain much higher levels in the subsoil, presenting a significant constraint to crop production. This work showed that whilst high levels of salt could be found in many of the subsoils assessed, the distribution of salt was much more variable than for B (Holloway 1991). Physical disturbance of subsoils and incorporating nutrients or organic matter are two ways of ameliorating poor subsoils and improving crop performance. However, trials on the EP indicated that there was no economic advantage in mechanical intervention to ameliorate compacted subsoil layers. Although limited benefits were seen at some sites (mostly sandy soils but not Calcarosols), these did not last longer than two seasons (Doudle 2004). Another trial reported that fluid fertiliser applied into the subsoil using a deep ripper increased yield but was not economic (Hancock 2003). In summary, mechanical interventions and soil ameliorants can increase biomass and grain yield but results are highly variable and it can take some time following modification to see benefits (Masters 2019). Responses to the interventions and ameliorants depend on season, site and treatment selected, indicating the management strategy needs to be tailored to the characteristics of the soil profile at depth (Masters and Davenport 2015). If a holistic approach of managing more than just one subsoil constraint is used, that may improve the longevity of benefits and of overall returns on investment.

GAPS IN CURRENT KNOWLEDGE

The weight of evidence from low rainfall south-eastern Australia is that productivity on these soils is well below potential and that opportunities for improving productivity in the region, using multiple strategies, are likely to be greater on sands than finer-textured soils. Similar opportunities also exist in the finer-textured calcareous soils of high rainfall SE of SA.

Highlighted gaps in our knowledge of cropping on calcareous to very highly very calcareous soils and opportunities for improvement include:

- Understanding and quantifying water holding characteristics of highly calcareous soils in order to manage water supply more effectively.
- Rhizoctonia is still a major constraint. Fungicides recently released for reducing rhizoctonia on cereals are proving less effective on highly calcareous soils.
- Potential effects of fungicides on soil biological functions in calcareous soils are not well understood.
- Effectiveness of cereal variety-based variation in rhizoctonia inoculum levels for reducing disease impacts in the following crops has not been investigated in Calcarosols on the EP.
- There is no information on management effects on rhizosphere microbial communities in calcareous soils.
- Our understanding of seasonal mineral N accumulation in the calcareous soils under different cropping systems is limited and it may be this cycle that is constraining the development or expression of a disease-suppressive community.
- A mechanistic knowledge of the soil N cycle and the various biological processes involved, especially seasonal changes and in relation to plant requirements, is critical to understand N-immobilisation capacity of calcareous soils, but has not been adequately quantified and management effects are not known.
- There is limited understanding of the activity, composition and diversity of soil bacterial and fungal communities in calcareous soils.
- There is a paucity of knowledge on the role of improved soil organic matter stocks in highly calcareous soils.
- Biochar is a soil conditioner which has been successful in other soils and environments but has been rarely tested on calcareous soils.
- Optimal soil P reserves, rates and types of P fertilisers are all uncertainties for SE farmers on calcareous soils.
- The impacts of physical interventions with or without ameliorants in calcareous soils are uncertain.
- Subsoils in highly calcareous soils can have extremely high pH, high bicarbonate concentrations, be sodic and/or saline and sometimes have toxic levels of B. It is not well understood how much these conditions affect crop performance nor how they can be effectively and profitably overcome.

Methodology

This project was addressing constraints to crop productivity and profitability by undertaking activities in five major areas with controlled environment (CE) and field experimentation. The five major areas were:

1. MECHANISM STUDIES

- a) Incubation studies and glass house experiments evaluated a range of amendments (inorganic, including gypsum, and organic, including stoichiometrically optimised C inputs) on subsoil properties (especially organic C pools, aggregation) and plant growth in hostile calcareous soils. The soils were characterised for pH buffer capacity (pHBC). The purpose of this work was to inform the field studies.
- b) Soil microbial functionality and functional resilience against stress (methods developed under CRC project 4.1.003) were evaluated in field soils collected by SARDI. Key functions assessed included the cycling of C, N, P and sulphur (S). These were assessed using traditional enzyme assays and compared with a new mid-infrared (MIR) methodology developed by NSW DPI. Traditional nutrient cycling was used to determine the soil/ organic amendment contribution to crop N and P nutrition.
- c) Novel amendments based on the ability to adjust soil redox potential, and the ability to adsorb labile soil organic matter onto iron (FeOOH) were evaluated using incubation studies, both with and without rhizodeposition. ¹³C studies investigated the role of these amendments in increasing SOC, and the development of aggregates. A range of soil fractionation, soil C pool and stable isotope methodologies were employed. This work also served to inform the field studies.

2. FIELD VALIDATION

Upper Eyre Peninsula

A small field demonstration trial was conducted in 2020 to showcase the project at Poochera on upper EP, prior to the major field trials being started in 2021. The trial included a range of treatments addressing both top and subsoil constraints and was maintained for the duration of the project to monitor residual effects from the treatments and as a resource of amended soils for CE studies.

Six field trials were implemented on the upper EP in 2021 and continued into 2022 and 2023 to investigate the impact of strategies and amendments on crop production and soil conditions under conditions typical of commercial crops. Four of these trials were located on highly calcareous grey sandy soils (near Poochera and Port Kenny) and the other two on a red calcareous sandy loam (near Minnipa). Three of the six trials were set up to investigate long-term subsoil strategies (Table 1), and the other three, short-term topsoil strategies (Table 1). Treatments focussed on best-bet options for improving establishment and/or early crop vigour, or on amendments to the subsoil. All trials were run for the length of the project (i.e., for three seasons) and their designs allowed for additional treatments to be added in later years as informed by the other components of this project and also the linked one. The Minnipa site was chosen to represent soils more typical of the SA and Victorian mallees, where carbonate levels are much lower than occur in the highly calcareous sandy soils of the upper EP. This gave us the opportunity to test strategies which were effective in the grey

highly calcareous sandy soils for their relevance to other mallee regions. These trials were being conducted in conjunction with Agricultural Innovation and Research Eyre Peninsula (AIR EP).

Table 1: Summary of topsoil and subsoil treatments

Topsoil treatments	Details
Carbon-coated minerals_100 kg/ha	Carbon-coated minerals @ 100 kg/ha, banded under seed rows
Carbon-coated minerals_500 kg/ha	Carbon-coated minerals @ 500 kg/ha, banded under seed rows
Residual CCM_500 kg/ha	Applied in 2021 only and benefit in 2022 and 2023 measured
Broadcasted_urea	Broadcasted urea pre-seeding
Continuous_P	Fluid P + trace elements at seeding + 2 in-crop foliar P sprays
Fungicide_bandedN_phosacid_TEs	Uniform fungicide + banded urea + phos acid + TEs (Zn, Cu, Mn)
Fungicide_GranNP_TEs	Uniform fungicide + granular N&P + TEs (Zn, Cu, Mn)
Granfert_N&P_carbon coated minerals_match	Granular N&P + nutrients to match Carbon-coated minerals_500 kg/ha
High_seedrate_fungicide_bandedN_phosacid_TEs	High seed rate (75) + uniform fungicide + urea + phos acid + TEs (Zn, Cu, Mn)
Phosacid_TEs	Phos acid + TEs (Zn, Cu, Mn)
SE14_Wetter	SE14 wetter banded with the seed
Seed_coating	Seed coating of microbes
Sweep_cultivation	Sweep cultivation (pre-seeding)
Typical practice	Typical practice (control)
Subsoil treatments	Details
Deep_rip	Deep rip
DR_carbon-coated minerals	Deep rip + carbon-coated minerals @ 5 t/ha
DR_granfert_carbon-coated minerals_match	Deep rip + granular fert to match nutrients in carbon-coated minerals
DR_granfert_NEUTROG*_match	Deep rip + granular fert to match nutrients in Neutrog
DR_Inclusion_plates	Deep rip + inclusion plates
DR_Inclusion_plates_NEUTROG	Deep rip + inclusion plates + Neutrog @ 5 t/ha
DR_Neutrog	Deep rip + Neutrog @ 5 t/ha
DR_Phos_acid	Deep rip + Phos acid
DR_Phos_acid_TEs	Deep rip + Phos acid + trace elements (Zn, Cu, Mn)
Typical practice	Typical practice (No deep ripping)

Neutrog – pelletised composted chicken manure

Carbon-coated mineral (CCM) is a carbon-rich material (biochar) post-treated with phosphoric acid. It is produced in oxygen-limited conditions generally between 400–700 °C and has been proposed as a geoengineering solution that has potential to actively reduce the atmospheric concentrations of greenhouse gases and enhance the sustainability of agriculture through improving soil conditions.

Subsoil treatments were implemented on 14 May 2021 at Minnipa, on 18 May 2021 at Poochera, and on 19 May 2021 at Port Kenny.

All but one of the topsoil treatments (Table 1) were intended as annual strategies implemented at sowing. Sweep cultivation was implemented on 15 April 2021 at all sites to simulate a practice common in these districts and as a strategy to reduce rhizoctonia.

In 2021, all the trials were sown to Scepter wheat at a standard rate of 60 kg/ha and 50 kg/ha DAP; in 2022 all trials were sown to 50 kg/ha Maximus CL barley and 50 kg/ha DAP;

and in 2023 trials were sown to 100 kg/ha GIA Kastar field peas and 60 kg/ha MAP. Plant measurements included crop establishment, crop vigour, early (GS31, stem elongation) and late (GS60, flowering) biomass, root health, plant nutrient analysis at flowering, grain yield and quality. Soil sampling to depth of key treatments was undertaken in March 2024 to assess the 'legacy effect' on soil chemical health, and the results from the EP Analysis lab are due in early April 2024.

Statistical analysis of data was performed using standard ANOVA models in R.

South East

A field trial was conducted in 2021 to investigate nutritional constraints in a heavier calcareous soil of the SE region of SA (Lake Hawdon) under high rainfall conditions. This trial was conducted with guidance from local consultants and MFMG reps and investigated Mn and Fe deficiency in broad beans. After discussions with local farmers and advisers, it was clear that yellowing in broad beans was a major issue on the highly calcareous loamy clays. The cause of this problem was believed to be a combination of Fe and Mn deficiency brought on by cold wet conditions during winter. The treatments in this trial were designed to test the value of adding Fe and/or Mn early in crop growth to prevent yellowing and stunted growth in Aquadulce broad beans.

There were 11 treatments, replicated four times, in the trial that were designed to assess:

- The value of new approaches to adding Mn and Fe to a bean crop compared to the district practice of foliar sprays during the season after the yellowing has appeared.
- Whether coating Mn and/or Fe on the seed is effective.
- Whether adding Mn and/or Fe at seeding is effective.
- Whether chelates are a more effective source of Mn or Fe than sulphates at either seeding or as foliar sprays.
- Whether adding phosphorous as a fluid is more effective than as granular MAP.

Aquadulce broad beans were sown at 311 kg/ha after inoculating with peat-based rhizobia on 19 May 2021. All plots received 100 kg/ha of MAP or a fluid equivalent under each seed row. Foliar sprays of Mn and/or Fe were applied on 9 August (6–7 nodes) and 15 September (flowering). Plant measurements included crop establishment, crop vigour, flowering biomass, nodulation, plant nutrient analysis at flowering, grain yield and quality.

Another field trial with broad beans was conducted in a neighbouring paddock in 2022. Treatments were revised after reviewing the results from 2021 by doubling the rates of trace elements and focussing more on strategies which had Fe-EDDHA as the major source of Fe (a chelated formulation of Fe which is stable at alkaline pH and normally used through fertigation or as a soil drench in more intensive plant industries).

Aquadulce broad beans were sown in 2022 at 277 kg/ha after inoculating with peat-based rhizobia on 12 May. All plots received 100 kg/ha of MAP or a fluid equivalent under each seed row except the no nutrient control. Boomspray applications of Mn and/or Fe were applied on 27 June, 8 August (first flowers) or 29 September (mid-flowering). Establishment, early crop vigour, nodulation and grain yields were assessed.

Both field trials had a treatment labelled 'typical practice'. This treatment was managed in a way typical of best practice for the local area and was the benchmark for comparing the performance of all amendments or novel strategies.

Statistical analysis of data was performed using standard ANOVA models in Statistix 10.

During the last two years of the project, attempts were made by the SARDI team to produce Fe deficiency in broad beans so that investigations into sources and application strategies of Fe could be pursued more rapidly than in field trials.

Attempt 1.

Topsoil (0–10 cm) from the 2021 field trial at Lake Hawdon was sieved and placed in freely draining pots, seeded to Aquadulce broad beans and kept outside in July and August 2022 at the Waite campus in Adelaide. No treatments were applied. Plants were harvested at early flowering.

Attempt 2.

Topsoil (0–10 cm) and subsoil (10–20 cm) from the 2022 field trial at Lake Hawdon was sieved and placed in freely draining pots, subsoil below topsoil in equal amounts, and seeded to Aquadulce broad beans and kept in a shade house in August and September 2022 at the Waite campus in Adelaide. Fe-EDDHA was applied to one half of the pots as a soil drench when potting up. Plants were harvested at early flowering.

Attempt 3.

Topsoil (0–10 cm) and subsoil (10–20 cm) from the 2022 field trial at Lake Hawdon was sieved and placed in freely draining pots, subsoil below topsoil in equal amounts, and all plots were placed in saucers to keep the bottom of the pots in water for the duration of the trial. Pots were seeded to either lentils or chickpeas. Half of the pots were kept in a glasshouse and the other half in a CE cabinet which approximated temperatures and daylength similar to Lake Hawdon in July. Fe-EDDHA was applied to one half of the pots as a soil drench midway through the trial. Plants were harvested at early flowering.

Attempt 4.

Topsoil (0–10 cm) and subsoil (10–20 cm) from the 2022 field trial at Lake Hawdon was sieved and placed in freely draining pots, subsoil below topsoil in equal amounts, and all plots were placed in saucers to keep the bottom of the pots in water for the duration of the trial. Pots were seeded to either chickpeas or Aquadulce broad beans and kept in a CE cabinet which approximated temperatures and daylength similar to Lake Hawdon in July. Plants were harvested at early flowering with the growing points (leaf material above the youngest fully expanded blade) removed for nutrient analysis.

The following treatments were imposed with Fe-EDDHA and replicated three times:

Treatment	Rate (Iron)
Nil control	0
Low rate – soil drench prior to seeding	1kg Fe/ha
High rate – soil drench prior to seeding	5kg Fe/ha
Low rate – foliar application, three weeks after seeding	0.25kg Fe/ha
High rate – foliar application, three weeks after seeding	1kg Fe/ha
Seed coating	2kg Fe/tonne seed

3. SOIL WATER RELATIONS

SARDI, in collaboration with CSIRO as part of the complementary project, assessed water-holding characteristics at the four experimental sites to characterise plant available water capacity (PAWC). CSIRO characterised profiles for LL15 and field capacity and examined the drying characteristics of these soils (including crop establishment tests under varying moisture treatments) in the glasshouse. Soil samples from all sites were fully characterised for chemical fertility and constraints, carbon chemistry, microbial biomass and activity.

More details of the methodology, findings and outcomes for Section 3 can be found in the reporting of project, CSP2009-003RTX.

4. SOIL HEALTH AND NUTRIENT CYCLING

CSIRO, as part of the linked project, undertook CE experiments using intact cores and disturbed soils from field experimental sites to determine responses in microbial activity, nutrient (N and P) mineralisation and rhizoctonia growth to changing available soil moisture.

The aim of these experiments was to quantify responses in microbial activity and specific microbial populations involved in C, N and P cycling, mineralisation and immobilisation to help better estimate crop nitrogen supply, rhizoctonia survival and growth, disease incidence and early crop growth.

N mineralisation and immobilisation (using ^{15}N as a tracer), early crop growth and fertiliser toxicity issues was investigated using intact cores and disturbed soils in CEs as part of the complementary project.

The effect of saturating key soils with phosphorus on the chemical behaviour and plant response was also being investigated. The effect of varying placement and nutrient combination on crop uptake of nutrients and biomass indicators will be followed with dual labelling ($^{15}\text{N}/^{33}\text{P}$).

More details of the methodology, findings and outcomes for Section 4 can be found in the reporting of project, CSP2009-003RTX.

5. DECISION SUPPORT TOOLS

Data from the project will be supplied to Program 4 Output 3 of the Soil CRC to inform the machine-learning models so that the impact of best bet strategies can be simulated for different farm businesses and new environments.

Communication

Project progress and findings were conveyed to end users through a range of tools at different events summarised in the table below.

Deliverer	Event	Date	Audience
Cook A, Davenport D	Presentation at Minnipa and Poochera farmer groups	Aug 2020	26, farmers and private consultants
Cook A, Davenport D, Bates A	Field visit - Poochera	Sept 2020	4 SAGIT trustees
Cook A	Field visit – Port Kenny	Oct 2020	35 farmers and advisers
Cook A	Field visit – Mt Cooper	Oct 2020	29 farmers and advisers
Wilhelm N	<i>Lifting crop productivity on crushed limestone.</i> Soils CRC Webinar, +YouTube	July 2021	approx 40, researchers and academics
Wilhelm N	Presentation. Soil testing workshop - Naracoorte	Sept 2021	20 farmers and advisers
Wilhelm N, Dzoma B	Minnipa Ag Centre field day	Sept 2021	130 farmers and advisers
Wilhelm N	Field visit - Millicent	Oct 2021	100 farmers and advisers
Wilhelm N, Dzoma B	Presentation at Lake Hawdon as part of a MFMG Crop walk	Nov 2021	15 farmers and advisers
Wilhelm N	Presentation at Univ of Adelaide - Honours seminar	Nov 2021	20 students and researchers
Wilhelm N	Online presentation to SAGIT trustees	Feb 2022	6 SAGIT trustees
Dzoma B	EP farmer meetings presentations (Ceduna, Port Kenny, Lock, Rudall, Cowell, Kimba)	Feb 2022	142 farmers, researchers, and advisers
Wilhelm N, Dzoma B, Tavakkoli E, Gupta V, McBeath T, Pearce A	More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints. https://www.youtube.com/watch?v=R39kPBTahal	Aug 2022	Internet
Wilhelm N, Dzoma B, Tavakkoli E, Gupta V, McBeath T, Pearce A	More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints. Presentation at Soils CRC Annual Conference, Adelaide.	Aug 2022	CRC researchers and staff, soils R&D colleagues
Dzoma B	Field visit, Minnipa	Sept 2022	4, SAGIT reps
Wilhelm N	Field visit, Poochera	Sept 2022	20, SAGIT reps, farmers, advisers
Wilhelm N	Summary of field trials presentation, Minnipa Ag Centre Annual Field Day	Sept 2022	148 farmers and advisers,

			expert panel, SAGIT reps
Gupta V	Microbial activity and nutrient cycling presentation, Minnipa Ag Centre Annual Field Day	Sept 2022	148 farmers and advisers, expert panel, SAGIT reps
Wilhelm N	Poochera sticky beak day	Sept 2022	20 farmers, advisers, researchers
Wilhelm N	MAC sticky beak day	Sept 2022	10 farmers, advisers, researchers
Dzoma B, Wilhelm N, Cook A, Richter I and Standley C	More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints. YouTube	Sept 2022	Farmers and advisers
Wilhelm N	ABC Rural interview	Nov 2022	ABC rural listeners
Wilhelm N	Field visit, Lake Hawdon	Oct 2022	16 Farmers and advisers
Wilhelm N	Adelaide Grains Research update	Feb 2023	100 researchers, advisers, farmers
Dzoma B	EP farmer meetings presentations (Lock, Rudall, Cowell)	Feb 2023	50 Researchers, advisers and farmers
Lillecrap J	Presentation at HYC Innovators group (Hatherleigh and Coonawarra)	March 2023	33 growers and agronomists
Wilhelm N, Dzoma B	Minnipa Ag Centre field day	Sept 2023	140 farmers and advisers
Wilhelm N, Dzoma B	SARDI Crop sciences Research Forum	Oct 2023	40 Researchers
Wilhelm N	High Performance Soils CRC Board	Feb 2024	CRC board and staff
Dzoma B	EP farmer meetings presentations (Lock, Rudall, Cowell)	Feb 2024	80 Researchers, advisers and farmers
Wilhelm N, Gupta, V.	Presentations to GRDC National Grower Network Forums on Eyre Peninsula at Ceduna, Wirrulla, Pt Kenny and Wangary	Mar 2024	75 farmers, researchers and advisers

List of Publications

- Dzoma B (2022) 'Findings from the first season of the Calcareous soils project', *AIR EP Newsletter*, 29 April 2022. <https://airep.com.au/news/air-ep-e-newsletter-29-april-2022/>
- Dzoma B, Wilhelm N, Cook A, Richter I and Standley C (2021) 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints' *Eyre Peninsula Farming systems Summary*, 2021.
- Dzoma B, Wilhelm N, Cook A, Richter I, and Standley C (2022) 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints', *Eyre Peninsula Farming systems Summary*, 2022.
- Dzoma B (2023) 'Calcareous soils: What we've learnt in 2 years', *AIR EP Newsletter*, October 2023. <https://airep.com.au/news/calcareous-soils-what-weve-learnt-in-2-years/>
- Dzoma B, Wilhelm N, Cook A, Richter I and Standley C (2023) 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints', *Eyre Peninsula Farming systems Summary*, 2023.
- Evans M, Dzoma B, Gupta VV, Masters B, McBeath T, Tavakkoli E, Wilhelm N and Van Zwieten L (2020) 'The nature of highly calcareous soils and their impact on crop productivity. Literature review', *GRDC report*.
- Fang Y, Tavakkoli E, Weng Z, Collins D, Harvey D, Karimian N, Luo Y, Mehra P, Rose MT, Wilhelm N and Van Zwieten L (2022) 'Disentangling carbon stabilization in a Calcisol subsoil amended with iron oxyhydroxides: A dual-¹³C isotope approach', *Soil Biology and Biochemistry*, 170:108711.
- Gupta VVSR, McBeath T, Kroker SK, Hicks M, Johnston C, Dzoma B and Wilhelm, N (2023) 'In-crop nitrogen supply capacity in wheat crops on highly calcareous soils', *Eyre Peninsula Farming systems Summary*, 2022.
- Gupta VVSR, McBeath T, Kroker SK, Hicks M, Regmi R, Dzoma B and Wilhelm N (2023) 'Functional microbiology and plant-microbe interactions in Calcareous soils', *Soil Science Australia Conference*, Darwin, June 2023.
- Wilhelm N (2020) 'Summary of calcareous project. Ground Cover - Nutrient efficiency supplement'.
- Wilhelm N (2021) 'Hyperyielding field day article. Millicent'.
- Wilhelm N (2021) 'MFMG crop walk article. Lake Hawdon'.
- Wilhelm N and Dzoma B (2021) 'Minnipa Agricultural Centre field day article. Minnipa'.
- Wilhelm N (2022) 'Project update: Overcoming soil constraints in highly calcareous soils', *CRC Newsletter*, Oct 2022.
- Wilhelm N, Armstrong R, Dzoma B, Fraser M, Gupta V, Mason S, Masters B, McBeath T, Tavakkoli E, Trengove S, Uddin S, Verburg K and Van Zwieten L (2023) 'Recent developments in managing crops on calcareous soils, detecting K deficiency in crops and applying P fertiliser', *GRDC Research Update*, Adelaide, Feb 2023.
- Wilhelm N, Cook A, Richter I, Fraser M and Davenport D (2020) 'Constraints to barley production highlighted in a very calcareous grey sand at Poochera', *Eyre Peninsula Farming systems Summary*, 2020.

Wilhelm N, Fraser M and Pearce A (2022) 'Improved management of iron and manganese deficiency in broad beans at Lake Hawdon', *2021 MFMG Trial Results Book*.

Wilhelm N, Fraser M and Pearce A (2023) 'Improved management of iron and manganese deficiency in broad beans at Lake Hawdon. Take 2', *2022 MFMG Trial Results Book*.

AUTHORS, ORGANISATIONS

¹Wilhelm N, ¹Dzoma B, ¹Cook A, ¹Pearce A, ¹Fraser M, ⁵Evans M, ⁶Davenport D, ⁷Armstrong R, ²Gupta V, ²McBeath T, ⁴Van Zwieten L, ^{3,4}Tavakkoli E, ⁴Fang Y, ¹Masters B

¹SARDI

²CSIRO, Waite Campus

³University of Adelaide

⁴NSW Department of Primary Industries (Agriculture)

⁵Evans Consulting

⁶Davenport Consulting

⁷Agriculture Victoria Research

Results

1. MECHANISM STUDIES

Study 1

This study focussed on the grey highly calcareous sandy soil from Poochera on upper EP and sought to quantify the effects of P fertilisers (DAP, liquid phosphoric acid [H₃PO₄], and biochar-blended P fertilisers) on mineralisation of soil inorganic and organic C, and root exudates surrogate (glucose), and the underlying abiotic and biotic mechanisms. Results showed that nutrients (P fertilisers with or without biochar blending) increased native SOC mineralisation by 50–80% in bulk soils but lowered glucose-induced SOC mineralisation by 11–23%, possibly due to the shifted microbial preference to glucose-C as indicated by the decreased fungi-to-bacteria ratio (Appendix A, Study 1, Fig 3B). The loss of SIC (0.04–0.37%) and glucose-C (39–43%) was increased by nutrient inputs, dominantly due to the increased microbial biomass N/C, lowered pH, and increased alkaline phosphatase. Nutrients decreased microbial C use efficiency (Appendix A, Study 1, Fig 5A) due to greater increases in bacterial compared to fungal community, which may have increased turnover of microbial necromass (Appendix A, Study 1, Fig 5B)

Full results in Appendix A, Study 1: Deciphering carbon cycling affected by phosphorus fertilisers in calcareous subsoil: Comparisons of biochar-blended and mineral phosphorus fertilisers.

Study 2

This long-term controlled study showed that the effects of goethite and biochar on labile C stabilised after two months. At 21 months, goethite decreased loss of labile C from the

lucerne stubble for 3.5%, and the bespoke biochar formulation applied to soil facilitated lucerne stubble decomposition for 5–6%.

Full results in Appendix B, Study 2: Inorganic and organic carbon dynamics in a calcareous subsoil: The abiotic and biotic drivers.

Study 3

Iron oxyhydroxides significantly increased the stabilisation of organic C, both the simulated rhizodeposit glucose (which increased by 14–80 kg C ha⁻¹), and native SOC. However, this stabilisation needs to be weighed against the increased loss of CO₂ from the carbonate fraction. The large increase in redox potential by glucose addition (250–330 mg CO₂-C kg⁻¹ soil, equivalent to 6.7–8.9% of native SOC) presented in the current study suggests that root exudates can result in a relevant quantity of native SOC degradation, which could partly explain the low SOC stock in Calcarosol subsoils.

Our study showed that glucose increased the stable fraction of SOM as shown by the decreased SOC/TN ratio (Appendix C, Study 3, Table 3), due to increased microbial growth and turnover (Appendix C, Study 3, Figs. 3 and 4). This result reinforces the importance of labile C inputs in building stabilised soil organic matter in Calcarosol subsoils (i.e., through increasing microbial residues).

Full results in Appendix C, Study 3: Disentangling carbon stabilisation in a Calcarosol subsoil amended with iron oxides: a dual-13C isotope approach

The initial pot studies to evaluate the effects of amendments (stubble, biochar, and Fe minerals) on improving soil physicochemical and biological properties and nutrient availability in hostile Calcarosols led to the development of the carbon coated mineral product (CCM) used in the field trials.

2. FIELD VALIDATION. UPPER EYRE PENINSULA ACTIVITIES

Soil strength

Initial soil penetration resistance was measured at all sites in August 2021 to a depth of 50 cm, using a Rimik cone penetrometer. Figure 1 shows that soil compaction is not a constraint likely to limit crop root growth at Poochera because penetration resistance was always below the level considered to inhibit root growth, 2500 kPa. There appeared to be a compacted layer at Minnipa at a depth of 25 cm and at Port Kenny at 15 cm. Deep ripping reduced resistance throughout the top 40 cm but may have increased it in the layer just below where the ripper tines reached at Port Kenny.

Follow up measurements of penetration resistance were carried out using a cone penetrometer in December 2023, after 72 mm of rain. Data showed that there were signs of re-compaction of the ripped treatments because penetration resistance was slightly higher in 2023 than in 2021 initial measurements. For example, in 2021 at 35 cm soil depth, penetration resistance was 524 kPa at Poochera and 964 kPa at Port Kenny, and at the same depths in 2023 it increased to 1216 kPa at Poochera and 1684 kPa at Port Kenny respectively.

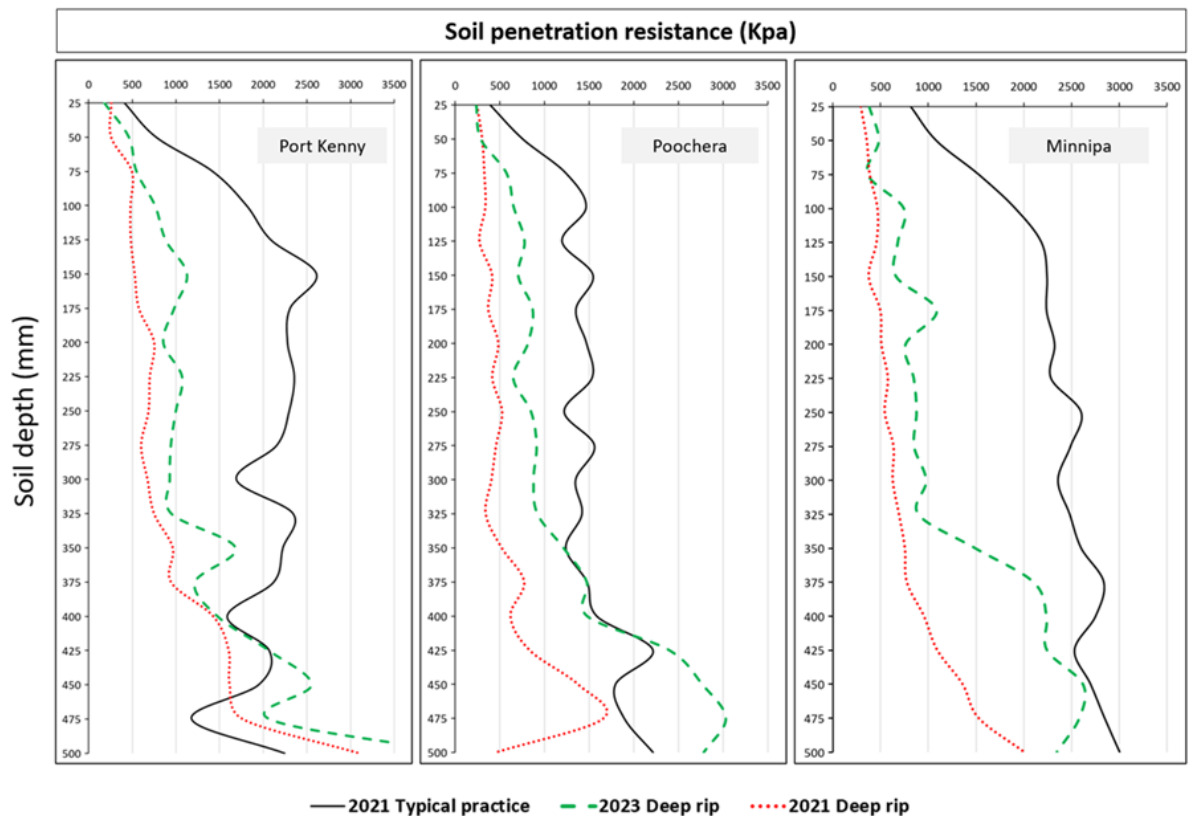



Figure 1: Soil penetration resistance (kPa) in 2021 and 2023 comparing deep ripping with unripped typical practice at Minnipa, Poochera, and Port Kenny

2023 Deep rip means that soil penetration resistance measurements were taken in 2023 (after ripping in 2021).

Soil characteristics


On the EP, calcareous soils are largely represented by the highly calcareous sandy loams (Table 2) and sandy clay loams (with varying depth to substrate) with smaller areas of deep carbonate sand often containing shell fragment (soil subgroup H1). Additionally, there are large areas of loamy textured surface soils over highly calcareous subsoil clays (Table 3).

Table 2: Soil characteristics of a very highly calcareous sandy loam

		Soil Chemistry							
Very Highly Calcareous Sandy loam (Wookata) (EW060) 	Depth (cm)	Layer Description	pH (H ₂ O)	CaCO ₃ (%)	ECe (dS/m)	OC (%)	ESP (%)	CEC	Boron (mg/kg)
	0-30	Brown massive highly calcareous sandy loam	8.3	70	1.2	0.95	1.5	12	2.8-4.8
	30-43	Rubby class IIIc carbonate	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	43-100	Light yellow brown massive very highly calcareous sandy loam (abrupt calcrete)	9.0	78	0.9	0.25	6.3	5	2.3
	132-170	Yellow massive very highly calcareous light sandy clay loam	9.2	85	1.3	0.13	12.3	8	3.9
Desired value			6.0-7.0	<3.0%	<2.0 dS/m	>1.5% in 0-10 cm	<6%	>10	<15 mg/kg

General profile Description
Very highly calcareous sandy loam over rubby carbonate becoming less rubby and slightly more clayey with depth

Table 3: Soil characteristics of a calcareous sandy loam over clay (Minnipa)

		Soil Chemistry							
Calcareous sandy loam over clay (Minnipa/Wiabuna) (EC054) 	Depth (cm)	Layer Description	pH (H ₂ O)	CaCO ₃ (%)	ECe (dS/m)	OC (%)	ESP (%)	CEC	Boron (mg/kg)
	0-25	Dark brown soft slightly calcareous sandy loam	8.0	1-4	1.1-1.4	0.9	1	15	3
	25-42	Yellowish red very highly calcareous light sandy clay loam	8.3	14	3.4	0.4	3	14	4
	42-70	Orange very highly calcareous light sandy clay loam with 20-50% Class IIIB carbonate nodules into hard laminar carbonate	8.7	54	11.6	0.3	7	9	31
	90-200	Reddish yellow highly calcareous light clay	9.5	37-52	10.5	<0.1	38-53	9	40
Desired value			6.0-7.0	<3.0%	<2.0 dS/m	>1.5% in 0-10 cm	<6%	>10	<15 mg/kg

General Description
Calcareous sandy loam grading to a very highly calcareous sandy clay loam over rubby carbonate with clayey substrate within 120 cm


Soil analysis identified medium to very high carbonates, which increased with depth at all three sites (Table 4). Port Kenny was classified as very highly calcareous with water repellence in the top 10 cm, Poochera as highly calcareous and Minnipa site as calcareous. Port Kenny and Poochera had high levels exchangeable sodium in subsurface layers while boron was high in subsurface layers at Minnipa.

Table 4: Surface (0–10 cm) and subsurface (60–80 cm) soil characterisation at three sites in 2021

Site	Depth	pH	Colwell P	Nitrate N	Exch Na	Boron	MED (0-5 cm)	Carbonates
	cm	CaCl ₂	mg/kg	mg/kg	mg/kg	mg/kg	molar	%
Minnipa	0 - 10	7.9	36	45	0.5	2.7	0	15
	60 - 80	8.5	<10	18	10.7	24		40
Poochera	0 - 10	7.9	31	33	40	2.1	0	40
	60 - 80	8.4	<10	25	665	9.1		50
Port Kenny	0 - 10	7.9	45	52	127	2.2	1.2	75
	60 - 80	8.4	<10	19	524	9.6		82

In the higher rainfall zone of the SE, Calcarosols are represented by heavier textured clay loams to clays, often with shallow surface soils on highly calcareous subsoil layers (calcrete or marl) (Table 5). Due to the clayey textures and the fact that these soils are usually found in low-lying situations in the landscape, drainage is poor and can result in waterlogging and increased salinity levels in the lower subsoils (DWLBC 2002).

Table 5: Soil characteristics of a calcareous clay on Marl

Calcareous Clay on Marl	Depth (cm)	Layer Description	Soil Chemistry ^A						
			pH (H ₂ O)	CaCO ₃ (%)	ECe (dS/m)	OC (%)	ESP (%)	CEC	Boron (mg/kg)
 <p>(SE054) General profile Description Calcareous dark coloured clay loam to clay becoming more clayey and calcareous at depth with variable rubble over marl</p>	0-17	Black firm calcareous medium heavy clay (2-10% carbonate concretions)	7.9	16	1.12	6.8	1.1	48	2.7
	17-26	Dark firm moderately calcareous medium heavy clay	8.2	37	0.64	2.3	2.1	25	2.1
	26-81	Dark grey firm highly calcareous light medium clay (10-50% carbonate nodules)	8.6-8.9	45-55	0.5-0.8	<0.7	3.9-8.0	9-17	0.6-1.2
	81-160	Grey friable (wet) highly calcareous light clay (2-20% carbonate nodules)	8.6	24-42	0.9-1.1	<0.3	6.4-8.0	14-24	0.8
	Desired value			6.0-7.0	<3.0%	<2.0 dS/m	>1.5 % in 0-10 cm	<6%	>10

Soil-borne pathogens

Soil-borne pathogens are one of the critical bottlenecks to plant growth and production in crops grown on calcareous soils. The commonly observed soil-borne fungal and nematode pathogens include rhizoctonia root rot (*Rhizoctonia solani* AG8), take-all (*Gaeumannomyces graminis* var. *tritici*), fusarium crown rot (*Fusarium pseudograminearum*), *Fusarium culmorum*, pythium root rot (*Pythium* spp), common root rot (*Bipolaris sorokiniana*), and *Pratylenchus* species. However, under conservation cropping systems rhizoctonia root rot caused by *R. solani* AG8 has been found to be the most dominant disease.

DNA-based soil testing (Predicta B) was carried out in March 2020 at all trial sites to help identify which pathogens posed a risk to plants before seeding. The results (Table 6) showed that the Minnipa site was at low risk of infection by the five pathogens tested. Highest risk to rhizoctonia was at Poochera, and Port Kenny had the highest risk to crown rot. Risk to yellow leaf spot was medium at Poochera and Port Kenny, and low at Minnipa.

Table 6. Mean levels of soil borne pathogens at Minnipa, Poochera and Port Kenny in 2020

	Rhizoctonia <i>R. solani</i> AG8 pgDNA/g Sample	Crown rot <i>F. pseudograminearum</i> pgDNA/g Sample	Yellow leaf spot <i>Pyrenophora tritici-repentis</i> kDNA copies/g Sample	Bipolaris <i>Bipolaris</i> spp pgDNA/g Sample	Root-lesion nematode <i>Pratylenchus thornei</i> nematodes/g soil
Minnipa	3.2	0.4	19.9	11.2	48.9
Poochera	139.1	0.0	406.7	227.8	33.2
Port Kenny	6.8	1246.7	535.1	17.7	0.1
Low risk	3–32	4–32	2–71	4–16	0.1–15
Med risk	32–100	32–316	71–759	16–126	15–60
High risk	>100	>316	>759	>126	>60

Crop establishment

None of the subsoil strategies investigated improved plant population (plants/m²) at any of the three sites in 2021, 2022 and 2023. However, in 2021 when the trials were established, deep ripping with inclusion plates caused a lot of soil disturbance which resulted in a rough and cloddy seedbed (Figure 2), and consequently the lowest plant densities at all sites. In comparison to typical practice (control), deep ripping with inclusion plates resulted in a 50% (Minnipa), 34% (Port Kenny) and 25% (Poochera) reduction in plant population (wheat). Furthermore, deep ripping without inclusion plates reduced plant density by 14% at Port Kenny, 20% at Poochera and 33% at Minnipa compared to the control.



Figure 2: Rough and cloddy seedbed as a result of deep ripping at Minnipa in 2021.

In 2021 and 2022, the use of a higher seeding rate improved plant populations at all sites, but none of the other topsoil strategies significantly improved plant numbers. In 2022, the use of a higher seeding rate increased plant population by 126% at Poochera, 96% at Minnipa and 65% at Port Kenny.

Early crop vigour

In 2022, subsoil strategies at Minnipa and Port Kenny did not affect early crop vigour, however, at Poochera, deep ripping with inclusion plates plus Neutrog improved crop vigour by 30% when compared with typical practice. Fresh CCM @ 500 kg/ha consistently improved early plant vigour in topsoil trials at all three sites (Table 7).

There were no early crop vigour assessments in 2021 and 2023.

Table 7. Crop vigour (g DM/plant) in topsoil trials at Poochera, Minnipa and Port Kenny, 2022

Topsoil treatment	Poochera	Minnipa	Port Kenny
CCM_100 kg/ha	1.50 abc	1.43 a	1.19 bcd
CCM_500 kg/ha	1.71 a	1.44 a	1.36 ab
Broadcasted_urea	1.35 abcde	1.07 bcd	0.87 efg
Continuous_P	1.44 abcd	1.40 ab	1.20 abcd
Fungicide_bandedN_phosacid_TEs	1.45 abcd	1.20 abcd	1.45 a
Fungicide_GranNP_TEs	0.78 f	1.14 abcd	0.88 efg
Granfert_N&P_carbon-coated minerals_match	1.48 abc	1.19 abcd	0.97 defg
High_seedrate_fungicide_bandedN_phosacid_TEs	1.01 ef	0.90 d	1.08 cdef
Phosacid_TEs	1.01 ef	0.98 cd	1.24 abc
Residual CCM	1.63 ab	1.24 abc	1.11 bcde
SE14_Wetter	1.16 cdef	1.16 abcd	0.86 efg
Seed_coating	1.00 ef	1.01 cd	0.83 fg
Sweep_cultivation	1.05 def	1.18 abcd	0.74 g
Typical_practice	1.23 bcde	0.98 cd	1.07 cdef
LSD (0.05)	0.42	0.34	0.3
P-value	0.001	0.03	<0.001
Mean (g DM/plant)	1.25	1.15	1.05

Rhizoctonia

Seminal root health and crown root infection by *Rhizoctonia* were assessed in August 2021 and 2022. Scores were quite variable and there were few clear trends with topsoil treatments. Overall root health at Poochera and Port Kenny was not affected by topsoil treatments. However, at Minnipa, all three CCM treatments had healthier roots.

Seminal root health was not affected by the subsoil strategies at Port Kenny and Minnipa, however, at Poochera, seminal root health was improved by subsoil organic matter (Neutrog) and deep ripping. Crown root infection was not affected at Port Kenny and Poochera. However, at Minnipa, typical practice had the highest crown root infection (52%), while 'DR_CCM' (17%), 'DR_Phos_acid' (22%) and 'DR_Phos_acid_TEs' (23%) had much lower levels.

Infection by *Rhizoctonia* was not affected by any of the topsoil strategies implemented at Port Kenny. However, at Poochera, the percentage of crown roots infected was lower in the two treatments which had fungicides (20%), when compared to the control (48%). High seeding rate resulted in the highest percentage of infected crown roots at Poochera (70%),

Port Kenny (70%) and at Minnipa (77%). At Minnipa, the lowest crown root infection occurred with sweep cultivation (52%). The control had 64% infection.

Root health assessments were not carried out on field peas in 2023.

Plant nutrient status at flowering

Whole shoot samples were collected during flowering in both growing seasons to assess the impact of the strategies on key plant nutrient concentrations. Responses were variable and inconsistent for both growing seasons. Shoot P concentrations were still low at all three sites despite improvements with some treatments.

In 2021, shoot P at Minnipa, and Mn at Poochera was higher in treatments where CCM was applied in the subsoil, when compared to the control. Treatments with Neutrog had higher Zn and copper (Cu) at Port Kenny and Poochera when compared to typical practice. N status was highest after deep ripping with inclusion plates and Neutrog at Poochera. In the topsoil trials, 'continuous P' resulted in significantly higher levels of shoot P at Minnipa and Port Kenny but did not affect P levels at Poochera. The 'High_seedrate_fungicide_bandedN_phosacid_TEs' treatment resulted in significantly higher levels of leaf K, S and Mn at Minnipa, when compared to typical practice.

In 2022, nitrate_N, B and Cu concentrations in whole shoots were not affected by subsoil treatments at any site. However, at Minnipa the highest shoot P concentrations occurred in 'DR_CCM' (0.11%), and highest K concentrations in 'DR_Inclusion_plates' (2.7%). Zinc shoot concentrations were higher in 'DR_Neutrog' at Poochera and Port Kenny only. In the topsoil trials, nitrate_N concentration (mg/kg) in shoot biomass was higher with 'Fungicide_bandedN_phosacid_TEs' at Minnipa (348 mg/kg) and at Poochera (197 mg/kg). Shoot P was higher with 'Continuous' at both Poochera (0.16%) and Port Kenny (0.08%). The highest shoot zinc concentrations were in 'Fungicide_GranNP_TEs' at Poochera (20 mg/kg) and at Port Kenny (12 mg/kg). At Minnipa, 'High_seedrate_fungicide_bandedN_phosacid_TEs' had the highest zinc concentration (14 mg/kg).

In 2023, shoot concentration of P at Minnipa and Port Kenny was not different from the control, however, at Poochera shoot P was significantly higher with CCM (0.22%) than typical practice (0.18%). Neutrog in the subsoil resulted in higher concentration of Zn in the plants at all three sites. Shoot zinc concentration with Neutrog was 17.5 mg/kg (Minnipa), 41.3 mg/kg (Poochera) and 25.1 mg/kg (Port Kenny). Shoot N (%) and Mn (mg/kg) was not affected by amendments and deep ripping at all three sites.

Topsoil 2023: Though adequate in controls, the combination strategy (fungicide + N + P + TEs) resulted in higher concentration of Zn in the plants at Minnipa (24.5 mg/kg) and Port Kenny (18.5 mg/kg). Shoot N (%), P (%) and Mn (%) were not affected by topsoil strategies at all three sites.

Flowering biomass

Subsoil: In both growing seasons, flowering biomass was not affected by any of the subsoil strategies implemented at Minnipa and Poochera, and physical disturbance through deep ripping (with or without inclusion plates) did not improve flowering biomass at any site. However, in 2021 at Port Kenny, deep ripping with CCM (4.5 t/ha) or Neutrog (4.3 t/ha) had higher flowering biomass than the control (3.3 t/ha); and in 2022, at Port Kenny, deep ripping

with Neutrog (with or without inclusion plates) resulted in 30% more biomass than typical practice.

Deep ripping with CCM and Neutrog had high cumulative flowering biomass over two growing seasons at Poochera and Port Kenny (Table 8). Results also suggest a P response at Poochera shown by the extra 3.2 t/ha of biomass when phosphoric acid was applied in the subsoil. None of the subsoil strategies implemented at Minnipa performed better than typical practice over the two growing seasons.

Table 8. Cumulative biomass and change (t/ha) relative to 'typical practice' at Minnipa, Poochera, and Port Kenny over two growing seasons (2021 and 2022)

SUBSOIL	Minnipa		Poochera		Port Kenny	
	Cum. biomass	Change (t/ha)	Cum. biomass	Change (t/ha)	Cum. biomass	Change (t/ha)
Deep rip + biochar	11.83	-1.1	13.21	3.6	13.52	2.2
Deep rip + Phos acid	11.72	-1.2	12.82	3.2	11.78	0.4
Deep rip + Neutrog	12.55	-0.3	12.61	3.0	14.44	3.1
Deep rip + gran fert (Biochar match)	9.92	-3.0	12.14	2.5	11.91	0.5
Deep rip + Phos acid + TEs	11.44	-1.5	11.85	2.2	12.28	0.9
Deep rip + inclusion plates + Neutrog	11.53	-1.4	11.8	2.2	14.25	2.9
Deep rip	11.75	-1.1	11.53	1.9	11.22	-0.1
Deep rip + gran fert (Neutrog match)	11.25	-1.6	11.47	1.9	12.65	1.3
Deep rip + inclusion plates	10.06	-2.8	10.57	0.9	11.68	0.3
Typical practice	12.89		9.62		11.36	

Bold figures are the top three performing treatments at each site, over two seasons, relative to typical practice.

Topsoil: In 2021, 'High_seedrate_fungicide_bandedN_phosacid_TEs' and CCM consistently yielded more biomass than typical practice. At Port Kenny, high seeding rate increased biomass by 22% compared to typical practice. In 2022, late flowering biomass was affected by many, but isolated, rhizoctonia patches across the Poochera trial; by take-all and crown rot patches at Port Kenny; and by B toxicity, net form of net blotch and leaf rust at the Minnipa site. Nonetheless, 'high_seedrate_fungicide_bandedN_phosacid_TEs' consistently produced higher flowering biomass than the typical practice at all three sites. Fresh and residual CCM @ 500 kg/ha (Figure 3) yielded more biomass at Minnipa, when compared to typical practice. There was a 20% reduction in late flowering biomass at Poochera from the use of 'SE14 wetter' and from the 'seed coating' when compared to typical practice.

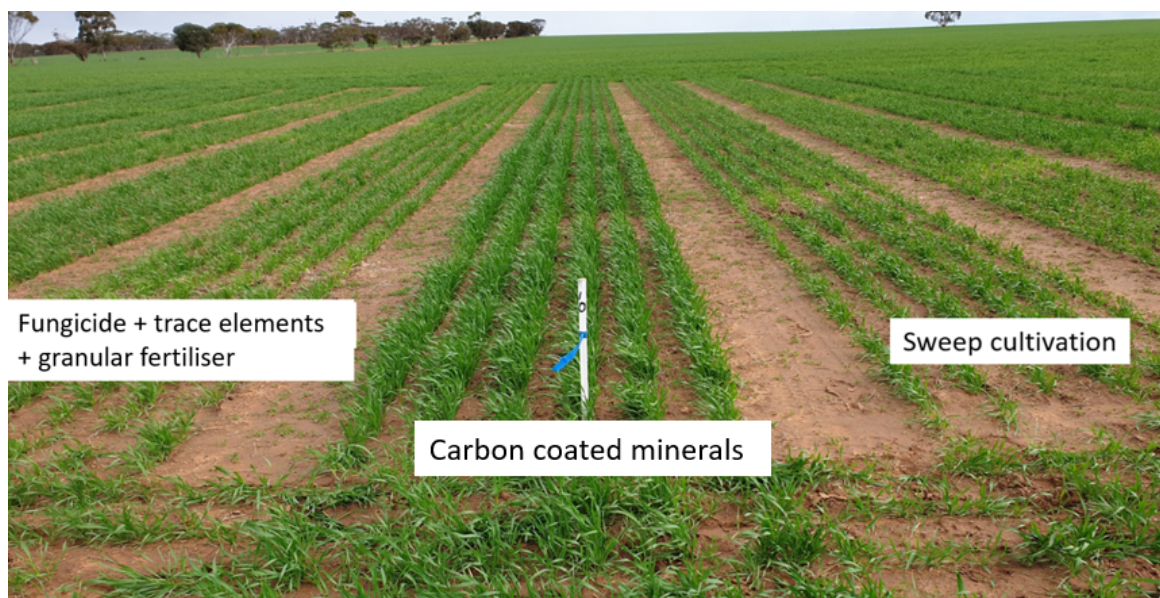


Figure 3: Better crop growth and vigour from carbon coated minerals (CCM) @ 500 kg/ha. Minnipa 18 August 2021

In 2021, the applied CCM @ 500 kg/ha and 'high_seedrate_fungicide_bandedN_phosacid_TEs' strategies resulted in more cumulative flowering biomass in two growing seasons consistently at all three sites (Table 9). Seed coating and the SE14 wetter are the only two topsoil strategies that resulted in reduced biomass at flowering at all three sites over the two growing seasons.

Table 9. Cumulative biomass and change (t/ha) relative to typical practice at Minnipa, Poochera, and Port Kenny over two cereal growing seasons (2021–2022).

TOPSOIL	Minnipa		Poochera		Port Kenny	
	Cum. biomass	Change (t/ha)	Cum. biomass	Change (t/ha)	Cum. biomass	Change (t/ha)
Broadcasted_urea	11.5	0.6	11.0	-0.9	11.6	0.2
Continuous_P	12.0	1.1	11.3	-0.5	11.9	0.5
Fungicide_bandedN_phosacid_TEs	10.7	-0.2	13.0	1.1	13.1	1.7
Fungicide_GranNP_TEs	11.8	0.9	11.9	0.0	12.4	1.0
Granfert_N&P_CCM_match	11.5	0.6	12.0	0.2	12.6	1.2
High_seedrate_fungicide_bandedN_phosacid_TEs	14.7	3.8	14.5	2.7	14.6	3.2
Phosacid_TEs	12.2	1.3	10.5	-1.4	13.0	1.6
Residual_CCM	13.0	2.1	13.4	1.5	14.6	3.2
SE14_Wetter	10.4	-0.5	9.7	-2.1	9.5	-1.9
Seed_coating	10.7	-0.2	9.8	-2.1	9.8	-1.6
Sweep_cultivation	11.9	1.0	11.0	-0.9	9.5	-1.9
<i>Typical practice</i>	<i>10.9</i>		<i>11.9</i>		<i>11.4</i>	

Bold figures are the top three performing treatments at each site, over two seasons, relative to typical practice. 2021 high seedrate, and 2022 applied CCM at 100 kg/ha and 500 kg/ha are not included in the table as these treatments were only investigated during one season.

In 2023, shoot biomass of field peas at flowering was not improved by subsoil strategies and trial site averages ranged from 2.1 t/ha (Port Kenny), 1.3 t/ha (Poochera) and 0.9 t/ha (Minnipa). At Minnipa and Poochera, none of the topsoil strategies improved biomass at flowering, however, at Port Kenny in 2023 applied CCM @ 500 kg/ha improved flowering biomass by 15% when compared to 'typical practice' (2.45 t/ha).

Grain yield

All sites were harvested during November in 2021 and 2022. Higher cereal grains yields were achieved in 2022 compared to 2021, with the subsoil Minnipa trial having the highest site mean grain yield of 4.3 t/ha. In 2023 only Poochera and Port Kenny were harvested.

Subsoil: In 2021, none of the strategies at Minnipa improved grain yield above typical practice (3.51 t/ha). Deep ripping with inclusion plates had the lowest yield (2.58 t/ha). At Poochera, deep ripping and incorporating 5 t/ha of CCM (3.13 t/ha) or Neutrog (3.04 t/ha) had higher yields than typical practice (2.57 t/ha). Deep ripping with inclusion plates and Neutrog, and deep ripping with phosphoric acid and trace elements (Zn, Cu, Mn) also yielded higher than the control. At Port Kenny, deep ripping with inclusion plates and incorporating Neutrog resulted in the highest yield (2.51 t/ha). Deep ripping with CCM (2.24 t/ha) or with Neutrog (2.26 t/ha) also yielded more than the control (1.86 t/ha).

In 2022, none of the subsoil treatments changed grain yields at Minnipa, when compared to typical practice. At Poochera, all amendments applied into the subsoil increased yields above typical practice, with CCM (4.41 t/ha) and Neutrog (4.28 t/ha) being the highest yielding treatments. At Port Kenny, only Neutrog incorporated by inclusion plates yielded higher than typical practice.

In 2023, a lack of moisture during late spring resulted in an early finish and generally very poor yields. Pod filling was poor at Minnipa and the site was not harvested. Site mean for grain yield was 0.2 t/ha at Poochera and 1.1 t/ha at Port Kenny. In the subsoil trial, deep ripping alone did not improve grain yield at Poochera and Port Kenny, however, all ripping with amendments improved grain yield at Port Kenny (Figure 4). Typical practice was 0.87 t/ha, and the two highest yielding treatments were deep ripping with inclusion plates and Neutrog (1.12 t/ha) and deep ripping with CCM (1.10 t/ha).

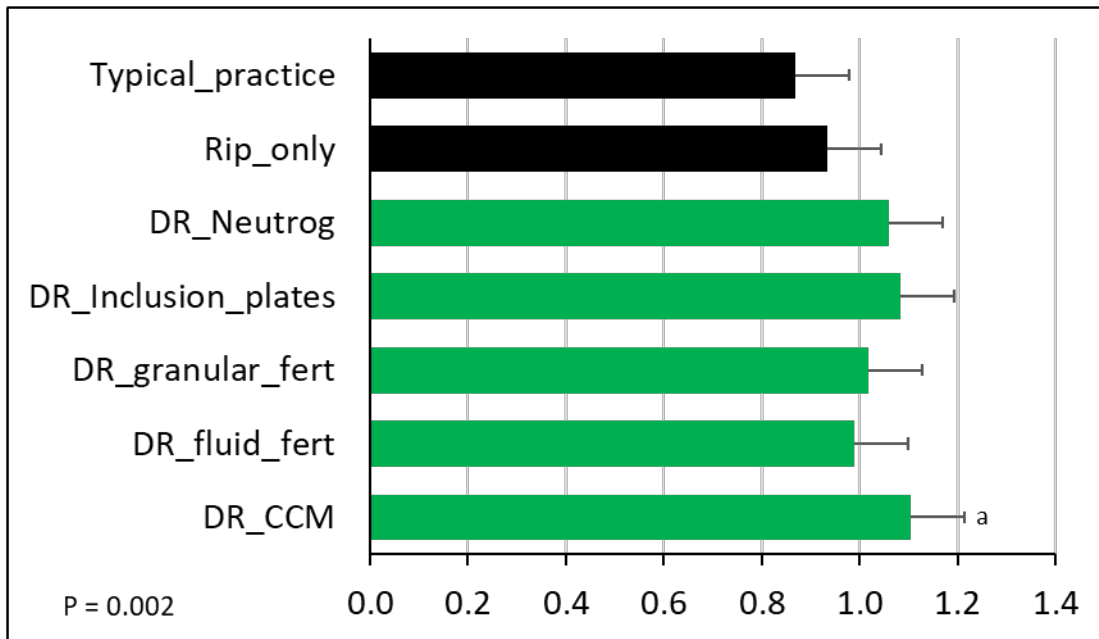


Figure 4: Field peas grain yield (t/ha) at Port Kenny-subsoil in 2023 for different treatments.

Table 10 shows the change in grain yield at all sites over the two growing seasons relative to the cumulative (2021 + 2022) grain yield of the typical practice. Typical practice had the highest cumulative grain yield at Minnipa; 7.66 t/ha and all other strategies reduced cumulative yield over the two seasons. At Poochera, 'DR_CCM' had the highest cumulative change in grain yield (1.58 t/ha) compared to typical practice. 'DR_Inclusion_plates_Neutrog' resulted in cumulative changes in grain yield over 15% of typical practice at both Poochera and Port Kenny.

Table 10. Cumulative change in cereal grain yield (t/ha) compared to typical practice in the topsoil and subsoil trials, relative to typical practice at Poochera, Minnipa and Port Kenny from 2021 and 2022.

SUBSOIL	Minnipa	Poochera	Port Kenny
Deep_rip	-0.31	0.31	-0.15
DR_carbon-coated minerals	-0.18	1.58	0.39
DR_granfert_carbon-coated minerals_match	-0.50	0.76	0.07
DR_granfert_NEUTROG_match	-0.46	0.76	0.50
DR_Inclusion_plates	-0.95	0.06	0.08
DR_Inclusion_plates_NEUTROG	-0.19	1.07	1.14
DR_Neutrog	-0.05	1.35	0.60
DR_Phos_acid	-0.29	0.85	-0.01
DR_Phos_acid_TEs	-0.45	0.82	0.08
<i>Typical practice</i>	7.66	5.97	5.79
TOPSOIL	Minnipa	Poochera	Port Kenny
Broadcasted_urea	0.04	0.52	0.12
Residual CCM_500 kg/ha	0.85	1.99	0.97
Continuous_P	0.73	0.74	0.48
Fungicide_bandedN_phosacid_TEs	0.20	0.87	0.33
Fungicide_GranNP_TEs	-0.07	0.36	-0.43
Granfert_N&P_CCM_match	0.26	1.02	0.42
High_seedrate_fungicide_bandedN_phosacid_TEs	1.13	1.54	1.34
Phosacid_TEs	0.71	1.01	0.25
SE14_Wetter	0.09	-0.03	-0.34
Seed_coating	-0.36	0.01	-0.81
Sweep_cultivation	0.08	-0.24	0.06
<i>Typical practice</i>	6.51	5.19	6.10

2021 high seed rate, and 2022 applied CCM at 100 kg/ha and 500 kg/ha are not included in the table as these treatments were only investigated during one season.

Topsoil: In 2021, at Minnipa, CCM had the highest yield (3.44 t/ha), while continuous P (3.24 t/ha), 'high_seedrate_fungicide_bandedN_phosacid_TEs' (3.27 t/ha) and phosphorus acid and trace elements (3.19 t/ha) also yielded higher than typical practice (2.82 t/ha). At Poochera, only the 'high_seedrate_fungicide_bandedN_phosacid_TEs' treatment (3.27 t/ha) and CCM (3.09 t/ha) had higher yields than the control (2.66 t/ha). At Port Kenny, CCM (2.42 t/ha) and the 'high_seedrate_fungicide_bandedN_phosacid_TEs' treatment (2.38 t/ha) were the highest yielding treatments. The high seeding rate (2.07 t/ha) also yielded better than the control (1.7 t/ha).

In 2022, Port Kenny was the highest yielding topsoil trial with mean barley grain yield of 4.09 t/ha. 'High_seedrate_fungicide_bandedN_phosacid_TEs' produced the highest barley grain yields at Minnipa (4.37 t/ha) and Poochera (4.17 t/ha). At Port Kenny, CCM 500 kg/ha resulted in the highest barley grain yields (4.76 t/ha) and consistently yielded better than typical practice across all three sites. A positive P response was evident at Minnipa and Port Kenny because 'Continuous_P' and 'Phosacid_TEs' yielded better than typical practice at

these two sites. Microbial seed-coating was the only treatment in the topsoil trials that yielded lower than typical practice.

'High_seedrate_fungicide_bandedN_phosacid_TEs' had more accumulated cereal grain yield at all three sites. Cumulative change in grain yield relative to typical practice was 1.13 t/ha at Minnipa, 1.54 t/ha at Poochera and 1.34 t/ha at Port Kenny (Table 10). CCM applied in 2021 at 500 kg/ha resulted in the highest change in grain yield (1.99 t/ha at Poochera) of all treatments.

In 2023, topsoil strategies did not improve grain yield at the two harvested sites.

3. FIELD EVALUATION AND POT STUDIES. SOUTH EAST ACTIVITIES

2021

Establishment: Broad beans emerged and established well, averaging 19 plants per m² regardless of treatments. Coating seed with chelated Fe and/or Mn did not affect emergence.

Vegetative growth: Bright yellowing and interveinal chlorosis in young leaves, extending to the whole plant in some cases, appeared in the trial within the first two months after seeding. The worst affected plants were also very stunted. None of the treatments that had trace elements (Fe and/or Mn) applied early (either on the seed and/or banded at seeding) appeared to have prevented the development of yellowing or improved crop vigour.

Crop vigour and nutrient status of the crop was assessed at early flowering (two weeks after the first foliar spray had been applied). No treatment improved crop vigour compared to either typical practice (which was one foliar spray of Fe and Mn sulphate) or no trace element application (Table 6). While there were some differences in Fe and Mn concentrations between treatments, treatments in which multiple applications of Fe and/or Mn had been applied did not necessarily result in the highest concentrations in young leaves. Levels of Fe and Mn were also above those we would expect to find in plants suffering from those deficiencies, despite symptoms typical (and extreme) for Fe and Mn deficiency being present in the trial at the time of sampling. P concentrations in young leaves were very consistent across all treatments and in the adequate range for pulses at flowering which suggests that 20 kg P/ha delivered in either granular or fluid products at seeding was sufficient to avoid deficiency at this site which had a soil Colwell P at sowing of 51 mg/kg.

Yellowing and stunted growth continued in the trial through spring although growth improved as conditions warmed up. There was still little visual impact of treatments on crop performance through spring.

Grain yield: The trial was harvested on 11 January 2022 after the whole paddock was desiccated. Despite the lack of clear benefits from any treatments to crop performance during most of the growing season, grain yield showed trends that suggest some treatments had provided benefits. There were two clusters of treatments which had either low or high grain yields relative to the rest of the trial (Table 11). One cluster yielded in the range of 5.1 to 5.4 t/ha and all treatments in this cluster had received either no Fe or Mn or only had foliar sprays of Fe and Mn applied as sulphates and all had received granular P. The other cluster yielded from 6.1 to 6.3 t/ha and all treatments in this cluster had received chelated Mn and Fe at seeding and as two foliar sprays. Most of them had also received P from a fluid source. The rest of the treatments in the trial yielded between these two clusters. This clustering

suggests that not treating for Fe and Mn resulted in poor grain yields but that repeated applications of Fe and Mn (but especially Fe) as chelates were required to improve grain yields, possibly with fluid P as part of that package.

Table 11. Effect of Mn and Fe applications on growth, nutrition and grain yield of broad beans at Lake Hawdon in 2021 (TEs were both Mn and Fe).

Treatment	Early Flowering				Grain yield (t/ha)
	Dry wt of shoots (kg/ha)	Nutrients in young leaves			
		Fe (mg/kg)	Mn (mg/kg)	P (%)	
No TEs	608	91	36	0.27	5.4
Local Practice	720	115	39	0.26	5.4
Fluid P	913	97	38	0.28	5.6
Fluid P + Sulphate TEs	987	80	46	0.29	5.7
Fluid P + EDTA TEs	821	80	37	0.30	6.3
Fluid P + chelated TEs	725	76	35	0.32	5.9
Mn only	675	79	40	0.28	5.1
Fe only	697	91	34	0.29	6.1
Chelated TEs 3 ways	789	87	37	0.27	5.5
Chelated TEs 3 ways & fluid P	852	74	36	0.30	5.8
0.5 x Fluid P + chelated TEs	730	89	38	0.27	6.1

LSD ($P=0.05$)

ns

21

5

ns

0.7

The pattern in the performance of treatments suggest that yellowing, stunting and lower grain yields were due more to Fe deficiency than to Mn deficiency.

2022

Establishment: The crop emerged and established well, averaging 19 plants per m² regardless of treatments, except for very stunted plants with Mn-only coated seeds. Coating seed with chelated Fe and/or Mn did not affect establishment or vigour but testing of the seed after seeding revealed that most of the trace element products may have fallen off the seeds prior to seeding.

Vegetative growth: Bright yellowing and interveinal chlorosis in young leaves, extending to the whole plant in some cases, appeared in the trial within the first two months after seeding but only in plots which had not received Fe-EDDHA by that time (Table 12). This pattern continued throughout the season; the greenest and most vigorous plants were in the triple treat Fe (Figure 4), then there were a group of treatments with Fe-EDDHA and Mn which were performing well and a third cluster which had not received Fe-EDDHA and were yellow and stunted (Table 12).



Figure 4: Response of broad beans to Fe applications at Lake Hawdon, 2022. LH plot – typical practice, RH plot – Triple treat Fe.

While the best performing treatment was triple treat Fe, boomspray applications of Fe-EDDHA also showed a lot of promise. These treatments produced vigorous plants and substantially reduced yellowing once the product had been applied and required lower rates of Fe product than the triple treat option. Fe-EDDHA is not recommended for use as a foliar application and the benefits of these boomspray applications may have been through uptake by the crop after the product had been washed into the soil. Lower rates are valuable because Fe-EDDHA is a very expensive source of Fe.

At mid flowering, DM production of shoots was nearly three times higher with triple treat Fe compared to NP (nitrogen and phosphorus only, received no trace elements) and typical practice. Where Fe and Mn were applied in combination, dry matter was still substantially better than typical practice but not as good as with Fe alone. Extent of nodulation was also assessed at mid flowering for a subset of treatments which represented a wide range of early TE strategies. Treatments that received seed coats of Fe or Fe+Mn had at least 40% more nodules per root system than the no TE control (Table 12). An application of Fe or Fe+Mn at seeding had little impact on nodule numbers.

Grain yield: The trial was harvested at maturity in January 2023 and the pattern of poor and well performing treatments seen during the growing season was also largely present in grain yields (Table 12). Broad beans in treatments without Fe or Mn were very stunted at maturity and the plots were largely overwhelmed by ryegrass; they only yielded between 1.6 and 2.6 t/ha. Yields with typical practice (two foliar sprays of Fe and Mn sulphate) were also very poor at 2.3 t/ha. The highest yielding treatment was triple treat Fe, which was more than 5.3 t/ha better than the no TE control and these beans almost completely outcompeted the background population of ryegrass. All other treatments that received multiple applications of Fe and Mn yielded in the range of 6.2 to 6.5 t/ha, suggesting that the addition of Mn partly suppressed the benefits of Fe-EDDHA. Beans in the Mn only treatment had an early setback from the Mn seed coat and struggled all season, yielding no better than the no TE treatments.

Applying a foliar spray of Fe-EDDHA very early in crop growth reduced yellowing and promoted crop growth during winter and spring but resulted in a modest grain yield of only 4.5 t/ha. In contrast two foliar applications of Fe-EDDHA at more traditional timings produced a grain yield of 6.1 t/ha, similar production to treatments with Fe applications starting at seeding.

The only difference between triple treat combo and chelated TEs two ways was that chelated TEs two ways did not receive a seed coating of Fe and Mn and yet it produced similar grain yields to triple treat combo (Table 12). This suggests that the seed coating had little impact on crop performance apart from improved nodulation.

Table 12: Effect of Mn and Fe applications on growth of broad beans at Lake Hawdon in 2022.

Treatment	Early crop colour (1: yellow - 5: green)	Mid Flower		Grain yield (t/ha)
		Dry wt of shoots (kg/ha)	Nodules per plant	
No P, N or TEs	3.1	1059		1.56
NP only	2.6	890	55	2.27
Fluid P	2.6	990		2.56
Typical Practice	2.8	944		2.32
Extra N	3.2	881		1.61
Extra N + Fe	4.1	1219		6.31
Triple Treat Mn	1.7	590		2.06
Triple Treat Fe	4.4	2488	95	7.60
Triple Treat Combo	4.0	1358	79	6.41
Triple Treat Combo + Fluid P	4.1	2091		6.53
Chelated TEs 2 ways	4.0	1244	64	6.23
Early Fe EDDHA	3.2	1292		4.49
Fe EDDHA foliar	2.4	1049		6.14
<i>LSD (P=0.05)</i>	<i>0.8</i>	<i>925</i>	<i>22</i>	<i>1.13</i>

Pot studies

Attempts 1–3: Fe deficiency could not be produced in plants grown in Lake Hawdon soil until the pots were kept in shallow trays of water so that the bottom portion of soil in every pot was kept saturated. Once the pots were kept in shallow water, the growing points of plants became yellow and growth slowed. Alternative pulses to broad beans were used to see if they became more Fe deficient than beans and hence a better indicator plant for pot trials.

Attempt 4: Chickpeas became paler, developed yellow growing points and shoot growth slowed after several weeks in conditions typical for Lake Hawdon in winter without added Fe. Beans eventually became paler without added Fe before harvest, nine weeks after sowing (see Figure 5 for chickpeas at harvest).

Shoot growth of chickpeas increased by more than 50% with a low rate of foliar Fe (see Table 13 for lentils and broad beans) but shoot growth of broad beans did not increase with any Fe treatment. The high rate of soil drench appeared to be toxic to growth of chickpeas because shoot growth was lower than for the low rate of soil drench. Shoot tip concentrations were very low in chickpeas in the nil and increased in all Fe applications. The high rate of soil drench produced the highest shoot concentrations of Fe in chickpeas. Young shoot concentrations were higher in the nil for broad beans compared to chickpeas and changed little with Fe applications.

Chickpeas were more sensitive to Fe deficiency in this experiment compared to broad beans and may be a better indicator plant for future pot studies. This study is consistent with the field trials in that both soil or foliar strategies of Fe-EDDHA can be effective at correcting Fe deficiency but also that rates and timing are important. Seed coating in pots was more effective than in the field. This trial suggests that a pot-based approach could be effective at testing Fe application strategies but that Fe deficiency is still not as severe as experienced in the field.

Table 13: Growth of chickpeas and broad beans after nine weeks in Lake Hawdon soil with different Fe applications

Treatment	Chickpeas		Broad beans	
	Shoot wt (g/pot)	Fe in shoot tips (mg/kg DW)	Shoot wt (g/pot)	Fe in shoot tips (mg/kg DW)
Nil Fe	1.35	28	4.6	41
Low rate of soil drench	1.75	95	4.4	58
High rate of soil drench	1.18	123	5.3	63
Low rate of foliar	2.05	63	5.3	50
High rate of foliar	1.93	60	4.4	57
Seed coating	1.65	71	4.8	62
<i>LSD (P=0.05)</i>	<i>0.37</i>	<i>28</i>	<i>ns</i>	<i>ns</i>

3 & 4. SOIL WATER RELATIONS. SOIL HEALTH & CYCLING

Summarised in the reporting requirements of the CSIRO led project, CSP2009-003RTX, 'Understanding constraints to crop profits on highly calcareous soils'.

5. DECISION SUPPORT TOOLS

Despite contact with Dr Keith Pembleton early in the project delivery, no further progress was made.

Discussion

The aim of this project was to improve early crop vigour, their ability to overcome soil-based constraints and to increase grain production on challenging highly calcareous soils using either long-term (subsoil) or year-on-year short-term topsoil strategies. The Minnipa site, though less calcareous than the other two sites, was an important link to other similar cropping regions. This is because the soil type at Minnipa is much closer to the Calcarosols

in other mallee regions (SA and Vic), and the topsoil strategies which worked on the highly calcareous grey soils also worked on the Minnipa soil.

After three years of conducting these trials, crop responses to the more costly subsoil strategies were smaller and less likely in highly calcareous soils with underlying physical, biological and chemical constraints.

Ameliorating high soil strength by deep ripping has proven to be less effective on these types of soils than on other sands. There can be a penalty from physical disturbance using deep rippers and inclusion plates, as a result of a rough seed bed. Even where crop establishment following ripping has been reasonable, benefits from deep physical disturbance have been small or absent. This is supported by only moderate penetration resistances of under 3000kPa to a depth of 40 cm at all the sites. Values much higher than this are common in siliceous sands where deep ripping is producing large crop production increases compared to the control.

Our results have demonstrated that on very highly calcareous soils, while improving the fertility of the subsoil has potential to improve grain yield, the responses can take several years to appear and were quite modest in size compared to their cost. These modest responses might be a result of the poor finish in 2023 and the response of these interventions in the longer term under different growing season conditions is unknown. Therefore, these response needs to be validated over more situations to determine their wider impact on longer-term crop productivity and profitability.

The development of a CCM formulation represents a significant advancement in addressing the challenges associated with P nutrition in highly calcareous soils. This innovative product was crafted through a process involving the slow pyrolysis of pre-treated oaten hay, with FeSO_4 as a feedstock. The slow pyrolysis, conducted at 450°C within a continuous reactor, led to the creation of CCM. To enhance its P-loading capacity (at a rate of 5%), these CCMs underwent post-treatment with 85% phosphoric acid. Subsequently, the biochar-based CCMs were carefully dried and ground to a particle size of less than 125µm. The rationale behind the development of this novel CCM product lies in its exceptional ability to facilitate the slow release of phosphorus while concurrently exerting an acidifying influence on the rhizosphere. This dual functionality addresses the persistent challenge of P availability in highly calcareous soils, promoting optimal plant growth and nutrient uptake. Furthermore, the incorporation of Fe in the form of FeSO_4 during CCM production offers additional benefits. Iron plays a pivotal role in soil redox processes. Its presence aids in maintaining the appropriate balance of electron transfer reactions in the soil, fostering a conducive environment for plant nutrient acquisition. Subsequent investigations employing DGT (Diffusive Gradients in Thin-films) sampling techniques have underscored the remarkable efficacy of this CCM product, revealing significantly higher concentrations of P in the soil compared to conventional treatments. This pioneering CCM formulation developed by NSW DPI (E Tavakkoli/L Van Zwieten) represents a promising solution to enhance P availability in calcareous soils via its iron-mediated impact on redox processes.

Several short-term and cheaper topsoil strategies have good potential to increase crop vigour and productivity in upper EP environments. Strong winds in these marginal environments often results in erosion on lighter soil types and sandblasting of emerging crops. This causes the tops of sandy rises to be bare and drifting. Therefore, early crop vigour is particularly important in these grey fragile sands because it provides adequate surface cover to protect against wind erosion, narrows the window of vulnerability and improves crop establishment. Plant populations, crop biomass and grain yields can be

improved through the use of higher sowing rates, providing that the denser plant populations are supported by improved nutrition (N and P and trace elements). Carbon-coated minerals have also boosted crop vigour and biomass production when placed just below the seed. We believe that at least part of the benefits from this carbon-coated mineral is to deliver P to the crop in a more effective way than current mineral fertilisers. The availability of P in these challenging soils is always low and several other treatments with higher P also improved crop production. Carbon-coated minerals at a lower rate (100 kg/ha) did not perform as well as the initial higher rate of 500 kg/ha but the residual benefits of carbon-coated minerals were very good. Further investigation is needed to determine how best to apply CCM, how low rates can be applied to improve crop growth and productivity, what forms of CCM are effective and how important is P enrichment to their performance.

Our results have been inconsistent in terms of strategies that can improve cereal root health, however there is an indication from one of the sites (Poochera) of less crown root infection when fungicides are used at seeding with adequate nutrition.

Elimination of yellowing and large increases in growth and grain yield of broad beans occurred on a very calcareous clay loam with applications of Fe. These findings suggest that yellowing and stunting of broad beans on calcareous soils of the SE is due to Fe deficiency and not Fe plus Mn deficiencies. Fe-EDDHA is a formulation of Fe which can correct Fe deficiency in broad beans grown on highly calcareous soils of the SE but the strategies used so far had high rates of Fe per ha (either as banding applications at seeding or foliar sprays) which are very expensive to apply. The Fe applications in the Triple treat Fe are currently costed at nearly \$1,000/ha. Applications of Mn did not alleviate yellowing and did not improve crop growth. In 2022, Mn appeared to have an antagonist effect on the response of broad beans to Fe applications. Nutrient analysis of young leaves was not a reliable diagnostic tool for Fe deficiency in broad beans in our field studies.

While being able to correct yellowing and stunting of broad beans on a highly calcareous clay loam was a breakthrough, there is much more work required before economically attractive management packages can be recommended to local growers and advisers. The strategies with Fe-EDDHA were cost-prohibitive to most growers so more work is needed to investigate application strategies of Fe which continue to be effective, but at reduced cost. These opportunities include:

- Reduced rates of Fe-EDDHA.
- Different strategies for applying Fe-EDDHA to crops (e.g., incorporating boomspray applications by seeding).
- The timing and frequency of boomspray applications.
- Identifying how much Fe is taken up directly through crop canopies.
- Seed coat formulations which keep Fe on bean seeds.
- Genotypic variation within popular crops.
- Different formulations of Fe which are stable at high pH and deliver plant-available Fe to crops.

A controlled-environment system has been developed which results in Fe deficiency in pulses in Lake Hawdon soil (Figure 5). This system will allow more rapid progress towards the opportunities listed above than can be achieved with field trials.



Figure 5: Iron deficiency in chickpeas and lentils in Lake Hawdon soil. LH pots – untreated, RH pots – Soil drench of Fe-EDDHA.

Conclusion

This project has demonstrated that there is potential to overcome soil constraints and improve early crop vigour and crop yield on challenging highly calcareous soils. The project identified multiple constraints and strategies to address these using integrated solutions to ensure an increase in crop productivity and subsequently profitable and sustainable farming systems.

KEY FINDINGS

Upper Eyre Peninsula

- High soil strength is an issue in calcareous soils, however, positive responses to deep ripping are less likely and usually limited by the hostile subsoil.
- Short-term topsoil strategies resulted in better gains in cereal crop biomass and yield when compared to the more longer-term subsoil strategies.
- A carbon-coated mineral (bespoke biochar) in the topsoil improved cereal crop vigour, biomass and grain yield as well as providing good benefits into the second crop.
- Increasing seeding rates and nutrition at sowing was effective at achieving high plant densities, crop biomass and grain yield.
- On very highly calcareous soils, improving the fertility of subsoils has the potential to improve grain yield, but responses only started showing in the third crop after application compared to the control.

South East

- Yellowing of broad beans during winter and early spring on highly calcareous clay-loams is caused by Fe deficiency alone, Mn deficiency does not seem to be present.
- Current local practice of using multiple foliar applications of iron and manganese sulphates or EDTA chelates had little impact on the condition.
- Applications of iron-EDDHA (a chelate stable under high pH) was effective at correcting the yellowing and resulted in large grain yields increases in broad beans.
- A controlled environment system has been developed which results in iron deficiency in pulses in the Lake Hawdon soil. This system will allow more rapid progress into rates, formulations, application strategies and alternative sources of iron than can be achieved with field trials.

Mechanism studies

- Broadcast applications of nutrients increase CO₂ emissions originating from both SOC and SIC in non-rooting zones, as well as new C input (rhizodeposits), but stabilise 'old' SOC in rooting zones, suggesting the necessities of band applications for better C capture and storage in highly calcareous soils.
- Goethite and biochar increased carbon use efficiency of plant-derived carbon, suggesting such amendment applications might offer additional potential for soil carbon storage.
- The decreased loss of OC (glucose and native SOC) following goethite and ferrihydrite amendment reinforces the role of Fe minerals in OC stabilisation and offers opportunities to better manage soils for increased SOC content.
- These studies advance the fundamental understanding of C cycling and stabilisation in Calcarosols, presenting a pathway forward to re-engineer hostile subsoils. Iron oxyhydroxide amendments may provide an important solution for increasing SOC in these challenging soils.

Our findings may have direct applicability to the other similar regions, despite considerable differences in farming systems and agroclimatic conditions.

Our field trials were used by several other projects because they were well managed and included a wide range of management strategies which increased crop production substantially:

- CSP2009-003RTX, 'Understanding constraints to crop profits on highly calcareous soils'.
- GRDC Rhizoctonia Management – Poochera.
- Herbicide residues project – Minnipa
- Enzo Lombi et al. for synchrotron and DGT studies – Poochera.

IMPLICATIONS

Cereal crop responses to the more costly subsoil strategies in the low rainfall environments of upper EP were smaller and less likely in highly calcareous soils with underlying physical, biological and chemical constraints. Ameliorating high soil strength by deep ripping has

proven to be less effective on these types of soils than on other sands. The incorporation of organic amendments (Neutrog, CCM) into subsoils has shown potential to improve crop production but is still economically dubious. However, this response needs to be validated over more situations to determine their general impact on longer-term crop productivity and profitability.

Plant population, crop biomass and grain yield were improved by higher sowing rates, providing that the denser plant populations are supported by improved nutrition (N and P and trace elements). CCM boosted crop vigour and biomass production when placed just below the seed. We believe that at least part of the benefits from this CCM is to deliver P to the crop in a more effective way than current mineral fertilisers. Residual benefits of CCM were evident which is very valuable for their economic value. However, further investigation is needed to determine how best to apply CCM and how low they can be applied to improve crop growth, productivity and overall farm profitability. CCM has shown great potential but is still not commercially available. In the meantime, the recommendation for growers to improve early vigour and ground cover as well as final cereal production on highly calcareous soils is to increase seed and fertiliser rates above those typically used.

The growth and grain yield of broad beans growing on highly calcareous clay-loams of the SE of SA can be greatly improved with applications of iron-EDDHA. However, this product is very expensive for broad-acre farmers. Further investigations are required to test the effectiveness of reduced rates of iron-EDDHA, to develop more effective application strategies or to find alternative (and effective) sources of iron before an economically attractive package can be recommended to growers and advisers in the region.

Mechanism studies improved the understanding of C cycling and stabilisation in Calcarosols, presenting a pathway forward to re-engineer hostile subsoils. Iron oxyhydroxide amendments may provide an important solution for increasing SOC, and amendments like goethite and CCM might offer additional potential for soil carbon storage in these globally important soils that are prevalent in the arid and semi-arid (sub)tropics of both hemispheres.

Recommendations

Key recommendations after four years of work are:

1. Crop responses to the more costly subsoil strategies are smaller and less likely in highly calcareous soils with underlying physical, biological and chemical constraints. Therefore, the focus should be on short-term topsoil strategies to improve early crop vigour, biomass and grain yield.
2. The use of higher sowing rates is an effective strategy to improve plant population, crop biomass and grain yield, providing that the denser plant populations are supported by improved nutrition (N and P and trace elements).
3. CCM placed just below the seed is another effective strategy to improve early crop vigour, biomass and grain yield. Residual benefits were very good. Further investigations are needed to fully define their residual benefits, determine how best to apply them and how low they can be applied to improve crop growth and productivity. This product is not commercially available.
4. Ameliorating high soil strength by deep ripping, and applying organic amendments in the subsoil has proven to be less effective on these types of soils than on other

sands, therefore the recommendation is to identify and understand the underlying subsoil constraints before making the decision to deep rip.

5. Further investigations are required to test the effectiveness of reduced rates of iron-EDDHA at correcting Fe deficiency in pulses, to develop more effective application strategies for iron-EDDHA or to find alternative (and cheaper) sources of Fe.
6. Addressing multiple constraints in calcareous soils using integrated solutions will ensure an increase in crop productivity and subsequently profitable and sustainable farming systems.
7. Provide further information about the recommendations of the project, including what is needed, if anything, to further develop, disseminate, commercialise, or exploit the project outputs, and what additional research is recommended for fully address the project's objectives.

Acknowledgements

This project, 'More profitable crops on highly calcareous soils by improving early vigour and overcoming soil constraints', is supported by the Cooperative Research Centre for High Performance Soils (Soil CRC), whose activities are funded by the Australian Government's Cooperative Research Centre Program. The project is co-funded by Grains Research and Development Corporation (GRDC). The authors would also like to thank the landholders and families involved in this project: Shard Gosling, Simon Guerin and the Minnipa Agricultural Centre.

Also thanks to the Hurst family for allowing us to undertake the SE trial on their property, Daniel Badman for being our local contact and helpful host at Lake Hawdon, and the Struan SARDI Agronomy team for conducting the trial and local growers and agronomists who contributed ideas for management options to consider.

References

- Adcock DP (2005) *Soil water and nitrogen dynamics of farming systems on the upper Eyre Peninsula, South Australia* [PhD thesis], University of Adelaide.
- Angus JF and Grace PR (2017) 'Nitrogen balance in Australia and nitrogen use efficiency on Australian farms', *Soil Research*, 55:435-450, doi:10.1071/SR16325.
- Bertrand I, Holloway R, McLaughlin M (2000) Understanding soil/fertiliser chemistry. A necessity for efficient fertiliser application and formulation. Eyre Peninsula Farming Systems 2000 Summary, pp. 79-80.
- Chew J, Zhu L, Nielsen S, Graber E, Mitchell DRG, Horvat J, Mohammed M, Liu M, van Zwieten, L, Donne S, Munroe P, Taherymoosavi S, Pace B, Rawal A, Hook J, Marjo C, Thomas D, Pan G, Li L, Bian R, McBeath A, Bird M, Thomas T, Husson O, Solaiman Z, Joseph S and Fan X (2020) 'Biochar-based fertilizer: Supercharging root membrane potential and biomass yield of rice', *Science of the Total Environment*, 713:136431, doi:10.1016/j.scitotenv.2019.136431.
- Cook A and Wilhelm N (2019) 'Impact of fertiliser on wheat emergence under dry conditions', *Eyre Peninsula Farming Systems 2019 Summary*, 38-40.
- Davey R, McNeill AM, Barnett SJ and Gupta VVSR (2021) 'Potential suppression of Rhizoctonia root rot is influenced by nutrient (N and P) and carbon inputs in a highly calcareous coarse-textured topsoil', *Soil Research*, 59(3):239–252, doi:10.1071/SR20158.

- Dockerill J (2019) Developing an ecotoxicologically-based index for fertiliser toxicity. Honours Thesis, School of Agriculture, Food and Wine, The University of Adelaide, South Australia, Australia.
- Doudle S (2004) 'Subsoil nutrition – residual benefits?' *Eyre Peninsula Farming Systems 2004 Summary*, 118-120.
- DWLBC (2002) Soils of South Australia's Agricultural Lands, Handbook produced by the Soil and Land Information (SALI) Group, DWLBC.
- Fang Y, Singh BP, Luo Y, Boersma M and Van Zwieten L (2018) 'Biochar carbon dynamics in physically separated fractions and microbial use efficiency in contrasting soils under temperate pastures', *Soil Biology and Biochemistry*, 116:399-409, doi:10.1016/j.soilbio.2017.10.042.
- Gupta RK and Khosla BK (1982) 'Seasonal variations in salt and water content profiles in shallow and saline ground water table'. *Indian J. agric. Sci.* 52, 506-510
- Gupta VVSR, Rovira AD, Roget DK (2011) Principles and management of soil biological factors for sustainable rainfed farming systems. In: Tow P, Cooper I, Partridge N, Birch C (Eds.), *Rainfed Farming Systems*. pp. 149-184 (Springer: Berlin Heidelberg)
- Gupta VVSR (2011) *Better prediction and management of Rhizoctonia disease risk in cereals. GRDC project CSE00048 Final report*, Canberra, ACT.
- Gupta VVSR (2015) *Management of soilborne rhizoctonia disease risk in cereal crops. GRDC Final Report*, p. 61, Canberra, ACT.
- Hancock J (2003) 'Manipulating grain yield with strategic nitrogen application', *Eyre Peninsula Farming Systems 2003 Summary*, pp. 97-98.
- Hancock JM, McNeill AM, McDonald GK & Holloway RE (2011) 'Fate of fertiliser N applied to wheat on a coarse-textured highly calcareous soil under simulated semi-arid conditions'. *Plant and Soil* 348, 139–153 (2011). <https://doi.org/10.1007/s11104-011-0917-5>
- Holloway RE (1991) *Factors affecting the growth of wheat roots in the soils of Upper Eyre Peninsula, South Australia*, [Masters thesis], University of Adelaide.
- Holloway RE, Bertrand I, Frischke AJ, Brace DM, McLaughlin MJ and Shepperd W (2001) 'Improving fertiliser efficiency on calcareous and alkaline soils with fluid sources of P, N and Zn', *Plant and Soil*, 236:209-219, doi:10.1023/A:1012720909293.
- Holloway B, McLaughlin MJ, McBeath T, Kelly J and Brace D (2007) *Fluid Fertilisers: A South Australian Manual*, Arris, Adelaide.
- Lal R (2006) 'Enhancing crop yields in the developing countries through restoration of the soil organic carbon pool in agricultural lands', *Land Degradation & Development*, 17:197-209, doi:10.1002/ldr.696.
- Lombi E, McLaughlin MJ, Johnston C, Armstrong RA and Holloway RE (2004) 'Mobility, solubility and lability of fluid and granular forms of P Fertiliser in calcareous and non-calcareous soils under laboratory conditions', *Plant and Soil*, 269:25-34, doi:10.1007/s11104-004-0558-z.
- Masters B (2018) *Understanding soil biology: what lies beneath the surface. Final report submitted by Natural Resources Eyre Peninsula to the Australian Govt and the Dept Env Water and Natural Resources: Healthy Soils for Premium Food*, Pt Lincoln, SA.
- Masters B (2019) 'Validating research outcomes to treat production constraints on sandy soils of Eyre Peninsula', *Eyre Peninsula Farming Systems 2019 Summary*, 99-104.
- Masters B and Davenport D (2015) 'Overcoming subsoil constraints to increase soil carbon on Eyre Peninsula soils', *Eyre Peninsula Farming Systems 2015 Summary*, 205-208.
- McNeill A and Day P (2001) 'Biological activity of surface soils on Upper Eyre Peninsula', *Eyre Peninsula Farming Systems 2001 Summary*, 110-111. Minnipa, SA.
- Rath KM, Maheswari A and Rousk J (2019) 'Linking microbial community structure to trait distributions and functions using salinity as an environmental filter', *mBio*, 10, e01607-19, doi:10.1128/mbio.01607-19.

- Rengasamy P and Marchuk A (2010) 'Cation ratio of soil structural stability (CROSS)'. *19th World Congress of Soil Science, Soil Solutions for a Changing World*. 1-6 August. Pg 9-11. Available in the conference proceedings from the 19th WCSS.
- Roget D, Taylor A, Thompson A and Frischke A (2004) 'Eyre Peninsula fumigation trials 2003 – investigating the true potential of EP soils', *Eyre Peninsula Farming Systems 2003 Summary*, 69-71.
- Streeter TC, Rengel Z, Neate SM and Graham RD (2001) 'Zinc fertilization increases tolerance to *Rhizoctonia solani* (AG8) in *Medicago truncatula*', *Plant and Soil*, 228:233-242, doi:10.1023/A:1004874027331.
- Wakelin SA, Macdonald LM, Rogers SL, Gregg AL, Bolger TP and Baldock JA (2008) 'Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils', *Soil Biology and Biochemistry*, 40:803–813, doi:10.1016/j.soilbio.2007.10.015.
- Wall PC, Neate SM, Graham RD, Reuter DJ and Rovira AD (1994) 'The effect of rhizoctonia root disease and applied nitrogen on growth, nitrogen uptake and nutrient concentrations in spring wheat', *Plant and Soil*, 163:111-120, doi:10.1007/BF00033947.

APPENDIX A

Study 1: Deciphering carbon cycling affected by phosphorus fertilisers in calcareous subsoil: Comparisons of biochar-blended and mineral phosphorus fertilisers.

Overall highlight: This is the first study in this kind to quantify the effects of phosphorus (P) fertilisers (DAP, liquid H₃PO₄, and biochar-blended P fertilisers) on mineralisation of soil inorganic and organic C, and root exudates surrogate (glucose), and the underlying abiotic and biotic mechanisms. The results show that different P sources had similar impacts on the loss of soil inorganic and organic C, and root exudates surrogate. Like root exudates, loss of soil inorganic C was mainly due to biotic effect (increased microbial activities), rather than the abiotic effect (neutralizing). Further, different forms of phosphorus fertilisers including biochar-blended P fertiliser lowered root-exudates induced soil organic carbon loss, due to (1) less nutrient mining and (2) carbon preferences of microbial communities. The data analysis and interpretation have been progressed. A paper entitled as 'Phosphorus and biochar-blended fertilisers affected inorganic and organic carbon dynamics in a calcareous subsoil: Towards a quantitative and mechanistic understanding' is under drafting.

Calcareous soils suffer persistent phosphorus (P) deficiency due to the great P-immobilising capacity. Application of biochar-blended P fertiliser to Calcarosols may effectively increase and maintain P bioavailability, with co-benefits of carbon (C) sequestration for both soil organic C (SOC) and soil inorganic C (SIC). However, deciphering multiple C sources in the Calcarosols, particularly rhizosphere, remains overlooked, hampering quantification and mechanistic understanding of the response of soil C stocks on P nutrient management. The dual-¹³C isotope approach used in this study enabled the accurate differentiation of three C sources [SOC, SIC, and glucose (a surrogate for rhizodeposits)] as regulated by varied P fertilisers (diammonium phosphate, liquid H₃PO₄, biochar-blended H₃PO₄) and biochar (without H₃PO₄ blended). Over 42 days of incubation period, nutrients (P fertilisers with or without biochar blending) increased native SOC mineralisation by 50–80% in bulk soils but lowered glucose-induced SOC mineralization by 11–23%, possibly due to the shifted microbial preference to glucose-C as indicated by the decreased fungi-to-bacteria ratio. The loss of SIC (0.04–0.37%) and glucose-C (39–43%) was increased by nutrient inputs, dominantly due to the increased microbial biomass N/C, lowered pH, and increased alkaline phosphatase. Nutrients decreased microbial C use efficiency due to greater increases in bacterial compared to fungal community, which may have increased turnover of microbial necromass. In conclusion, varied P fertilisers had similar effects on CO₂ emissions from inorganic/organic C stocks linking to microbial attributes and possibly biological-induced acidification. Our study highlights that broadcast applications of nutrients increase CO₂ emissions originating from both SOC and SIC in non-rooting zones, as well as new C input (rhizodeposits), but stabilise 'old' SOC in rooting zones, suggesting the necessities of band applications for better C capture and storage in highly calcareous soils.

The experimental soils were collected from subsoil (20–40 cm depth) at Poochera, South Australia (32.7436°S, 134.9021°E). The site has an annual rainfall of mm and a mean annual temperature of 18°C (<http://www.bom.gov.au>). The soil was a sandy clay loam (65.9: 27.7: 6.4), classified as a Calcarosol as per the World Reference Base Classification (IUSS Working Group WRB, 2015), or a Calcarosol as per the Australian Soil Classification (Isbell, 2002). The relevant soil properties were: total C 51.7 g kg⁻¹ soil, SOC 3.7 g kg⁻¹ soil, SIC 48.0 g kg⁻¹ soil, δ¹³C of total C -6.0‰, δ¹³C of SOC -22.6‰, δ¹³C of SIC -3.6‰, TN 0.6 g kg⁻¹ soil, TP 0.12 g kg⁻¹ soil, Colwell P 1.5 mg kg⁻¹ soil, pH (1:5 soil/H₂O) 9.34, EC (1:5 soil/H₂O) 255 μS cm⁻¹, cation exchange capacity (CEC) 13.2 cmol(+) kg⁻¹, exchangeable calcium (Ca²⁺) 9.6 cmol (+) kg⁻¹, exchangeable magnesium (Mg²⁺) 2.4 cmol (+) kg⁻¹. The dominant clay minerals were 66.1% Calcite, 28.5% Gisonite (Hydrated alumino-silicate), 5.4% Ankerite

(calcium, iron, magnesium carbonate). Further details of soil physicochemical properties and the associated analytical methods are given in the SI and Fang et al. (2022).

Soil incubation experiment

There was a total of ten treatments with three replicates for each including (1) five no-glucose treatments: control (nil biochar or nutrients), DAP [(NH₄)₂HPO₄], phosphoric acid (H₃PO₄), Biochar, Biochar-blended P (BiocharP); (2) five glucose (Glu) treatments: Glu (no nutrients), Glu + DAP, Glu + H₃PO₄, Glu + Biochar, Glu + BiocharP. Biochar and BiocharP were applied at 1.8 g kg⁻¹ oven-dried soil, corresponding to 2.5 t ha⁻¹ (bulk density of 1.4 g cm⁻³). Different P fertilisers were applied to the soils at 59 mg P kg⁻¹ soil, equivalent to the total P amount in the BiocharP treatments (83kg P ha⁻¹). To match the total N amount in the DAP treatments, urea-N fertiliser (53mg N kg⁻¹ soil; 75kg N ha⁻¹) was applied to the treatments except the control and Glu only. Glucose was applied at 1.0 g C kg⁻¹ soil, representing a major C input from root exudates that are utilized by almost the entire microbial community (Gunina and Kuzyakov, 2015). To partition three C sources (SIC, nSOC±biochar, and Glu-C), glucose was applied at two enriched δ¹³C levels (28 and 81‰) with three replicates for each (Whitman and Lehmann, 2015; Fang et al., 2022). In the treatments, nutrient solution, and/or glucose solutions were uniformly mixed with air-dried soil (260g) by shaking several times in the individual plastic bags. The soils were adjusted to 65% of maximum water holding capacity (WHC: 27%) and being packed into four 70mL plastic containers (bulk density of 1.4g cm⁻³). These soil containers were placed together with a CO₂ trap (1 or 1.5 mol L⁻¹ NaOH), and a RO water container (20mL H₂O) in a 1.2 L air-tight incubation vessel (Fang et al., 2018a). To ensure the best capture of the small difference between treatments (Fang et al., 2022), NaOH solutions varied between 1 and 1.5 mol L⁻¹ (over time) and volumes (between with and without Glu treatments) as below: (i) 8 ml in the non-glucose treatments, and (ii) 20–30 ml in the glucose treatments. The CO₂ traps were replaced on days 1, 3, 5, 9, 14, 21, 28, and 42 for total CO₂-C and δ¹³C analyses. Moreover, to minimise the CO₂ contamination in the incubation vessels, the headspace of the incubation vessels was immediately flushed with a zero-air gas (CO₂ < 5 ppm; BOC Gas, Australia) after being closed at each time. The vessels were incubated in the dark at 21 ± 0.5 °C. The blank vessels (i.e., without soil) were set up in the same manner as above non-glucose and glucose treatments (three for each) to account for CO₂ in the headspace. The soils were sampled on days 3, 9, and 42 for chemical (pH and EC, and SOC), and biological (microbial biomass C/N/P, soil enzymes, and phospholipid fatty acids) analyses.

Three-C-source partitioning

Total CO₂-C trapped in NaOH solution was determined by titration method, and the δ¹³C of CO₂ trapped in NaOH solution was determined using the PICARRO G2131-*i* Analyzer (Fang et al., 2022). In the control and nil-glucose treated soils, the CO₂-C emitted from the SIC and SOC sources were differentiated by a two-pool isotope mixing model (Fang et al., 2019). In the glucose-treated soils, we differentiated the three C-sources (i.e., SIC, nSOC ± Biochar-C, and glucose-C) by the isotope mixing model, as Eqs 1–3. In the biochar treated soils, we did not consider partitioning CO₂ emitted from biochar and SOC, because (1) it was not practical due to the low biochar-C mineralisation; (2) the δ¹³C of nSOC and the biochars had a small difference in δ¹³C (ca. 5.2‰).

- $f_1 + f_2 + f_3 = 1$ (1)
- $\delta_{T1} = f_1\delta_1 + f_2\delta_2 + f_3\delta_3$ (2)
- $\delta_{T2} = f_1\delta_1 + f_2\delta_2 + f_3\delta_4$ (3)

where f_1 , f_2 , and f_3 represent the fraction of the emitted CO₂-C derived from SIC, native SOC (nSOC) ± Biochar-C, and glucose, respectively; δ_1 , δ_2 , δ_3 , and δ_4 represent the endmembers of SIC (-3.6‰), nSOC (-22.6‰) or SOC + Biochar-C (-23.4‰), low ¹³C-enriched glucose

(29‰), and high ¹³C-enriched glucose (81‰); δ_{T1} and δ_{T2} represent low and high ¹³C-enriched glucose-treated soils, respectively.

Results

Total C. In the no-glucose treatments, (i) the total soil CO₂ emission over 42 days ranged between 82 and 153 mg CO₂-C kg⁻¹ soil, in the order of the control < Biochar < H₃PO₄ < DAP < BiocharP (Fig. 1A); and (ii) the total C loss (emitted/total) on 42 days ranged between 0.16% and 0.27%. In the glucose treatments, (i) total CO₂ emission over 42 days ranged between 712 and 745 mg CO₂-C kg⁻¹ soil, in the order of Glu, Biochar, H₃PO₄, DAP < BiocharP (Fig. 1D), and (ii) the total C loss (mineralised/total) on 42 days ranged between 1.37% and 1.42% (Fig. S1A). In the no-glucose treatments and over time, the majority CO₂ emitted (relative to total CO₂) were from SOC, accounting for 66–87%. In the glucose treatments over time, the proportion of emitted CO₂ (relative to total CO₂) were 2–67% for SOC, 2–47% for SIC, and 17–68% for Glucose-C.

SIC. In the no-glucose treatments, (i) the proportion of SIC loss (relative to the initial SIC content; 48.0 g kg⁻¹ soil) on 42 days ranged between 0.04% and 0.09% (Fig. S1B); and (ii) addition of P fertilisers (DAP, H₃PO₄, BiocharP) over 42 days increased the SIC loss by 0.5–1-fold, while biochar only increased SIC by a quarter, compared to the control soil (21 mg CO₂-C kg⁻¹ soil) (Fig. 1B). In comparison, glucose addition resulted in (i) SIC loss between 0.31% and 0.37% over 42 days (Fig. S1B); (ii) increased SIC loss by 6.1–7.7-fold, with the lowest increase in the DAP and the highest increase in the BiocharP (Fig. 1E);.

SOC. In the no-glucose treatments, (i) the proportion of SOC mineralised (relative to initial SOC; 3.7 g kg⁻¹ soil) on 42 days ranged between 1.7% and 2.7%, and (ii) SOC mineralization increased by 60–82% with application of P fertilisers (DAP, H₃PO₄, BiocharP) and by 51% with Biochar, compared to the soil control (60 mg CO₂-C kg⁻¹ soil) (Fig. 1C). In the glucose treatments, SOC mineralization on 42 days ranged between 3.1% and 4.6%. Importantly, although glucose stimulated SOC mineralization (i.e., positive priming effect), the co-applied P fertilisers (DAP, H₃PO₄, BiocharP) and biochar stabilized glucose-induced SOC mineralisation. That is, glucose-induced native SOC mineralisation was decreased by 11% in the biochar-treated soil, and 15–23% in the P-treated soils (DAP, H₃PO₄, BiocharP), compared to the glucose only treatment where SOC mineralisation was 170 mg CO₂-C kg⁻¹ soil (Fig. 1F).

Glucose-C. The cumulative glucose-C mineralisation over 42 days ranged between 386 and 430 mg CO₂-C kg⁻¹ soil (Fig. 1G), equivalent to 38.6% and 43.0% of the added glucose-C. Addition of P fertilisers and biochar immediately increased glucose-C mineralisation, and such effects diminished over time. Across treatments, most glucose-C mineralisation (>80–90%) occurred at the first nine days after incubation (Fig. 1G). Over 42 days, P fertilisers (DAP, H₃PO₄, BiocharP) increased glucose-C mineralisation by 4.4–11%, i.e., 417 ± 2 ~ 430 ± 6 mg CO₂-C kg⁻¹ soil vs. 386 ± 6 mg CO₂-C kg⁻¹ soil in the glucose only; but biochar only had no impact on glucose-C mineralisation (404 ± 4 mg CO₂-C kg⁻¹ soil).

Table 1. The basic chemical properties of the biochar and Biochar-blended P.

Abbreviations: TC = Total C; TN = Total N; TP = Total P; DOC = Dissolved organic C (< 20 μm); DIC = Dissolved inorganic C (< 20 μm); DN = Dissolved N (< 20 μm). Data are the mean \pm standard error (n = 3). NA: Not Applicable

Properties	Biochar	BiocharP
TC (g kg^{-1})	400.4 \pm 2.6	395.4 \pm 3.1
TN (g kg^{-1})	13.4 \pm 0.3	12.1 \pm 0
TP (g kg^{-1})	10.4 \pm 0.3	33.1 \pm 0.3
Water extractable P _{1:10 w/v} (g kg^{-1})	0.14 \pm 0.01	3.30 \pm 0.07
C/N ratio	30 \pm 1	32 \pm 1
C/P ratio	39 \pm 0	12 \pm 0
DOC (g kg^{-1})	2.1 \pm 0.3	1.2 \pm 0.1
DN (g kg^{-1})	0.12 \pm 0	0.04 \pm 0
DOC/DN ratio	18 \pm 2	33 \pm 1
DIC (g kg^{-1})	<0.04	1.4 \pm 0
$\delta^{13}\text{C}$ (‰)	-27.8 \pm 0	-27.7 \pm 0.1
pH _{1:20}	10.25 \pm 0.02	5.07 \pm 0
EC _{1:20} (mS cm^{-1})	13.1 \pm 0.4	12.2 \pm 0.1
Carboxylic groups (mmole g^{-1})	1.71 \pm 0.19	NA
Total acidic functional groups (mmole g^{-1})	6.25 \pm 0.05	NA
BET surface area ($\text{m}^2 \text{g}^{-1}$)	5.5	5.4
Dominant minerals	Quartz: 32.4%; Dolomite: 49%; Kaolinite: 7.6%; Calcite: 2.7%; Sylvite: 8.3%	

Soil pH and EC: The P fertilisers (DAP, H₃PO₄, and BiocharP) and Biochar decreased soil pH by 0.1–0.2 unit after 42 days, but not at the first 9 days, relative to the control soil (pH 8.99–9.02) (Fig. 2a). When glucose was added alone, soil pH increased throughout the incubation period (pH 9.11–9.18). Such increase was alleviated by 0.1–0.2 unit when P fertilisers (DAP, H₃PO₄, and BiocharP) and biochar was co-added with glucose. In no-glucose treatments, EC was increased by P fertilisers (DAP, H₃PO₄, and BiocharP) and biochar for 20–180 $\mu\text{S cm}^{-1}$ throughout the incubation period, relative to the control soil (280–290 $\mu\text{S cm}^{-1}$) (Fig. 2b). On average, glucose addition decreased EC by 16%. In glucose treatments, BiocharP and biochar increased EC to 315–390 $\mu\text{S cm}^{-1}$, equivalent to 18–50% increases, compared to glucose alone treatment.

Table 2. The estimated CO₂-C emissions derived from soil inorganic C (SIC), soil organic C (SOC), and/or glucose-C in the bulk and Calcarosol rhizosphere (bulk density 1.4 g cm⁻³) after 42 days of incubation period. The values are calculated based on assumptions of inputs of 83 kg P ha⁻¹, and 75 kg N ha⁻¹, and 1.4 t rhizodeposits C ha⁻¹ in the respective treatments. Data are mean ± standard error (n=3). Abbreviations: Glu = glucose, DAP = Diammonium phosphate [(NH₄)₂HPO₄], BiocharP = biochar-blended P, NA = not applicable.

Treatments	Total C loss (kg C ha ⁻¹)	SIC loss (kg C ha ⁻¹)	SOC loss (kg C ha ⁻¹)	Glucose-C loss (kg C ha ⁻¹)	Remained total C (t ha ⁻¹)
<i>Bulk soil (without glucose input)</i>					
Control soil	114±7	28.8±1.5	86±5	NA	72.27±0.01
DAP	194±1	56.7±1.8	137±1	NA	72.19±0.01
H ₃ PO ₄	181±4	42.4±1.7	139±3	NA	72.20±0.01
Biochar	165±3	36.1±1.5	129±2	NA	72.21±0.01
BiocharP	214±4	58.0±2.5	156±1	NA	72.17±0.01
<i>Rhizosphere soil (with the input of glucose, e.g., a surrogate of rhizodeposits)</i>					
Glu	997±16	218±8	239±7	541±9	72.78±0.02
DAP+Glu	1010±5	205±4	202±2	603±3	72.77±0.01
H ₃ PO ₄ +Glu	999±16	232±16	184±13	583±8	72.78±0.02
Biochar+Glu	1003±11	227±9	212±7	565±6	72.78±0.01
BiocharP+Glu	1043±15	250±9	191±5	602±8	72.74±0.02

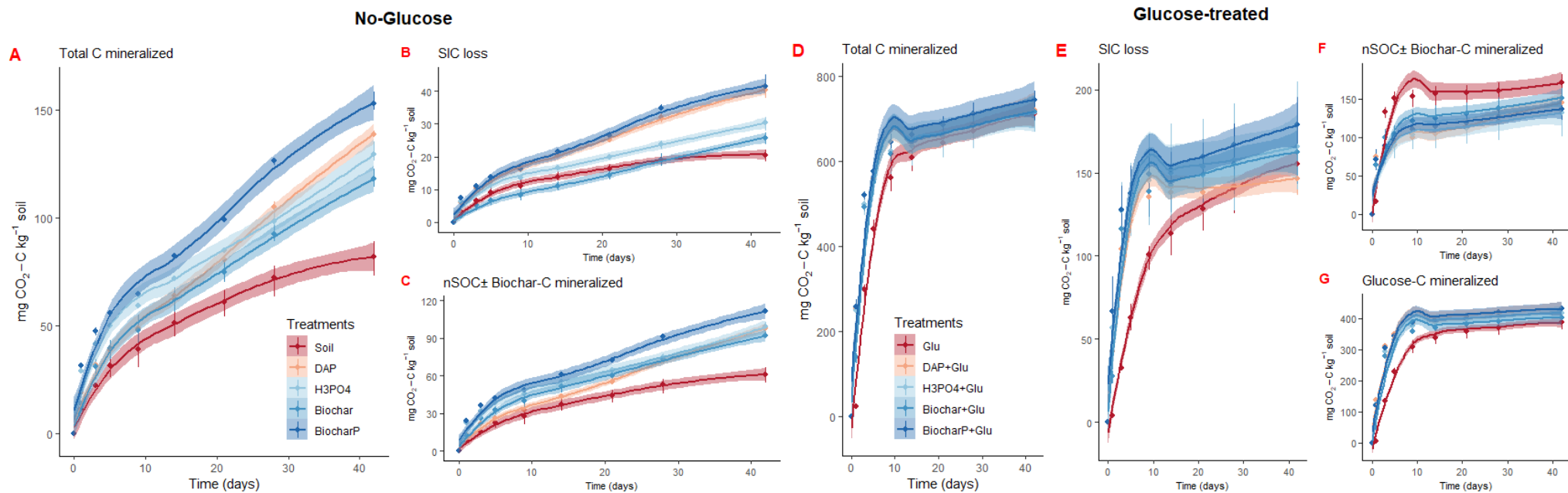
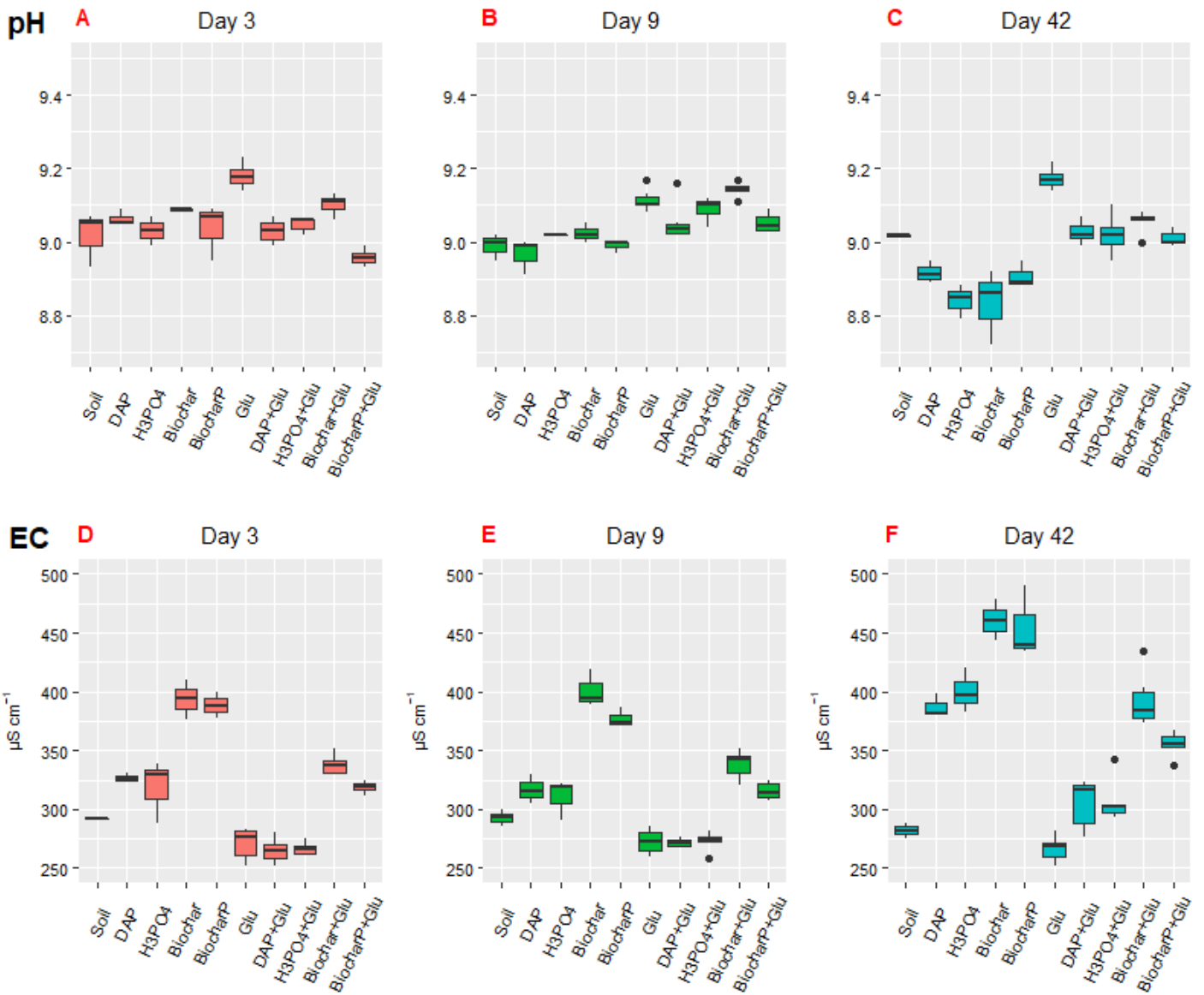
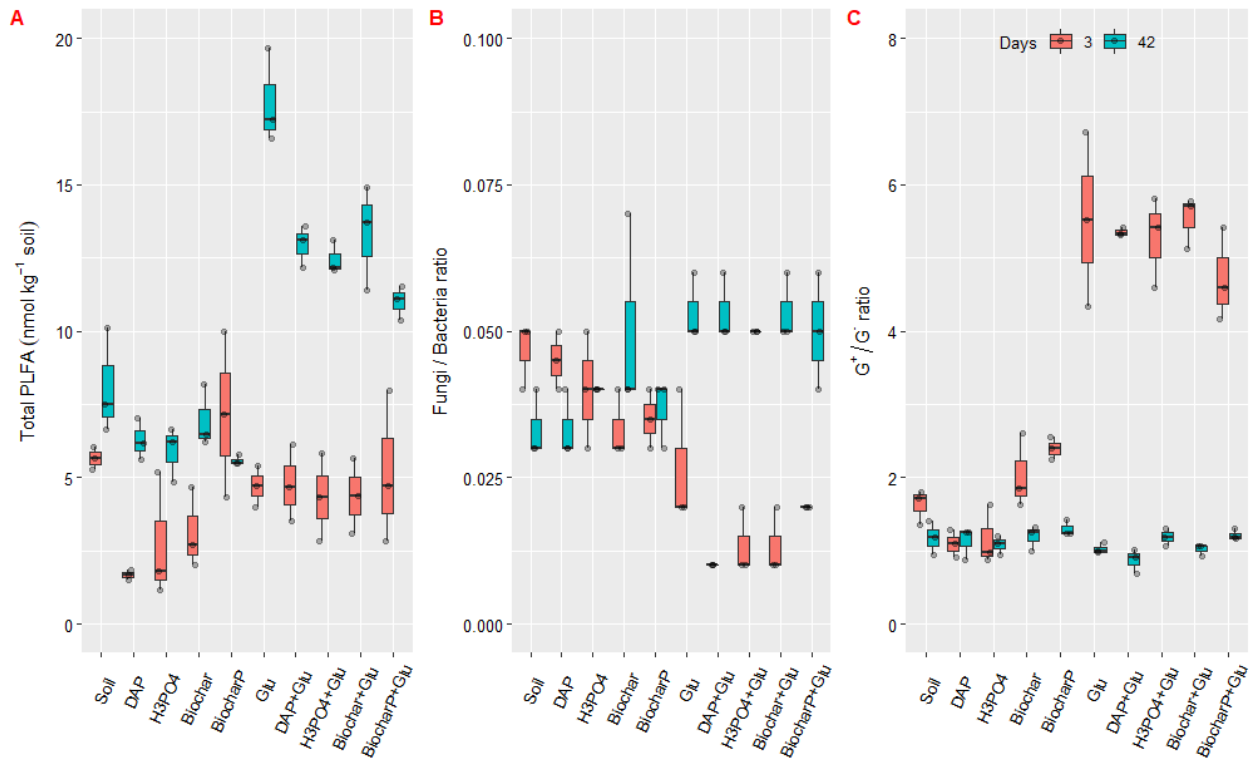


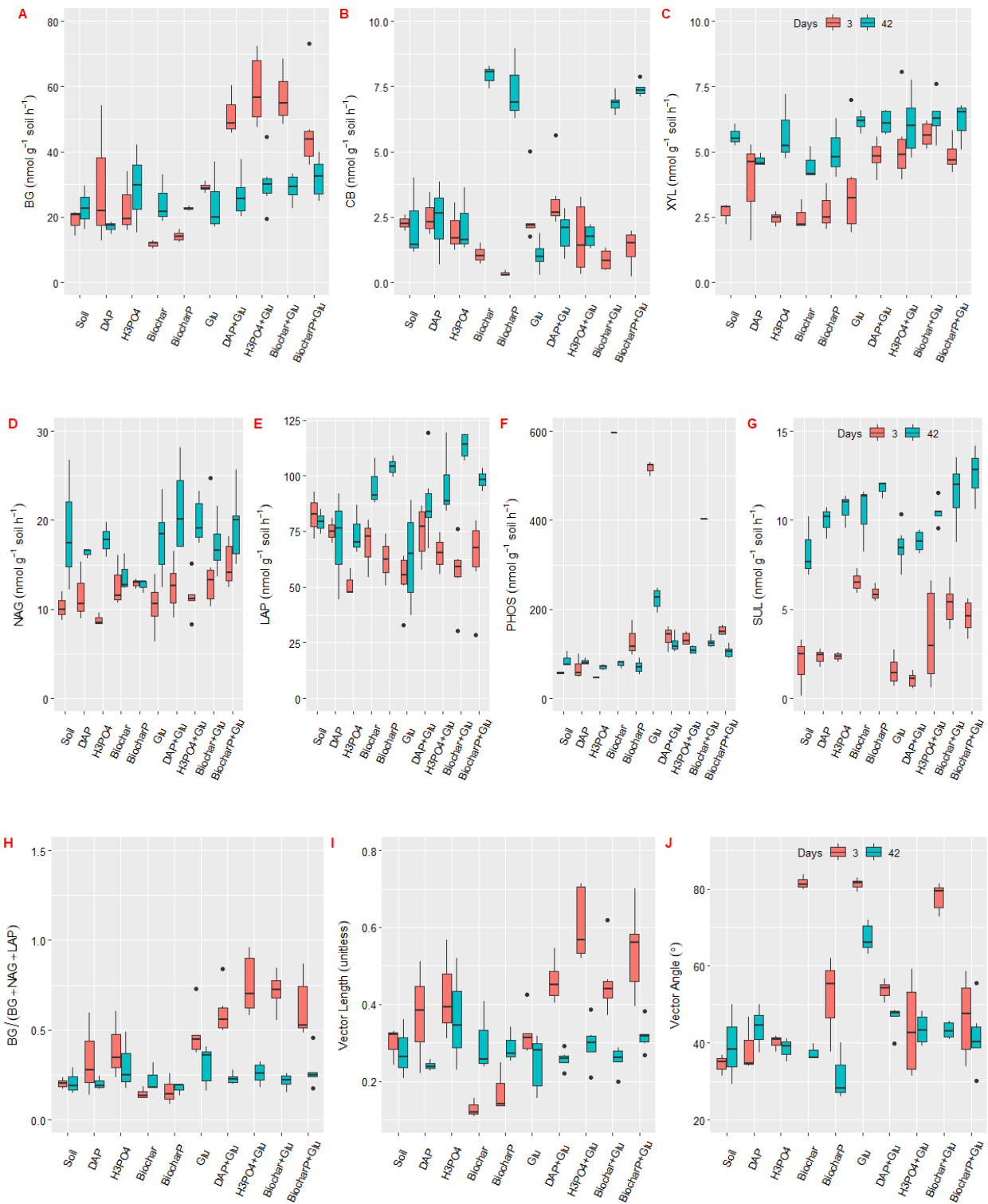
Fig. 1. Cumulative CO₂-C emission (mg CO₂-C kg⁻¹ soil) from the total C (TC) (**A, D**), soil inorganic C (SIC) (**B, E**), SOC ± 7C (**C, F**), glucose-C (GluC) (**G**) from the Calcarosol subsoil over the 42 days incubation period. The treatments include four phosphorous forms [Diammonium phosphate (DAP), phosphoric acid (H₃PO₄), biochar (Biochar), and biochar-blended P (BiocharP)] without and with glucose addition (1.0 g glucose-C kg⁻¹ soil). Error bars represent ± standard errors of the mean.



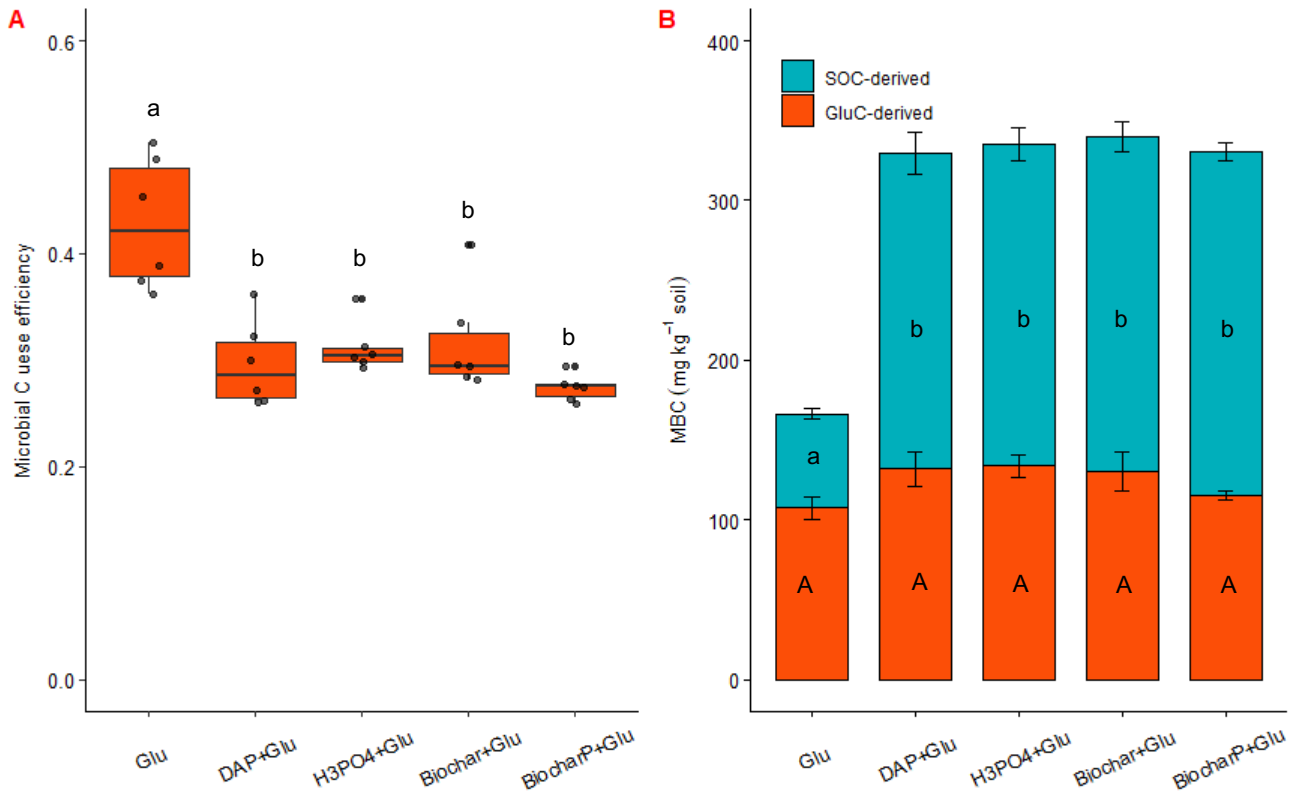
Error! Reference source not found. Soil pH and EC in the Calcarosol subsoil after 3, 9, and 42 days of incubation period. The treatments include four phosphorous forms [Diammonium phosphate (DAP), phosphoric acid (H₃PO₄), biochar (Biochar), and biochar-blended P (BiocharP)] without and with glucose (Glu) addition.



Error! Reference source not found. Total PLFA, Fungi/Bacteria ratio, and G⁺/G⁻ ratio in the Calcarosol subsoil on 3 and 42 days. The treatments include four phosphorous forms [Diammonium phosphate (DAP), phosphoric acid (H₃PO₄), biochar (Biochar), and biochar-blended P (BiocharP)] without and with glucose addition (1.0 g glucose-C kg⁻¹ soil).



Error! Reference source not found.. Soil enzyme activities (nmol g⁻¹ soil h⁻¹) on days 3 and 42, and Wild-type F statistics (*p*-values) of the fixed terms and their interactions on enzyme activities (univariate analysis). The treatments include four phosphorous forms [Diammonium phosphate (DAP), phosphoric acid (H₃PO₄), biochar (Biochar), and biochar-blended P (BiocharP)] without and with glucose addition (1.0 g glucose-C kg⁻¹ soil). Abbreviations: BG = β -Glucosidase; CB = β -Cellubiosidase; XYL = β -Xylosidase; NAG = Glucosaminidase; LAP = L-Leucine aminopeptidase; PHOS = Alkaline phosphatase; SUL = Arylsulfatase.



Error! Reference source not found. Microbial C-use efficiency (CUE) of glucose (A) and microbial biomass C (MBC)-derived from glucose and native SOC (B) on 3 days in the Calcarosol subsoil.

APPENDIX B

Study 2: Inorganic and organic carbon dynamics in a calcareous subsoil: The abiotic and biotic drivers

Overall highlight: The long-term controlled study (over 21 months) investigates the role of bespoke biochar and goethite on soil C stabilisation in calcareous subsoil has been completed. The analysis of CO₂ and the associated ¹³C from the gas samples at 21 months have been completed. This 21-month study has shown that the effects of goethite and biochar on labile C stabilised after two months. At 21 months, (1) goethite decreased loss of labile C from the lucerne stubble for 3.5%; (2) the bespoke biochar formulation applied to soil facilitated lucerne stubble decomposition for 5–6%. This study indicates that goethite and biochar increased carbon use efficiency of plant-derived carbon, suggesting such amendment applications might offer additional potential for soil carbon storage. This work is currently in the first draft for publication.

Methodology:

The experimental soil was sampled from subsoil (20–40 cm depth) at Poochera, South Australia (32.7436°S, 134.9021°E). The site has an annual rainfall of 326 mm and a mean annual temperature of 18°C (<http://www.bom.gov.au>). The soil was a sandy clay loam (65.9: 27.7: 6.4), classified as a Calcarosol as per the World Reference Base Classification (IUSS Working Group WRB, 2015), or a Calcarosol as per the Australian Soil Classification (Isbell, 2002). The relevant soil properties were total C 51.7 g kg⁻¹ soil, SOC 3.7 g kg⁻¹ soil, SIC 48.0 g kg⁻¹ soil, δ¹³C of total C -5.98‰, δ¹³C of SOC -22.56‰, δ¹³C of SIC -3.60‰, TN 0.6 g kg⁻¹ soil, TP 0.12 g kg⁻¹ soil, Cowell P 1.5 mg kg⁻¹ soil, pH (1:5 soil/H₂O) 9.34, EC (1:5 soil/H₂O) 255 μS cm⁻¹, cation exchange capacity (CEC) 13.2 cmol(+) kg⁻¹. The dominant clay minerals were 66.1% Calcite, 28.5% Gisonite (Hydrated alumino-silicate), 5.4% Ankerite (calcium, iron, magnesium carbonate).

Lucerne stubble, biochar, and goethite

The ¹³C-enriched lucerne stubble (*Medicago sativa* cv. 'Aurora') was obtained after multiple (four times) pulse labelling events (details in SI). Throughout the plant growth season, we applied 50L of ¹³CO₂ (99.0 atom%, Cambridge Isotope Laboratories, Andover, MA, USA) and 26 kg urea-15N ha⁻¹ (98.2 atom%, Cambridge Isotope Laboratories, Andover, MA, USA) was applied. The aboveground plants were harvested and dried at 60°C. Two biochars were produced from pre-treated δ¹³C-labelled sugarcane residues with different δ¹³C levels (Weng et al., 2020). The biochars were pyrolysed in a modified muffle furnace under a stream of pure N₂ gas, where pyrolysis temperature ramped to 450°C at 5–10 °C min⁻¹ and held for 45 min. Prior to the pyrolysis process, each of the δ¹³C-labelled sugarcane residues (<1mm) were treated with FeSO₄ solution and then air-dried, following by mixing with basalt rock dust and kaolinite minerals. The biochars were post-treated with 85% phosphoric acid. The post-treated biochars were dried at 60°C and grind to <125μm for the incubation experiment. The average conversion rate of residue biomass to biochar was 36% on a mass basis. The chemical properties of the biochars are shown in Table 1.

Lucerne stubble was cut into pieces and then ground with a mill (Retsch Ultra Centrifugal Mill ZM 200) to < 2 mm size for soil incubation. Lucerne stubble was finely ground (<125 μm) using a MM400 Mixer Mill grinder (Retsch GmbH, Haan, Germany), redried at 60°C, and analysed for chemical properties (Table 1). To assess the level of uniformity of δ¹³C of the lucerne stubbles and biochars, the labile metabolic organic components (LOC; < 20 μm) in the bulk materials was extracted by 60°C hot water (1:40 w/v) after shaking for 30 minutes (Haddix et al., 2016), and freeze-dried. The remaining residual structural organic components (recalcitrant organic C; ROC) were rinsed with Reverse Osmosis (RO) water and oven dried at 60°C. The weight proportion of LOC and ROC were 44.3 ± 1.0% and 55.7 ± 0.8%, respectively. Representative subsamples of the ground bulk materials, the LOC, or ROC (<125 μm) were analysed for C and δ¹³C by a LECO TruMac® Carbon and Nitrogen Elemental Analyzer (Michigan, USA) and a Combustion Module Cavity Ring-Down Spectroscopy system (PICARRO G2131-i Analyzer: California, USA), respectively.

Synthesized goethite was ground to <125µm. X-ray diffraction (XRD) scanned from 10 to 80°2θ with a 0.05°2θ step-size and a 2s count-time. The surface area of goethite was determined by Brunauer–Emmett–Teller (BET) analysis with N₂ gas. The goethite had specific surface area of 140 m² g⁻¹, and adsorption cumulative volume of pores (between 1.7 and 300 nm) of 0.22 cm³ g⁻¹.

Incubation experiment

The experimental treatments used a full-factorial design (total of eight treatments = 23 with four replicates for each): lucerne stubble (<2mm), biochar (<125µm), goethite (<125µm), and their interactions (Table S1). Lucerne stubble (LS), biochar (BC), goethite (Goe) and were applied to the Calcarosol at 3.6, 5.4, and 1.8 g kg⁻¹ soil (oven-dried basis) (corresponding to 5, 7.6 and 2.5t ha⁻¹) to a depth of 10 cm (bulk density of 1.4g cm⁻³). To mimic the district practice, nutrients (NH₄)₂HPO₄ were applied at 28.6mg kg⁻¹ soil (40kg ha⁻¹) across the control and treatments. In order to partition multiple C sources, two ¹³C-enriched levels lucerne stubble (LSL: 102‰ and LSH: 406‰) and two ¹³C-enriched levels biochar (BCL: 35‰ and LSH: 45‰) were used (Table 1). The soil amendments were uniformly mixed with the air-dried soil, nutrients and RO water to achieve 65% of maximum water-holding capacity. To measure the total CO₂-C and δ¹³C, the soils were incubated with CO₂ traps (NaOH solution) in air-tight vessels which are placed in a dark environment at 20 ± 0.5 °C. The detailed procedures for soil handling and incubation are presented in the SI and a previous study (Fang et al., 2018). The CO₂ traps were replaced on days 3, 10, 18, 30, 60, 140 and 210 for total CO₂-C and δ¹³C analyses. The soils were sampled on days 3, 10, 30, and 210 for chemical (pH and EC, and SOC), and biological (microbial biomass C and N) analyses.

Isotope mixing models

Soil total CO₂ emission and the associated δ¹³C trapped in the NaOH solutions were determined by titration method and the PICARRO G2131-i Analyzer, respectively (Fang et al., 2016). To partition the C sources of CO₂ emission in the treatments, we used mixing models. In the control and goethite-treated soils, the two C-sources (i.e., SIC and SOC) were determined by a two-pool isotope mixing model (Fang et al., 2019). In the LS/BC alone treated soils, the three C-sources (i.e., SIC, SOC, and LS-C/BC) were determined by a three-pool isotope mixing model (Fang et al., 2022), as Eqs. 1-6:

$$f_1 + f_2 + f_3 = 1 \quad (4)$$

$$\delta_{T1} = f_1 \delta_1 + f_2 \delta_2 + f_3 \delta_3 \quad (5)$$

$$\delta_{T2} = f_1 \delta_1 + f_2 \delta_2 + f_3 \delta_4 \quad (6)$$

$$f_3 = \frac{\delta_{T1} - \delta_{T2}}{\delta_3 - \delta_4} \quad (7)$$

$$f_1 = \frac{(\delta_{T1} - \delta_2) - f_3(\delta_3 - \delta_2)}{\delta_1 - \delta_2} \quad (8)$$

$$f_2 = 1 - f_1 - f_3 \quad (9)$$

where f_1 , f_2 , and f_3 represent the proportion of emitted CO₂-C from SIC, native SOC, and LS-C (or BC), respectively; δ_1 , δ_2 , δ_3 , δ_4 , δ_{T1} , and δ_{T2} represent δ¹³C of SIC, SOC, low ¹³C-enriched LS (LS_L or BC_L), high ¹³C-enriched LS (LS_H or BC_H), LS_L or BC_L treated soils, and LS_H or BC_H treated soils, respectively. In the LS and BC co-applied treatments (LS+BC, LS+BC+Goe), only LS-C mineralisation was differentiated based on Eq. 4, but not the other three sources (SIC, SOC, and BC), due to the small proportion (i.e., <3%) of CO₂ derived from biochar.

Microbial attributes

Soil microbial biomass C (MBC) on 3, 10, 30 and 210 days were determined using the chloroform-fumigation-extraction method (Vance et al., 1987) and the details are in the SI. The DOC in acidified soil extracts (carbonate removed) were analysed using a Total Organic Carbon Analyzer (TOC-V CSH/CPN; Shimadzu, Japan). The proportion of LS (or BC)-derived DOC in the soil extracts were calculated by a two-pool mixing model (as detailed in the SI). The total and LS (or BC)-derived MBC was the difference in

the respective DOC between fumigated and non-fumigated samples divided by the conversion factors (kec) of 0.45 (Vance et al., 1987). The CUE in the LS (or BC) alone ± Goe treatments on Day 3 was calculated using Eq. 7 (Geyer et al., 2016):

$$CUE = \frac{MBC_{LS}}{(MBC_{LS} + C_{min})} \quad (10)$$

where, MBCLS is LS (or BC)-derived MBC (mg kg⁻¹ soil), and Cmin is the cumulative LS-C (or BC) mineralized (mg kg⁻¹ soil).

Table 1 The design of the treatments.

No	Treatments	Goethite (g kg ⁻¹ soil)	Biochar (g kg ⁻¹ soil)	Lucerne stubble (g kg ⁻¹ soil)
1	Control soil (nil amendments)	NO	NO	NO
2	Soil + Goe	1.8	NO	NO
3	Soil + BC _L	NO	5.4	NO
4	Soil + Goe + BC _L	1.8	5.4	NO
5	Soil + BC _H	NO	5.4	NO
6	Soil + Goe + BC _H	1.8	5.4	NO
7	Soil + LS _L	NO	NO	3.6
8	Soil + Goe + LS _L	1.8	NO	3.6
9	Soil + LS _H	NO	NO	3.6
10	Soil + Goe + LS _H	1.8	NO	3.6
11	Soil + BC _L + LS _L	NO	5.4	3.6
12	Soil + Goe + BC _L + LS _L	1.8	5.4	3.6
13	Soil + BC _H + LS _L	NO	5.4	3.6
14	Soil + Goe + BC _H + LS _L	1.8	5.4	3.6
15	Soil + BC _H + LS _H	NO	5.4	3.6
16	Soil + Goe + BC _H + LS _H	1.8	5.4	3.6

Abbreviations: Goe = goethite; BC_L = biochar with low ¹³C; BC_H = biochar with high ¹³C; LS_L = lucerne stubble with low ¹³C; LS_H = lucerne stubble with high ¹³C.

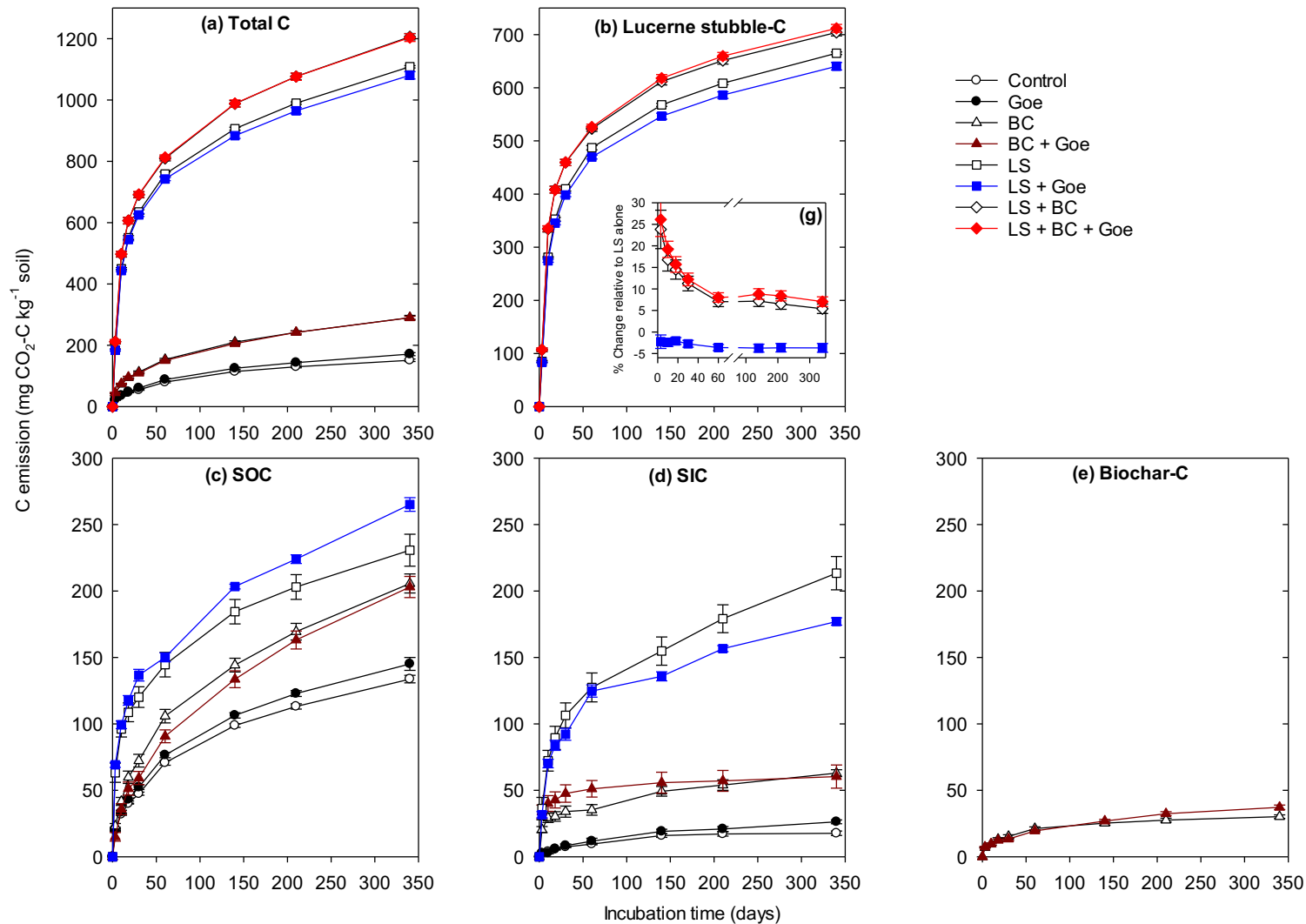


Figure 2. Cumulative mineralization of total C (a), lucerne stubble (LS)-derived C (b), soil organic C (SOC) (c), soil inorganic C (SIC) (d), and biochar-C (BC) (e) in soil amended with biochar, lucerne stubble, and/or goethite (Goe) over the 340 days incubation period. Subfigure e shows the temporal changes of cumulative LS-C mineralization in the presence of biochar and/or goethite, relative to lucerne stubble only treatment. Error bars represent the standard errors of the mean.

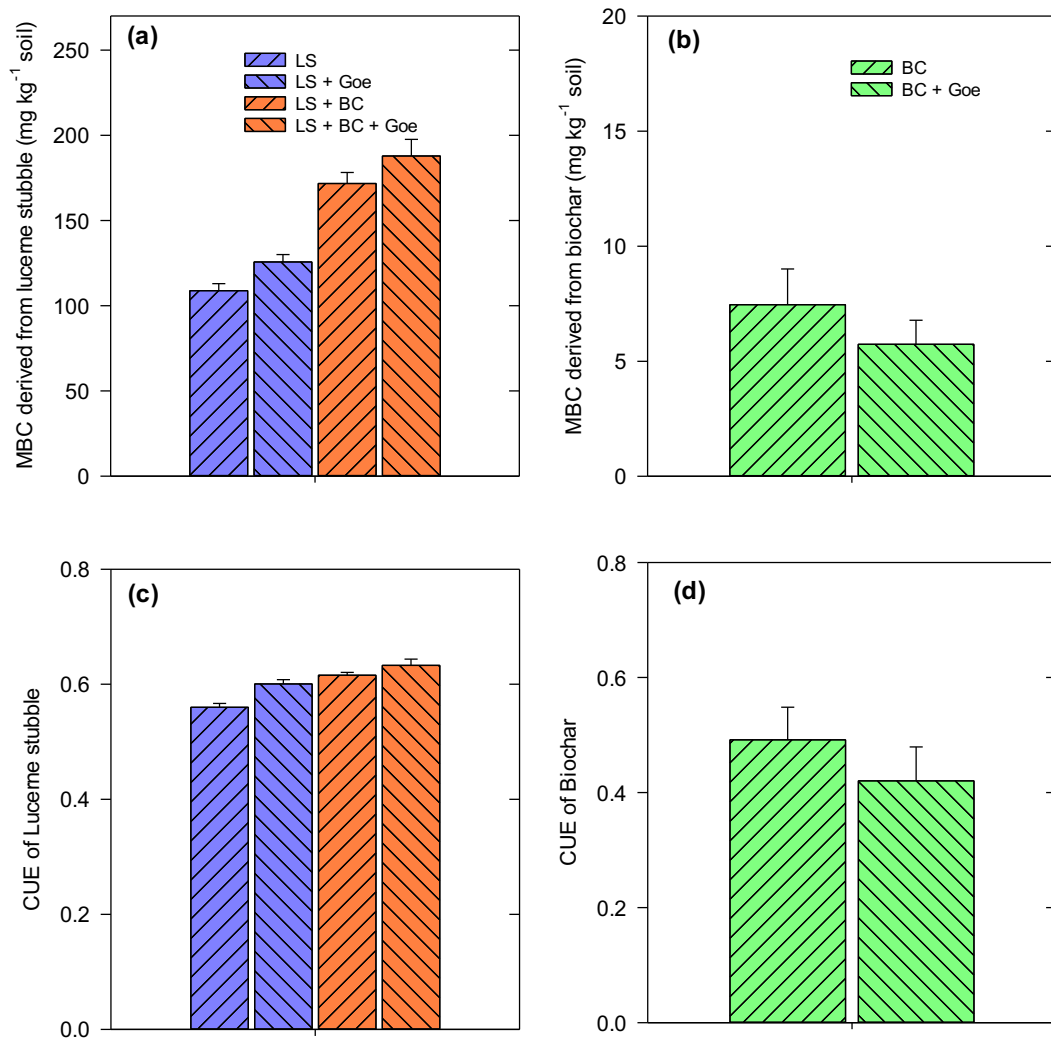


Figure 3. Microbial biomass C (MBC) derived from lucerne stubble (a) and biochar (c), and carbon use efficiency of lucerne stubble (b) and biochar (d) in amended soils after 3 days of incubation period. Abbreviations: LS = lucerne stubble; BC = biochar; Goe = goethite. Error bars represent the standard errors of the mean.

APPENDIX C

Study 3: Disentangling carbon stabilisation in a Calcarosol subsoil amended with iron oxides: a dual-¹³C isotope approach

Overall highlight: Our study provides new knowledge and evidence on the dynamics and sequestration of rhizodeposits and SOC in a Calcarosol following amendment with iron oxyhydroxides. Iron oxyhydroxides significantly increased the stabilisation of organic C, both the simulated rhizodeposit glucose (which increased by 14–80 kg C ha⁻¹), and native SOC. However, this stabilisation needs to be weighed against the increased loss of CO₂ from the carbonate fraction. In conclusion, our study advances the fundamental understanding of C cycling and stabilisation in Calcarosols, presenting a pathway forward to re-engineer hostile subsoils. Iron oxyhydroxide amendments may provide an important solution for increasing SOC in these globally important soils that are prevalent in the arid and semi-arid (sub)tropics of both hemispheres.

Calcarosols generally have a low SOC content which limits biotic and abiotic functions of the soil, and ultimately productivity. The findings from our study give insights to the potential of stabilizing SOC in Calcarosols through the application of Fe oxides amendments, i.e., 14–80 kg C ha⁻¹ from rhizodeposits were stabilised by Fe oxides amendments, by assuming 20% of photosynthetically fixed C (2.5 t C ha⁻¹) entered the soil via rhizodeposition (Gunina and Kuzyakov, 2015). The large positive PE by glucose addition (250–330 mg CO₂-C kg⁻¹ soil, equivalent to 6.7–8.9% of native SOC) presented in the current study suggests that root exudates can result in a relevant quantity of native SOC degradation, which could partly explain the low SOC stock in Calcarosol subsoils. Limiting this SOC loss resulting from positive priming would be an important measure to build SOC stocks. The decreased loss of OC (glucose and native SOC) following goethite and ferrihydrite amendment reinforces the role of Fe minerals in OC stabilisation and offers opportunities to better manage soils for increased SOC content. Notably, our study showed that glucose increased the stable fraction of SOM as shown by the decreased SOC/TN ratio ($p = 0.002$; Table 3), due to increased microbial growth and turnover (Figs. 3 and 4). This result reinforces the importance of labile C inputs in building stabilised SOM in Calcarosol subsoils (i.e., through increasing microbial residues).

Our study showed that the dual-¹³C isotope approach, for the first time, was able to quantify three-C sources in Calcarosol, in particular separating CO₂ emissions from organic and inorganic sources from soil. However, this method can result in some bias due to the nonuniformity and fractionation of C isotopes, which commonly occur during isotope source partitioning. The isotope sensitivity analysis showed that the C loss from different sources can vary when considering glucose fractionation ($\pm 5\%$ variation), i.e., 6.1–8.6% (vs. 8.5%) for SOC, 34.5–40.2% (vs. 38.2%) for glucose-C, and 0.1–0.4% (vs. 0.15%) for SIC. The values may potentially shift in the same direction across treatments but the differences between treatments would have remained relative.

Calcarosols pose some unique challenges, particularly relating to their low organic carbon (C) content and low C storage ceiling. To address this, we investigated the role of iron (oxyhydr)oxides – goethite and ferrihydrite (0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil) in the presence of a labile C substrate (glucose) to simulate rhizodeposition, on C-cycling. As the incubation of soil had three potential C sources: (i) glucose-C, (ii) native SOC, and (iii) soil inorganic C (SIC), a novel dual-¹³C isotope approach ($\delta^{13}\text{C}$ -enriched glucose of 29 and 81‰) was needed to accurately differentiate these C sources in the Calcarosols ($\delta^{13}\text{SOC}$, -23‰ ; $\delta^{13}\text{SIC}$, -3.6‰). Over 28 days, across the glucose and iron oxides treatments, 34.8–41.7% of the supplied glucose-C (1.0 g C kg⁻¹ soil), 7.5–9.6% of the native SOC (3.7 g kg⁻¹ soil), and 0.11–0.19% of the SIC (48 g kg⁻¹ soil) were lost as CO₂. Goethite and ferrihydrite generally stabilised organic C (including glucose-C and native SOC) which occurred primarily within the first 10 days after

iron oxides addition, and the stabilisation effect generally increased with increasing Fe oxide dose. This is likely due to rapid Fe-OC adsorption that protected the OC from microbial decomposition. Ferrihydrite (*cf.* goethite) had a smaller effect on suppressing positive priming of SOC mineralisation induced by glucose, possibly linked to the lower C use efficiency and less stable Fe-OC associations owing to the higher dissolution rate of ferrihydrite. The SIC loss increased after glucose addition, which was further enhanced by iron oxides. The quantification of three-C sources from soil CO₂ emissions demonstrated that iron oxides can present as possible amendments for increasing SOC in highly alkaline Calcarosol subsoils.

Methods

Goethite and ferrihydrite were applied to the Calcarosol at 0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil (oven-dried basis) (corresponding to 0.5, 1.0, 5, and 10t ha⁻¹) to a depth of 10 cm (bulk density of 1.4g cm⁻³). To each of these, including a zero Fe control, glucose was applied at 1.0 g C kg⁻¹ soil, with two glucose-¹³C levels (29 and 81‰; three replicates for each) (**Table S2**): For the Fe oxide amendments, soil + amendment was uniformly mixed using air-dried soil (235 g; equivalent to 220g oven-dried soils) by shaking several times in the individual plastic bags. Subsequently, the glucose (24 ml of 23.0 g L⁻¹; equivalent to 0.55 g for each treatment) was added to the soil or soil-Fe oxide mixture in the individual plastic bags, and uniformly mixed by shaking. For each treatment and the control, the soil was adjusted to 65% of maximum water-holding capacity (WHC: 27%), and packed into four 70-mL plastic containers with a bulk density of 1.4g cm⁻³, and these soil containers were placed in a 1.2L air-tight incubation vessel. Each air-tight incubation vessel, also contained a CO₂ trap (containing 20mL of 1 M NaOH) and a jar of RO water (15mL water) (Fang et al., 2018). The vessels were incubated in the dark at 20 ± 0.5 °C. Three closed blank vessels (i.e., without soil) were set up in the same manner as above to account for the headspace CO₂. The CO₂ traps were replaced on days 3, 5, 7, 8, 10, 14, 21, and 28 for total CO₂-C and δ¹³C analyses. The soils were sampled on days 3, 10, and 28 for chemical (pH and EC, Fe content, and SOC), and biological (microbial biomass C and N, and soil enzymes) analyses.

Results and Discussion

Our study using a novel dual-¹³C isotope approach has provided evidence of the stabilisation of organic C (native SOC and exogenous glucose-C) by Fe oxides amended to a Calcarosol, resulting from the protection offered through rapid Fe-OC adsorption (**Fig. 6**). Iron oxides lowered glucose-induced SOC mineralisation by ~22% (**Fig. 2a**), which is supported by the previous finding that Fe(III) phases suppressed SOC mineralization (e.g., by 47%) (Chen et al., 2020). Negatively charged functional groups in SOC (e.g., carboxyl and phenol) may have formed complexes with positively charged Fe oxides through ionic bonding, therefore, being protected from microbial decomposition. This mechanism is supported by the NEXAFS spectra – a greater intensity in the carboxylic peak in soil with a higher dose of goethite (3.6g kg⁻¹ soil) compared to the lower dose (0.36g kg⁻¹ soil) and the nil Fe oxides control.

We found 35–42% of the glucose-C was mineralised across the treatments (Fig. S4b), which is in the lower range compared to the reported values from a meta-analysis which showed 40–70% of added glucose-C mineralized in a month (Geyer et al., 2020). We suggest that this is because the Calcarosol subsoil used in the current study was highly alkaline pH (9.34) and had a very low SOC (3.7 g kg⁻¹ soil), resulting in hostile conditions for microbial growth and activity, compared to the other agricultural soils from semi-arid regions (Khan and Joergensen, 2010; Schmidt et al., 2012).

There was 3–17% lower glucose-C mineralisation in the presence of Fe oxides (goethite and ferrihydrite) (Fig. 2b), reflecting the findings from Chen et al. (2020) that Fe(III) phases suppress the mineralisation of dissolved OC (e.g., by 35%). The low OC/Fed ratio (< 1) in our study (Table 3) indicates that glucose-C was mainly stabilised via the adsorption process (Fig. 6), as suggested by previous studies (Lalonde et al., 2012; Wang et al., 2017). The negative linear relationship between glucose-C mineralization and Fe application doses (Fig. 2; Table

S4 and S5) is likely because the amount of glucose-C adsorbed increased with increasing application doses of the goethite or ferrihydrite. This is further supported by the results obtained with ferrihydrite (cf. goethite) at the highest dose that caused a small but significant decrease in glucose-C mineralisation (350mg kg⁻¹ soil vs. 370mg kg⁻¹ soil found with goethite amendment), and the lower glucose-derived MBC in the ferrihydrite treated soils at Day 3 (Fig. 3a). As ferrihydrite has a poorly crystalline structure but with greater surface area (Fig. S6) than goethite, it has a higher affinity to bind OC and thus is theoretically better at protecting glucose-C from mineralisation. This was supported with a higher OC/Fed in the ferrihydrite treatments ($p < 0.001$; Table 3). In summary, the greater stabilisation of glucose-C than native SOC by ferrihydrite (Fig. 2) is likely due to the preferential adsorption of glucose by ferrihydrite compared to the other forms of carbohydrates in the soil (Eusterhues et al., 2011; Gunina and Kuzyakov, 2015).

The stabilization of glucose-C and native SOC following goethite and ferrihydrite was generally greater soon after their application to soil (≤ 10 days). This effect decreased in some cases after 10 days (Fig. S3). These results confirmed that Fe-OC adsorption is a rapid process, whereas Fe-OC weakened or broke down to release Fe-bound OC during microbial reduction of Fe(III) (Adhikari et al., 2017), which can be equal to, or overtake the protection of Fe-OC adsorption over time (Fig. 6). The varied effects (increase or decrease) of Fe oxides on glucose-C (and SOC) after 10 days can also be partly explained by the hydroxyl radicals produced during Fenton reactions in concert with microorganisms, stimulating OC oxidation to release CO₂ from hotspots (Chen et al., 2020; Du et al., 2020), which countered OC protection by Fe-OC adsorption (Fig. 6).

Table 3. Soil physicochemical properties (20–40 cm soil depth)

Site	Poochera, South Australia
WRB order	Calcarosol
Australian soil order	Calcarosol
pH (1:5 H ₂ O)	9.34±0.01
EC (1:5) (μS cm ⁻¹)	255±1.7
CEC (cmol(+) kg ⁻¹)	13.2±0.2
Exchangeable Ca (cmol(+) kg ⁻¹)	9.59±0.32
Exchangeable K (cmol(+) kg ⁻¹)	0.61±0.02
Exchangeable Na (cmol(+) kg ⁻¹)	0.59±0.02
Exchangeable Mg (cmol(+) kg ⁻¹)	2.39±0.13
Soil texture (Sand: clay: silt)	Sand clay loam (65.9: 27.7: 6.4)
TC (g kg ⁻¹ soil)	51.7±0.4
SOC (g kg ⁻¹ soil)	3.7±0.1
SIC (g kg ⁻¹ soil)	48.0±0.104
δ ¹³ C-TC (‰)	-5.98±0.51
δ ¹³ C-SOC (‰)	-22.56±0.02
δ ¹³ C-SIC (‰)	-3.60±0.15
TN (g kg ⁻¹ soil)	0.6±0.1
TP (g kg ⁻¹ soil)	0.12±0.01
SOC: N: P	31: 5: 1
Cowell P (mg kg ⁻¹ soil)	1.5±0.2
Clay minerals	Calcite: 66.1%; Gismonite (Hydrated alumino-silicate): 28.5%; Ankerite (calcium, iron, magnesium carbonate): 5.4%

Data are mean ± standard error (n = 4). EC = electrical conductivity, CEC = cation exchange capacity, TC = Total C, SOC = Soil organic C, SIC = Soil inorganic C, TN = Total N, TP = Total P.

Table 4. Soil enzyme activities (nmol g⁻¹ soil h⁻¹) on day 28 and Wild-type *F* statistics (*p*-values) of the fixed terms and their interactions on enzyme activities (univariate analysis).

No	Treatments	BG	NAG	LAP	PHOS	SUL	NAG+LAP	Vector length	Vector angle
1	Control soil – nil amendments	62.2±5.3	72.8±8.5	1771±66	2506±392	3.9±0.6	1844±74	0.042±0.00	52.7±4.5
2	Glucose alone	139.8±6.6	81.7±3.8	1717±48	7576±383	5.3±0.8	1799±49	0.075±0.00	75.7±0.8
3	Glu + Goe_1	164.5±7.8	109.6±10.7	1809±58	7985±307	5.7±0.7	1918±54	0.082±0.00	75.6±0.7
4	Glu + Goe_2	165.6±11.2	81.7±2.3	1663±84	7928±196	6.0±0.8	1745±84	0.090±0.00	76.7±0.6
5	Glu + Goe_3	159.8±5.1	100.0±9.3	1636±49	7365±126	6.7±0.9	1736±54	0.087±0.00	75.8±0.5
6	Glu + Goe_4	180.1±7.8	91.4±4.2	1870±52	7685±253	7.4±0.9	1961±53	0.087±0.00	74.7±0.5
7	Glu + Fh_1	147.4±18.2	80.7±10.8	1763±76	6086±170	5.0±0.9	1844±83	0.077±0.00	72.3±0.9
8	Glu + Fh_2	142.6±8.5	91.5±11.1	1756±170	5956±273	5.5±0.6	1847±169	0.078±0.00	71.8±1.8
9	Glu + Fh_3	146.8±7.4	100.7±7.3	1834±87	6639±150	5.0±0.8	1935±86	0.074±0.00	73.0±0.5
10	Glu + Fh_4	128.5±4.4	109.5±7.3	1739±133	6063±227	4.8±0.7	1848±136	0.070±0.00	72.4±0.8
<i>Statistical significance</i>									
Glucose application (control vs. Glu treatments)		< 0.001	0.610	0.209	< 0.001	0.240	0.218	< 0.001	< 0.001
Fe oxides application (Glu only vs. Glu + Fe oxides)		0.153	0.296	0.808	0.046	0.811	0.719	0.186	0.208
Fe oxides type (Goe vs. Fh)		< 0.001	0.987	0.575	< 0.001	0.013	0.576	0.001	< 0.001
Fe oxides dose (linear)		0.901	0.155	0.440	0.604	0.358	0.352	0.463	0.632
Fe oxides dose (non-linear)		0.965	0.396	0.447	0.888	0.867	0.408	0.620	0.763
Fe oxides type × Fe oxides dose (linear)		0.076	0.041	0.357	0.279	0.137	0.490	0.247	0.335
Fe oxides type × Fe oxides dose (non-linear)		0.365	0.075	0.081	0.038	0.975	0.065	0.813	0.694

Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the treatments represent Fe oxides application doses of 0.36, 0.72, 3.6 and 7.2 g kg⁻¹ soil, respectively. ACE = Esterase, BG = β-glucosidase, NAG = Glucosaminidase, LAP = L-Leucine aminopeptidase, PHOS = Phosphatase, SUL = Arylsulfatase. Vector length = relative investments in C vs. nutrient acquisition. Vector angle = P vs. N acquisition. Data are mean ± standard error (n = 3 for the control, or n = 6 for the treatments).

Table 5. Citrate-dithionite extractable Fe content (Fe_d), the molar ratio of Fe-bound OC: Fe_d , SOC, and TN contents, and SOC/TN ratio after 28 days of incubation. Wald-type F statistics (p -values) of the fixed terms and their interactions on each parameter (univariate analysis).

NO	Treatments	Fe_d content (g kg ⁻¹ soil)	Fe-bound OC/ Fe_d	SOC (g kg ⁻¹ soil)	TN (g kg ⁻¹ soil)	C/N ratio
1	Control soil – nil	2.18±0.07	0.48±0.02	3.40±0.01	0.27±0	12.3±0.2
2	Glucose alone	2.18±0.02	0.43±0.03	3.30±0.02	0.29±0	11.6±0
3	Glu + Goe_1	2.21±0.03	0.40±0.03	3.20±0.01	0.28±0.01	11.6±0.2
4	Glu + Goe_2	2.48±0.04	0.40±0.04	3.48±0.05	0.30±0.01	11.5±0.4
5	Glu + Goe_3	3.62±0.07	0.26±0.02	3.18±0.01	0.28±0.01	11.5±0.2
6	Glu + Goe_4	5.37±0.21	0.18±0.02	3.31±0.03	0.28±0.01	11.7±0.3
7	Glu + Fh_1	2.22±0.05	0.48±0.03	3.30±0.01	0.29±0	11.3±0.1
8	Glu + Fh_2	2.28±0.02	0.44±0.02	3.23±0.05	0.29±0.01	11.2±0.3
9	Glu + Fh_3	3.55±0.02	0.29±0.03	3.40±0.01	0.29±0	11.7±0.2
10	Glu + Fh_4	5.03±0.04	0.26±0.03	3.31±0.02	0.29±0	11.5±0.2
<i>Statistical significance</i>						
	Glucose application (control vs. Glu treatments)	0.965	0.151	0.082	0.043	0.002
	Fe oxides application (Glu only vs. Glu + Fe oxides)	0.182	0.853	0.918	0.449	0.430
	Fe oxides type (Goe vs. Fh)	0.015	< 0.001	0.487	0.163	0.246
	Fe oxides dose (linear)	< 0.001	< 0.001	0.664	0.246	0.156
	Fe oxides dose (non-linear)	0.794	< 0.001	0.005	0.033	0.655
	Fe oxides type × Fe oxides dose (linear)	0.107	0.556	0.054	0.816	0.361
	Fe oxides type × Fe oxides dose (non-linear)	0.284	0.090	< 0.001	0.005	0.294

Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the treatments represent Fe oxides application doses of 0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil, respectively. Data are mean ± standard error (n = 3).

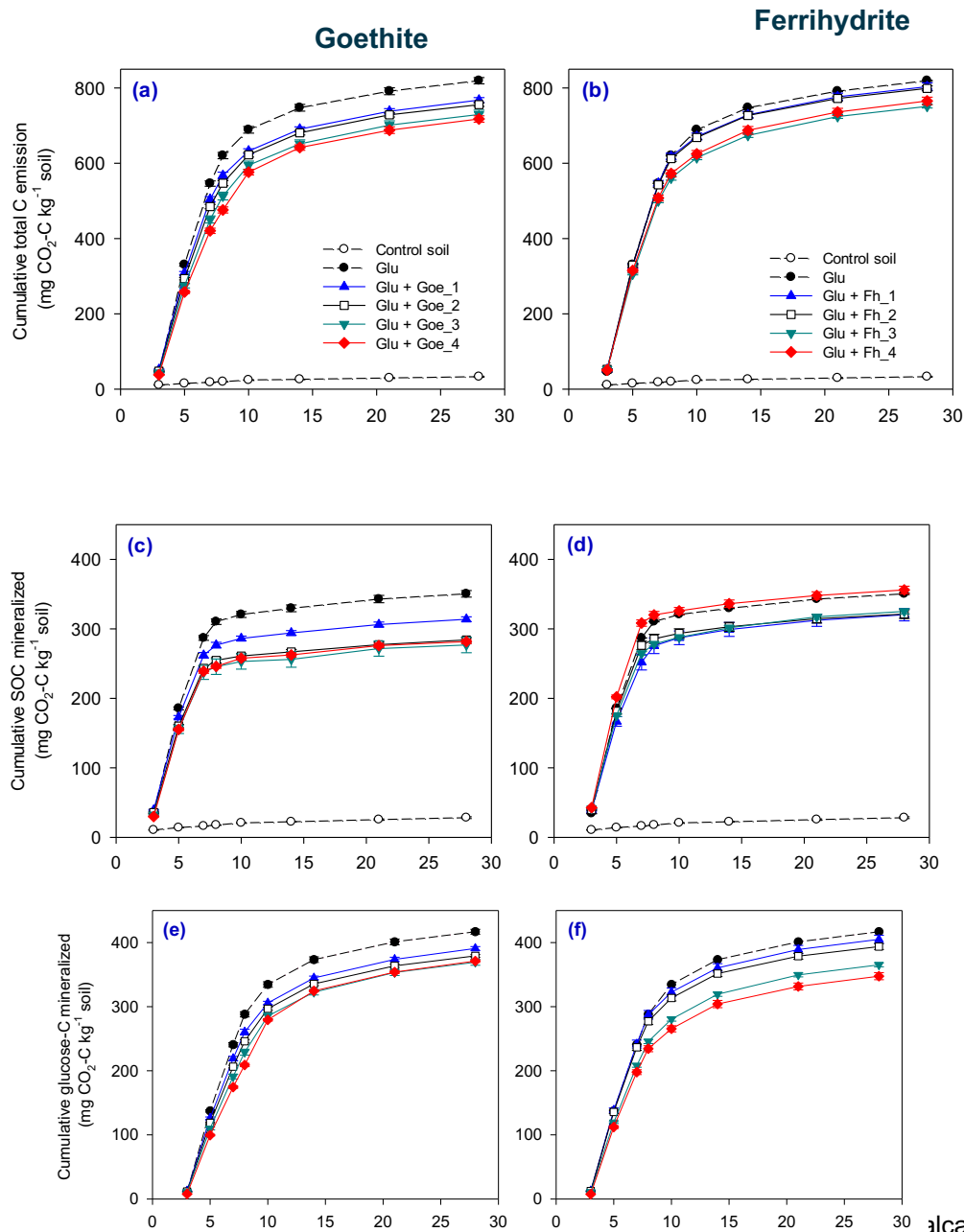


Fig. 4. Cumulative of the total C emission (a, b), SOC mineralised (c, d), glucose-C mineralised (e, f), and soil inorganic C (SIC) loss (g, h) (mg CO₂-C kg⁻¹ soil) over the 28 days incubation period. The treatments include glucose application (1.0 g glucose-C kg⁻¹ soil) without and with the application of Fe oxides (goethite and ferrihydrite). Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the legends represent Fe oxides application doses of 0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil, respectively. Error bars represent ± standard errors of the mean (n = 3 for the control, and n = 6 for the treatments).

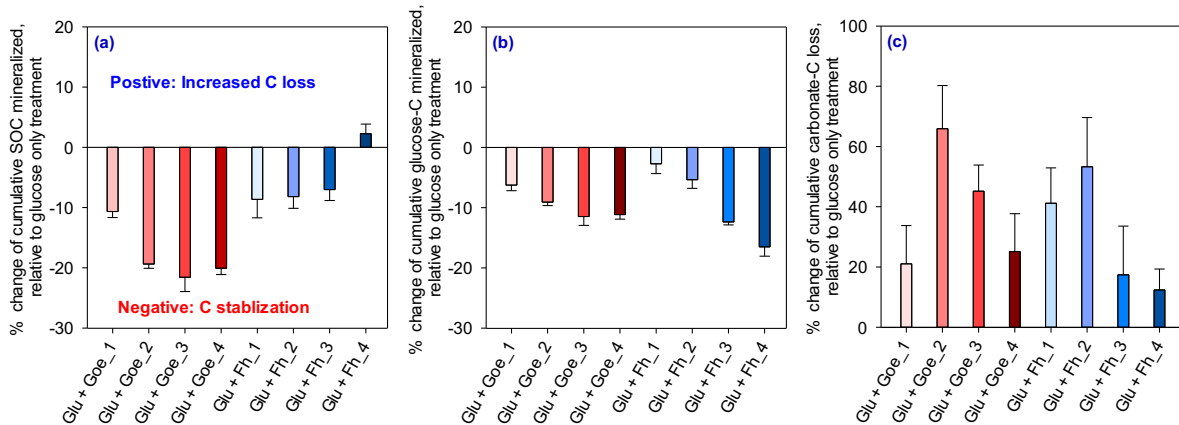


Fig. 2. Percentage changes of cumulative SOC mineralised (a), glucose-C mineralised (b), and soil inorganic C (SIC) loss (c) from the treatments of Glucose + Fe oxides, relative to Glucose only, after 28 days of incubation. The treatments include glucose application (1.0 g glucose-C kg⁻¹ soil) without and with the application of Fe oxides (goethite and ferrihydrite). Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the legends represent Fe oxides application doses of 0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil, respectively. Error bars represent ± standard errors of the mean (n = 6).

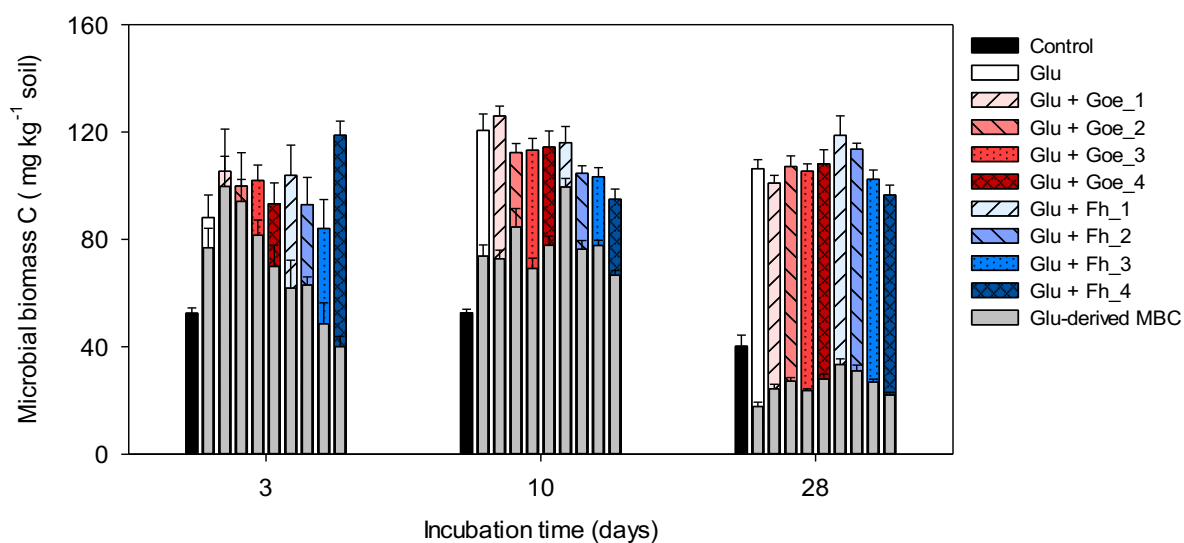


Fig. 5. Total and glucose-derived microbial biomass C (MBC) in the soils on 3, 10, and 28 days. The treatments include glucose application (1.0 g glucose-C kg⁻¹ soil) without and with the application of Fe oxides (goethite and ferrihydrite). Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the legends represent Fe oxides application doses of 0.36, 0.72, 3.6, and 7.2 g kg⁻¹ soil, respectively. Error bars represent \pm standard errors of the mean ($n = 3$ for the control, and $n = 6$ for the treatments).

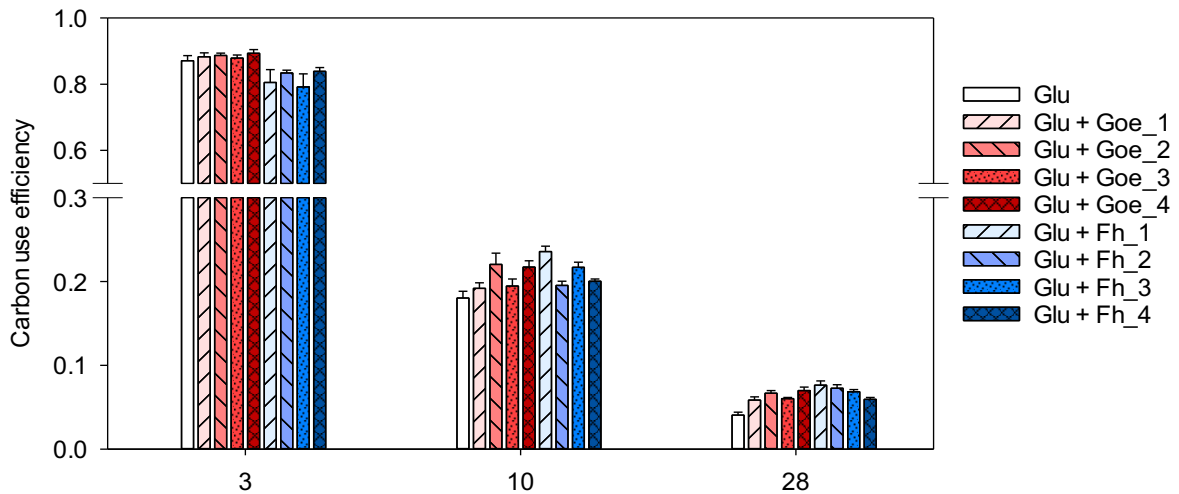


Fig. 6. Microbial C-use efficiency (CUE) of glucose on 3, 10, and 28 days. The treatments include glucose application ($1.0 \text{ g glucose-C kg}^{-1} \text{ soil}$) without and with the application of Fe oxides (goethite and ferrihydrite). Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1, 2, 3, and 4 in the legends represent Fe oxides application doses of 0.36 , 0.72 , 3.6 , and $7.2 \text{ g kg}^{-1} \text{ soil}$, respectively. Error bars represent \pm standard errors of the mean ($n = 6$).

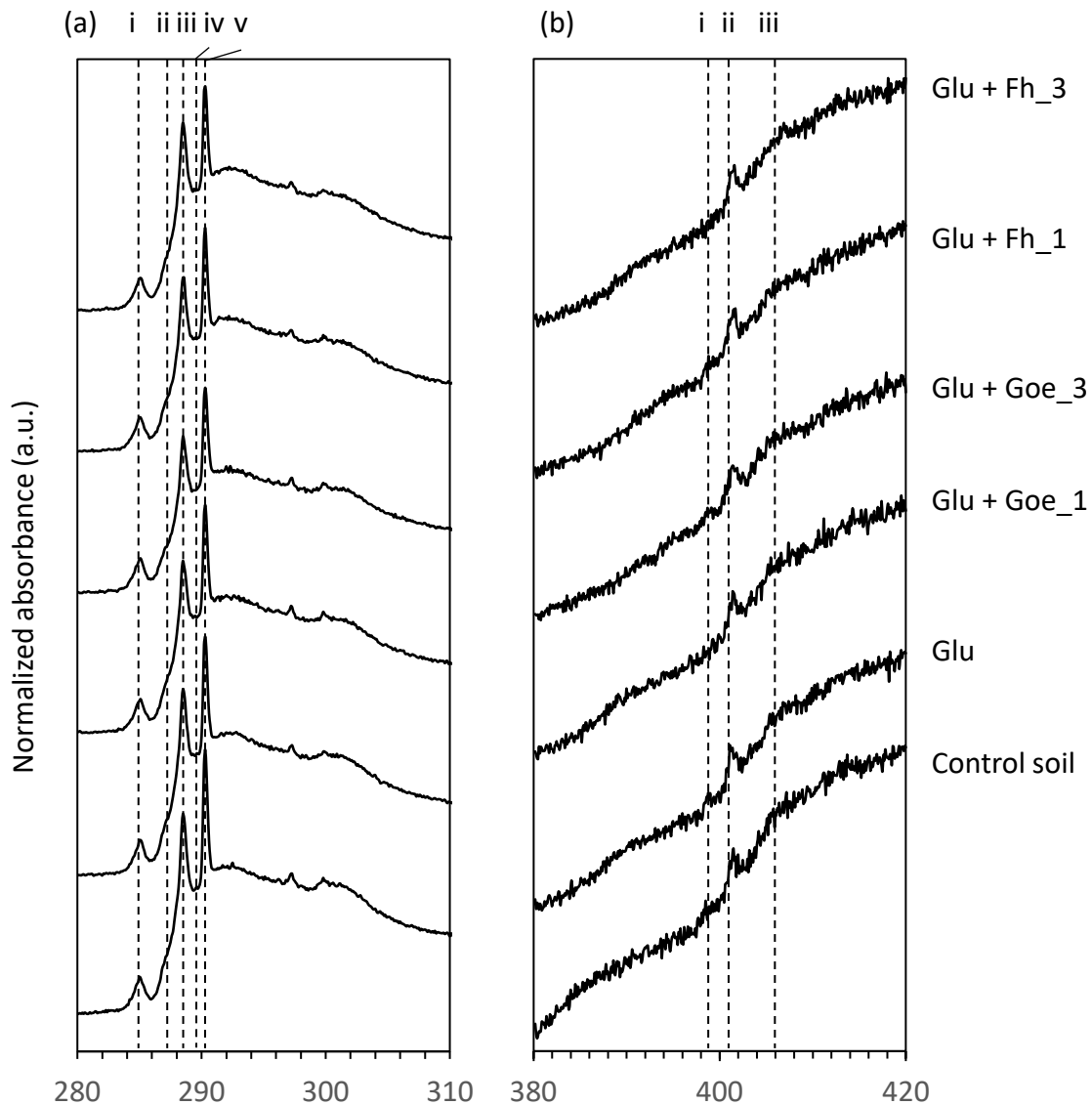


Fig. 7. Normalised absorbance of C **(a)** and N **(b)** K edge NEXAFS spectra of soils after 28-day incubation. The treatments include glucose application ($1.0 \text{ g glucose-C kg}^{-1} \text{ soil}$) without and with the application of Fe oxides (goethite and ferrihydrite). Abbreviations: Glu = Glucose, Goe = Goethite, Fh = Ferrihydrite. The numbers 1 and 3 in the legends represent Fe oxides application doses of 0.36 and $3.6 \text{ g kg}^{-1} \text{ soil}$, respectively. Carbon features were identified as: (i) aromatic C at 285 eV ; (ii) aliphatic at 287.5 eV ; (iii) carboxylic at 288.6 eV ; (iv) carbohydrate hydroxyl at 289.6 eV ; and (v) carbonate C=O at 290.2 eV . Nitrogen features were identified as: (i) aromatic N in 6-membered rings (pyridines, pyrazines) at 398.8 eV ; (ii) amide (protein) at 401.2 eV ; and (iii) alkyl-N at 406 eV .

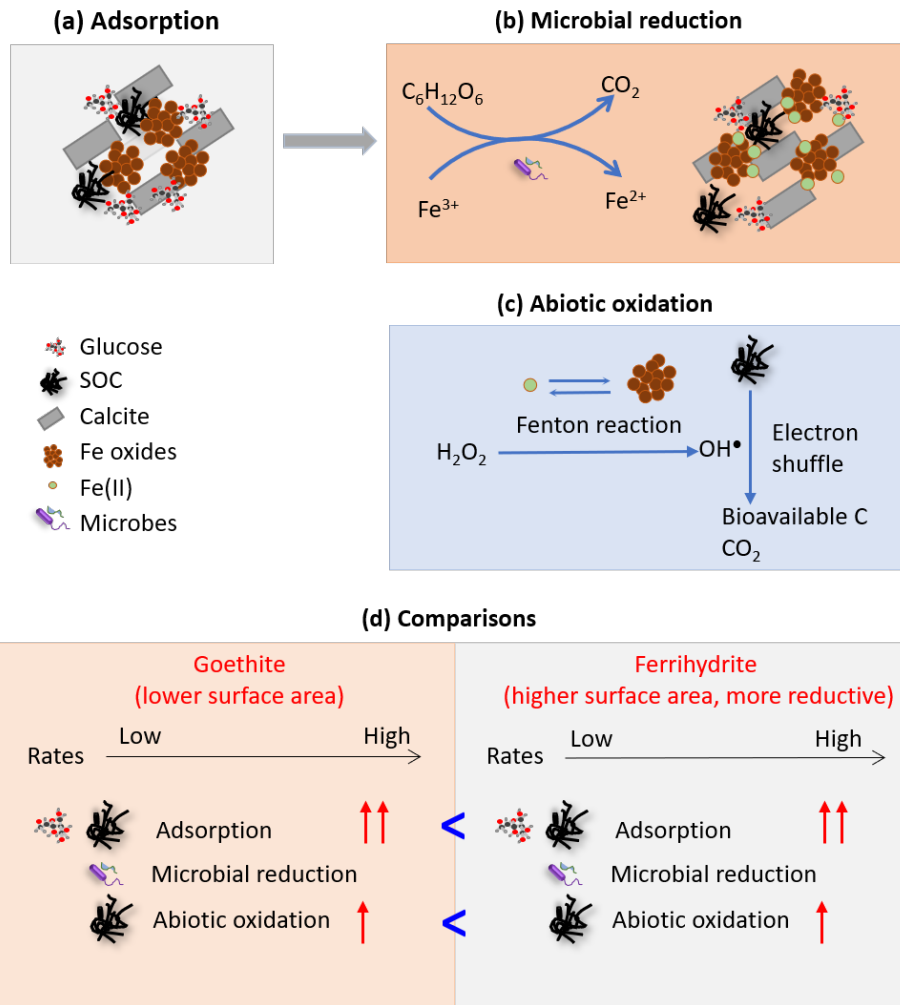


Fig. 8. A conceptual diagram showing glucose and native SOC stabilization as affected by Fe oxides (goethite and ferrihydrites) in the Calcarosol. (a) Fe oxides adsorb C to stabilise glucose-C and native SOC; (b) Microbial reduction increases glucose C mineralisation; (c) Fenton reaction and electron shuttling increases abiotic oxidation of native SOC; (d) Ferrihydrite (*cf.* goethite) are more microbially reducible and counteracts the greater protection from adsorption resulting from the higher surface area.

Appendix A, B, and C please contact:

Dr Ehsan Tavakkoli | Senior Mortlock Fellow

School of Agriculture, Food and Wine | The University of Adelaide, Waite Campus

T: 08 8313 7418 M: 0421018075 ORCID: orcid.org/0000-0002-2676-6018

E: ehsan.tavakkoli@adelaide.edu.au W: <https://www.adelaide.edu.au/directory/ehsan.tavakkoli>