

Final Technical Report

Understanding Biological Farming Inputs

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Abstract

Biological inputs have received much attention in recent years, but much of this research has been conducted overseas, in different systems (e.g. horticulture), or in laboratory and glasshouse experiments. The goal of this research was to investigate biological inputs including alternative fertilisers, biostimulants, microbial inocula, humates, composts, manures and biochars. We sourced our data from local reports and international peer-reviewed literature, as well as collating an inventory of over 60 biological inputs available within Australia and chemically characterising them. Despite finding a wide variability in biological inputs, both within and between product classes, few significant results were found either in the glasshouse, or in a series of eight field experiments testing 38 biological inputs across two growing seasons and five states. Further mechanistic research found no positive effect of biostimulants or humates on the capture of N by wheat, either from soil or from an isotopically labelled legume residue. Though the research was conducted only in the short term, we found limited evidence to support efficacy of biological inputs in the Australian broad acre dry-land grains context. We recommend that growers considering the use of biological inputs consider local constraints to production, and conduct controlled on-farm tests before full adoption.

Executive Summary

This project was designed to investigate the efficacy of biological farming inputs in the context of Australian broad-acre dry-land grains cropping. The definition of biological farming inputs is broad, encompassing alternative fertilisers, biostimulants, humates, microbial inocula, composts, manures and biochars – in essence, any non-conventional amendment, the purpose of which is to provide more enhancement to the farming system than just its pure nutrient value.

There are a wide range of biological inputs available within Australia, the main goal of the project was to investigate a wide gamut of amendments across the spectrum of those available. Focusing on coverage rather than in-depth investigation, the three year project chemically analysed well over 60 amendments, testing 38 of these in field experiments conducted at eight sites across the country.

Experimental and investigatory work was broken up into four discrete but complementary scientific components. 1) A thorough review of the literature was conducted, enabling the project to set its own results in the context of wider research findings. 2) Laboratory testing and glasshouse screening was conducted in order to quantify chemical variability of biological farming inputs both within and between product classes, and to examine growth responses of wheat in a controlled environment. 3) Field testing of biological inputs was conducted across eight sites in five states, covering all three GRDC grain growing regions. A total of 38 biological input treatments were investigated (14 per site, four replicated across all sites) relative to a conventional fertiliser response curve (eight treatments). Yield, grain nutrient content, and various soil biogeochemical and microbial properties were examined in order to assess impacts of amendments. 4) Laboratory and glasshouse experiments were conducted to probe key mechanisms underlying biological input modes of action. In addition to the investigatory research outlined, this project has provided the opportunity to collate a plain English review of biological farming inputs, accompanied by a practical guide to their on-farm testing, and two on-line calculators to examine data from on-farm experiments and economic scenarios in which biological inputs may improve farm profitability.

From the major review of nationally and internationally available literature, we found that there was a general lack of evidence for the efficacy of biological inputs, especially within the broad-acre dry-land grains context, both within Australia and internationally. Even for more established amendments such as manures and composts, it is apparent from this review of the literature that multiple years of re-application may be required before major changes are observed. A particular barrier to the clarity with regard to biological input efficacy testing is a lack of clarity over active ingredients and mode of action, particularly with regard to biostimulants and humates. Our review highlighted the need to take a productivity or soil constraints approach to evaluating biological farming inputs, with particular emphasis on understanding both the main constraints to production, and also likely longevity of effect for any amendment applied.

Laboratory chemical testing and glasshouse screening revealed that although there is huge diversity in chemical composition, both between and within biological input classes, there were very limited responses of wheat to any of the >50 amendments tested in controlled conditions within a research glasshouse. Across the 38 biological inputs examined over two years at eight field sites, only four significant results in terms of wheat yield were observed. In the first year of trials, two significant reductions in yield were observed after application of one biostimulant and one humate treatment at the Paskeville site in South Australia. In the second year of trials, those treatments did not negatively impact yield again relative to the control. More positively, a chicken litter treatment had a significantly positive effect on grain yield at two sites in New South Wales, though this was likely related to high nitrogen availability within that particular litter. More in-depth analysis of soils from several of the treatments at each of the field sites revealed only sporadic and generally minor responses in terms of various soil health and fertility indicators, including microbial community structure.

Given the limitations of short term (1-2 year) field experiments, we also conducted two mechanistic studies to investigate how biostimulants and humates impact on wheat capture of nitrogen from legume litter (simulating a previous legume rotation), and how biochars, humates, composts and manures impact on nutrient and carbon cycling and release as a function of biological input chemistry. The glasshouse experiment investigating nitrogen uptake utilised ¹⁵N isotopic labelling in order to establish whether nitrogen within the plant was coming from the legume litter or the bulk soil. Intriguingly, despite conditions optimised for growth, we saw no positive and indeed some negative impacts of biostimulants on capture both of bulk soil- and legume derived- nitrogen. Examination of nitrogen dynamics throughout the wheat growth period also failed to reveal any positive influence of



any of the ten amendments tested on nitrogen release. Thus, it is apparent that in the short term, neither biostimulants nor humates are likely to enhance uptake of nitrogen by wheat.

The second mechanistic experiment was a laboratory incubation in which carbon and nutrient (nitrogen and carbon) fluxes were investigated over a 56 day period following biological input addition, reflective of the early growth period of grains crops. We found that generally, only the more nutrient rich composts and manures enhanced nutrient availability relative to an unamended control soil treatment. Secondly, just as we had previously found amendment classes to be highly heterogeneous on the basis of their chemical composition, we also found that their behaviour upon application to soil was equally variable from a nutrient and C release perspective. Thus, knowledge of the chemistry of biological inputs is required in order to make informed predictions about the impact of such amendments on soil nutrient cycling in the short term.

In addition to the scientific research conducted, this project has produced several key outputs for growers, advisors, and potentially policy makers. A technical report was produced at the start of the project and provided to GRDC to aid in understanding the variety of amendments available, and our project team's approach to investigating their efficacy. A plain English review has been produced that is targeted at growers and advisors, and this collates and summarises key findings from a scientific review paper that has been submitted for publication in an international peer-reviewed journal. This plain English review reports the state-of-the-art of our understanding of biological farming inputs in the context of the Australian broad-acre dry-land grains industry.

In addition to the plain English and scientific reviews, the project produced a practical guide to on-farm testing of biological inputs. This document provides a summary description of the various biological farming input product classes, and focuses the reader to consider key limitations to production, and which amendments may be most likely to exhibit the modes of action required to ameliorate production constraints in their specific situation. The second part of the guide then details how on-farm field-scale testing of biological farming inputs with growers' equipment should be conducted, particularly with regard to replication.

To support the practical guide, two web-based calculators were developed. The first is a simple statistical analysis that allows for two treatments e.g. business as usual and a biological input to be compared, and provides the user with an output determining whether the treatment had a statistically significant impact on yield or other measured parameters. The second calculator provides a tool for economic scenario analysis. It enables the user to input conventional input rates and costs, biological input rates and costs, and any expected offset against conventional inputs, and expected change in yield as a result. This then allows the user to examine under what conditions a biological input may become economically viable from a grain yield perspective. The option to investigate impacts of amendment on out years (including re-application of the biological input) is also provided.

Collectively, the research conducted in this project benefits several stakeholders. In providing an overview of the diversity of biological inputs available in Australia, and providing a framework by which known constraints to production can be used to guide selection, we have provided a pathway for growers and advisers to take a targeted approach when considering the use of biological inputs. Our field and mechanistic experiments, indicating minimal short-term positive impacts on yield or soil fertility, provide a background level of expected results, at least in the short term. Whilst we acknowledge that we have not been able to test all amendments available, nor have longer-term field experiments been possible within the scope of the project, we are confident in stating that the efficacy of biological inputs must be tested *in situ* on farm in order to reliably predict whether positive economic or environmental outcomes will be seen. Secondly, this project is to our knowledge the largest controlled examination of a wide variety of biological amendments alongside one another in the Australian grain growing context, and thus it provides a valuable benchmark across the varied classes of biological amendments available within Australia. Further long-term research trials would be required in order to identify whether amendments might have significant cumulative effects in the medium term, and we do not discount this possibility where a biological input that is likely to have an effect beyond one growth season is re-applied annually.



Contents

Abstract.....	4
Executive Summary.....	5
Contents	7
Background.....	9
Project objectives.....	12
Methodology	14
Desk based activities	14
Analytical methods	15
Plant analyses	15
Soil and amendment chemical analyses	16
Soil biological analyses	17
Screening studies.....	18
Laboratory characterisation	18
Screening pot experiment.....	19
Field experimentation.....	21
Field set up and analysis	21
The APSIM model and the simulation of WLYP	23
Mechanistic experiments.....	24
Impact of biostimulants on the capture of legume-derived N	24
Impact of biological inputs on soil microbial function and structure.....	26
Statistical analysis.....	28
Location	29
Results.....	30
Chemical characterisation of biological inputs	30
Plant growth screening of biological inputs	36
Testing biological amendments in field conditions	41
Site characteristics	41
Response to conventional fertilisers.....	41
Response of crop and soil to biological inputs	42
Impact of biostimulants and humates on wheat capture of legume derived N.....	48
Plant responses.....	48
Soil responses	51
Impact of biological inputs on soil microbial activity	53
Discussion of Results	57
Overview.....	57
Approaches to on-farm testing	57
Inventory and screening	58
Responses in the field	58
Mechanistic probing.....	59
Conclusion.....	61
Implications.....	62
Recommendations.....	63



Glossary and Acronyms.....64
References65



Background

Pressures continue to mount on the farming community to maintain or increase outputs to feed a rising global population, while also reducing their impact on the land and wider environment, and potentially dealing with an increasingly uncertain climate. As a result of these many external factors, farmers continue to seek innovation to help maintain and potentially grow their businesses. Many biological inputs are marketed as an innovative way to extract more output from the farm while reducing impact, particularly on soil health, either as a substitution for, or in addition to conventional fertiliser or pest control inputs. In 2013-14, soil 'enhancers', including composts, manures and biochars, were applied across 800,000 ha, and the contribution of each sort can be seen in Figure 1 (ABS, 2015). Unfortunately, finer-grained detail on the use of other amendments such as biostimulants, humates etc. is not available.

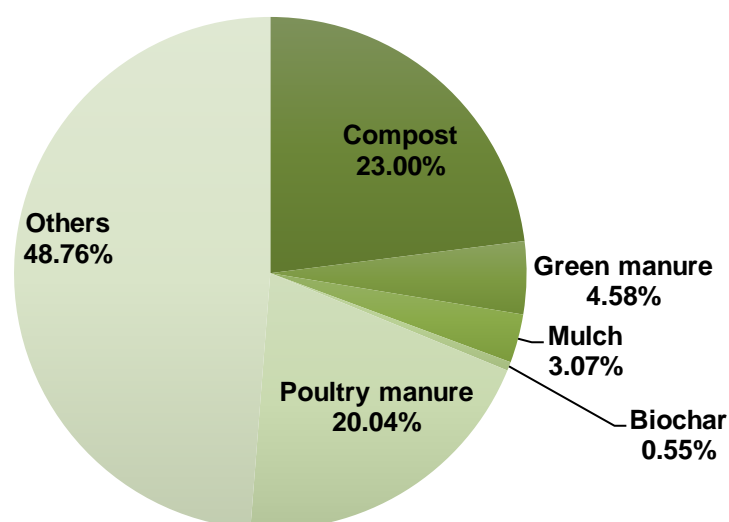


Figure 1: Soil 'enhancer' addition to Australian agricultural soils in 2013-14.

Biological amendments include inocula (mycorrhizal fungi / rhizobia), composts, manures, biochar, biosolids, biostimulants, humates and their value added products, including pelletised forms and extracts such as compost teas. There are also a number of alternative fertiliser products available that are offered with the aims of increasing nutrient use efficiency (NUE), or reducing losses to the environment or impacts on the biological health and function of the soil. These amendments are increasingly being used by growers, either as a replacement for or in addition to conventional farming inputs such as synthetic and mineral fertilisers and pesticides.

In the past decade, there has been an explosion in the understanding of the importance of soil microorganisms and the reactions they govern for soil fertility and health, particularly with regard to disease suppression, nutrient cycling, and carbon (C) storage and release (Graham et al., 2016). Within Australia, GRDC's Soil Biology Initiatives have investigated various aspects of soil biology in the context of the Australian grain growing industry (GRDC, 2009), as well as investment by other industries e.g. meat and livestock (Kahn, 2014) and cotton (Pereg, 2012).

Two major difficulties face growers who wish to better manage soil biology. Firstly, whilst some soil biological tests are available that provide specific insight into certain biological constraints such as disease (e.g. SARDI's Predicta-B tests), generic soil health tests that provide quantitatively meaningful data to growers in the same way as chemical fertility tests are lacking. Some providers do offer various assays ranging from simple microbial biomass values through to molecular profiling of the microbial community. However, relating these results to definite management practices that will improve yield or resilience of the farming system remains difficult.

The second barrier to targeted management of soil biology is the wide array of amendments available with stated modes of action that alter soil microbial structure and function. Some amendments, such

as manures and composts, have a long history of application to agricultural land, originally more for their direct fertiliser effect, rather than as a result of their impact on soil biology. Other amendments such as biostimulants, humates, and microbial inocula are more recent and often specifically target enhancements of soil microbial function as part of their stated modes of action to improve crop productivity. Whilst there is often evidence of the efficacy of various amendments available to growers and advisers, it is usually region and situation specific. As a consequence, broad recommendations are often difficult to make.

Instead of focusing on specifics of individual amendments, an alternative approach when considering the use of biological inputs is to consider specific constraints of the soil, climate, and agricultural system. Though there is much variability within various biological input product classes, it is likely that the mechanisms by which they operate can be categorised in broad terms. If specific constraints to production at a target site are correctly identified, an understanding of how different amendment types may alleviate these provides a potentially useful strategy for their selection (Figure 2).

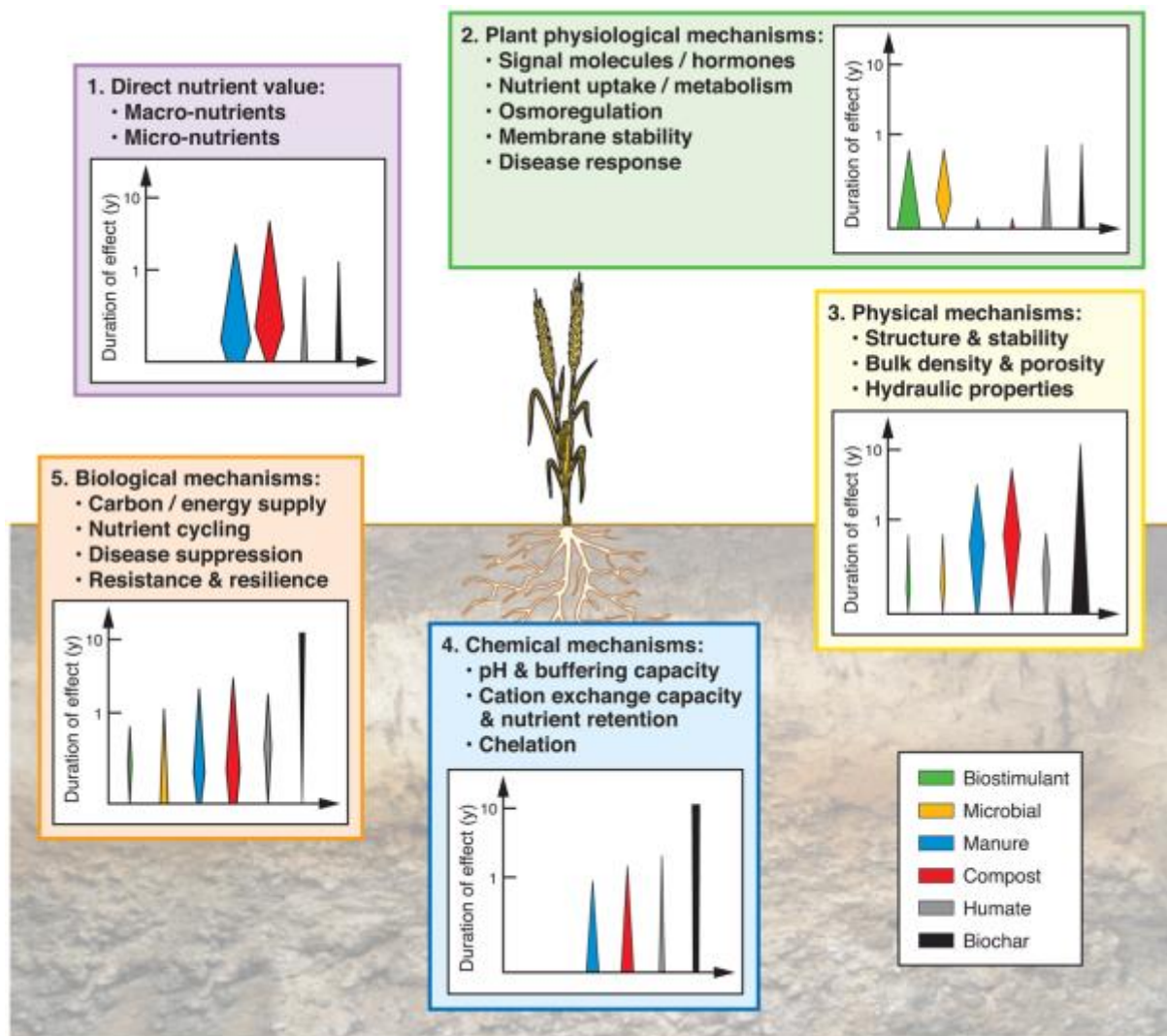


Figure 2: Potential benefits from the application of biological amendments in agriculture can be associated with direct nutrient contributions, plant physiological responses, and / or modifications in soil physical, chemical or biological components of soil health. The biological amendments are very varied but are categorised here as biostimulants (plant growth stimulants), microbial (including rhizobia for legumes and wider groups of microbial inoculants), manure and compost, humates (humic substances, some of which also fit the category of biostimulants), and biochar (includes biochars with a range of different properties). The width of bars indicates estimates of generalised intensity of response and the length of the bars indicates duration of response in years (y). Generalised effects include a range of methods of application and modes of action.

As an example, low soil organic matter (SOM) levels are a frequently observed biological problem soil in Australian grain-growing soils (Aye et al., 2016; Hoyle et al., 2013). Soil organic matter may be augmented by application of compost or manures, but this is not a common option in broad acre cropping due to limited availability of composts in many broad acre areas and high transportation costs, so protection of existing organic matter is a high priority. Biochars have also been heavily researched in recent years, but adoption by the broad acre community has been piecemeal (Macdonald et al., 2016)

Seasonal constraints associated with moisture stresses that contribute to yield loss in broad acre crops include frost and heat-stress (Smith et al., 2009) but the amount and distribution of rainfall can also result in drought-stress (Heng et al., 2007). For example, short dry spells can occur in any seasons more often than droughts and lead to significant reductions crop yield (Rockström et al., 2010). As low and erratic rainfall and temperature extremes become more common due to climate change (Hochman et al., 2017), a key consideration in these environments is to lower production risk by stabilising yields. Biostimulants and some biological amendments may offer the potential of improving the capacity of crops to tolerate such stresses.

The resurgence in interest in use of biological amendments in agriculture, including biostimulants, includes use of products and processes for which there is often little or no scientific research underpinning their effective use (Edmeades, 2002). An exception to this is successful inoculant industries that are underpinned by extensive research and tight regulation based on well-defined industry standards (e.g. legume inoculation (Howieson and Dilworth, 2016)). Scientific knowledge that enables confirmation of benefits attributed to biological amendments is often published in the peer-reviewed literature and is therefore not widely available for most marketed products. Where information is available, it may be developed through participatory on-farm research (Schut et al., 2016) or experimental field trials (Speirs et al., 2013). Nevertheless, the use of biostimulants in agriculture has been estimated to be growing at an annual rate of 12.5% with the global market set to reach US\$2.24 bn by 2018 (Calvo et al., 2014). The microbial inoculant industry is also rapidly expanding (Mordor Intelligence, 2017).

It is therefore clear that while many of these biological farming inputs have been available for a long time, questions about their effectiveness and return on investment remain. The purpose of this project was to investigate a broad range of biological inputs available to Australian grain growers, including examining their chemistry, their impacts on soil microbial communities, and testing their efficacy in the field.



Project objectives

The overarching objective of this project has been to develop an understanding of the hugely diverse array of biological farming inputs available to Australian grain growers. The end goal was to be able to provide Australian grain growers and advisors with up to date advice on how to approach the use of biological inputs.

In order to meet this overarching goal, several individual objectives were set and met throughout the course of the project, and these are laid out below:

1. Collate, interpret and synthesise publicly available information on the use of biological farming inputs.

This objective was met by reviewing published data (scientific peer reviewed papers, reports, product information) and producing two reviews. The first is a manuscript under review at an international scientific journal, and the second a “plain English” document that summarises the information in the scientific review in a more accessible form for stakeholders and also provides an overview of some primary data generated from the project. Whilst these reviews drew on extensive published information, it should be noted that the majority of available data is drawn from studies outside Australia, and generally not in the broad acre cropping industry. However, all interpretation of published data was taken from an Australian grain growing perspective.

2. Collate and analyse a broad range of biological farming inputs available within Australia.

A large number of amendments were obtained from a diverse range of suppliers for laboratory chemical analysis and ongoing incubation, glasshouse, or field-based experiments. This activity provided primary data from which we were able to identify the chemical diversity of the range of amendments available, both within and between product types. Whilst over 60 products were examined in total, it is clear that this represents only a fraction of the inventory of products available. Further, it should be clear that we focussed our analytical efforts primarily on metrics of traditional importance for soil fertility e.g. pH, C content and chemistry, nutrient content etc. An in-depth screening of potentially bioactive compounds within the products was beyond the scope of the present research. It should also be noted that individual products will not be identified in this report or other publications arising. Instead, we have worked to ensure that several products of each type were analysed in order to provide evidence of their chemical composition and where tested, their effect on soil microbial function and grains production.

3. Examine effects of biological inputs on crop growth and yield, and soil biological and chemical fertility.

Given the number of available biological inputs, this objective was split into two components. Firstly, a screening pot experiment was carried out under semi-drought conditions in the glasshouse in order to test over 50 products for their effects on wheat growth and water use under stressful conditions in which it was expected that many of the products purporting positive effects on drought tolerance to demonstrate effectiveness. An important caveat to results arising from this pot experiment is of course that being a pot experiment, findings may not be representative that may be observed in the field, despite the imposition of moderate moisture stress.

In order to address concerns about the suitability of pot experiments for testing biological inputs, in consultation and collaboration with regional grower groups and consultants within WA, SA, Vic, NSW and Qld, we conducted eight field experiments for two years in which a core of four biological amendments were tested at all sites, a selection of six products from a nationally available inventory was tested (with some overlap between sites), and finally four locally relevant biological inputs were tested (usually organic amendments such as composts). In addition to these 14 biological input treatments, a full nutrient response curve (ranging from 0-200% local district practice) was generated. We worked with the grower groups and consultants to identify the best products to test at each site, and in the second year a comprehensive analysis of available nutrients, microbial biomass and community structure was conducted for a number of treatments at each site. Combined, these measurements ensured that not only would we identify which treatments had an impact on



grain yield and nutrient quantity relative to what would have been expected from conventional fertiliser inputs alone, but also that we understand how soil fertility and biology was impacted by the application of biological inputs. Whilst these field experiments were (mostly) conducted for two years with repeated additions of amendments (and fertiliser), it should be borne in mind that where more subtle impacts generated over a timeframe greater than that of this project could not be identified.

In the course of the project, comments have been made from some external parties questioning the validity of plot-based field experimentation for biological input research. Notwithstanding the fact that in order to test a large number of products plot-scale research is the only feasible approach, soil microorganisms operate on the sub-millimetre scale. Thus, provided the application of biological inputs is carried out in a manner representative of that which would be employed were a full paddock being treated, there is no reason why biological treatments would be more advantaged / disadvantaged than conventional fertiliser or other chemical treatments applied at the plot scale.

4. Probe mechanisms by which biological inputs may alter soil processes and plant nutrient uptake.

Although field experiments provide the most realistic setting in which to test the performance of biological inputs, in order to extend results from the locations and seasons specific to the trial sites, it is necessary to build a mechanistic understanding of how products function. Consequently, a glasshouse plant growth experiment and a laboratory incubation experiment were carried out.

Many biological inputs purport to increase nutrient capture from the soil by plants. Noting that if increased nutrient capture from the soil were to occur over several seasons (with a concomitant reduction of conventional fertiliser) then growers would run the risk of mining their soil for nutrients, we instead focussed on ascertaining whether certain biological inputs increased capture of N from a previous legume crop. This was done using ¹⁵N isotopic techniques to clearly identify how much N from the legume was captured by plants. Whilst conducted as a glasshouse experiment, we designed the study in such a way to maximise the likelihood of observing effects of the biological inputs tested.

The second mechanistic experiment focussed on soil biological processes through conducting a laboratory incubation. We used a selection of solid biological inputs from a wide range of chemistries and focussed on how they impacted N and P release, and C mineralisation in relation to their chemistry. We also probed how they impacted on the soil microbial community composition in order to establish whether systematic and predictable patterns occurred that would enable guidance on likely outcomes to be provided in other situations.

5. Provide growers, advisors and other stakeholders with recommendations for the use and on farm testing of biological farming inputs.

In order to meet our end goal of providing the Australian grain growing industry with reliable and transferrable advice on the use of biological farming inputs, we established a rationale for understanding soil / environmental constraints on production and interpreting likelihood of positive outcomes from the use of products to address those constraints. Using this approach, a web-based practical guide for growers on how to consider candidate biological inputs and conduct on-farm tests was produced, accompanied by two calculators to examine results from on-farm experiments, and to explore economic scenarios where it may be advantageous to employ biological inputs. In order to ensure exposure of the research and extension tools to the grain growing industry, we have contributed to several grower group newsletters and extension papers, and presented at two Southern Region GRDC Advisor updates meetings. Following on from this, we have fielded many queries from growers, agronomists and advisors on the use of biological inputs. To provide a lasting outcome of growers and their advisors having the information they need to gain understanding of how biological inputs may best suit their production systems, the “plain English” review (Objective 1) is being targeted at this audience. A final fact sheet summarising the main findings of the project will also be produced after completion in collaboration with the GRDC.

Methodology

In order to complete this project, four types of research were undertaken, and the methodology is laid out below.

Desk based activities

Four main desk based activities were conducted in the completion of this project. An introductory technical report was produced in September 2017 in order to explore constraints to grains production in Australia and how biological inputs may alleviate some of these. This report also laid out some of the methodology to be used within the broader project, and provided an outline of the information that would be yielded by these analytical approaches.

A comprehensive review of the literature was conducted by project scientists. International peer-reviewed literature, and local and some international 'grey literature' sources including client or project reports were examined. The review was built around two main concepts:

1. Reported modes of action including direct nutrient value, plant physiological responses, and impacts on soil quality.
2. Highlighting the need to match potential amendments to the soil constraints to production in the target area.

Soil biological amendments were divided into three major categories (biostimulants, soil organic amendments, microbial inocula) with appropriate sub-categories identified within. Each sub-category discussed the main modes of action proposed and the associated evidence for impacts on plant growth. The review summarised knowledge to date, whether there was general consensus on the likelihood of success in broad-acre cropping, and highlighted where more research is needed. The review then considered the main modes of action through which the various amendment types impact on soil properties and crop growth, before drawing overarching conclusions and recommendations for further research.

The scientific review was led by Lyn Abbott, Lynne Macdonald, and Mike Wong, with other researchers contributing to sub-sections based on their background and expertise as follows:

1. Biological amendments:
 - a. biostimulants (amino acids & hydrolysates, humic substances, seaweed extracts and inoculum: Mike Wong / Mike Webb
 - b. Animal Manures: Sasha Jenkins / Lyn Abbott
 - c. Composts and Vermicomposts: Mark Farrell
 - d. Biochar & related products: Lynne Macdonald
2. Modes of Action:
 - a. Plant physiological responses: Mike Wong
 - b. Soil Physical condition: Lynne Macdonald
 - c. Soil chemical condition: Mike Wong
 - d. Soil Biological condition: Sasha Jenkins / Lyn Abbott

This manuscript is currently under consideration at the international peer-reviewed journal *Agriculture, Ecosystems & Environment*.

Abbott LK, Macdonald LM, Wong MTF, Webb MJ, Jenkins SN, Farrell M (2017) Potential roles of biological amendments for profitable grain production – a review. *Agriculture, Ecosystems & Environment*, in review

The plain English review represents a condensed version of the final scientific review, and incorporates some summary primary data from the current project to provide the best information available to growers at its time of production. Language styling was modified, and tables used to reduce lengthy scientific paragraphs to highlight the main findings and research contexts.



In addition to these reviews and the preliminary technical report, a practical guide to on farm testing of biological products and two web-based calculators were produced. The practical guide provides an introduction to concepts of applying biological inputs to agricultural land, before discussing the major constraints to production (both soil and climate), then provides an overview of the types of biological amendment available that may assist in ameliorating these stresses. The second section of the guide focuses on on-farm testing of amendments, with particular emphasis on the use of conventional farm machinery to generate robust data. Advice on interpreting results is also provided, along with a brief overview of economic considerations. The practical guide can be found on-line at:

<http://soilquality.org.au/factsheets/biological-farming-inputs-a-practical-guide-to-on-farm-testing>

To accompany the practical guide, an on-line t-test calculator was provided to allow statistical comparison between two treatments when analysing yield (or other) data from on-farm tests. This was especially designed to provide clear output for users. The t-test calculator can be found on-line at:

http://soilquality.org.au/calculators/t_test

In addition to the practical guide and t-test calculator, a web-based economic calculator was also produced. Based upon a previous biochar calculator (<http://soilquality.org.au/calculators/biochar>), this allows users to test scenarios in which biological amendments may be used in order to verify required yield increases (or conventional input offsets) in order to see an increase in profit. The calculator allows the user to experiment with changing conventional inputs, and also to indicate whether an effect of the amendment is expected in out-years. The biological input calculator can be found on-line at: http://soilquality.org.au/calculators/bio_product

Analytical methods

Throughout the following three sections that describe the screening, field, and mechanistic investigations carried out as part of this project, a number of analyses (mainly soil chemical and biological) have been carried out. In order to reduce repetition, each analysis is explained here, and then referred to where appropriate in the following sections.

Plant analyses

1. Grain yield – In the case of field experiments, this was determined *in situ* at harvest on the field plot header. These data are supplied as fresh weight, and no moisture correction has been applied. The exception to this was the screening pot experiment where the plant was dried at 60°C for a period of >72 h prior to manual threshing and grain yield quantification.
2. Grain moisture content – Quantified gravimetrically on a sub-sample of grain before and after drying at 60°C for a period of >72 h.
3. Biomass measurements – Quantified gravimetrically upon plant materials dried at 60°C for a period of >72 h. Where measured, root biomass was quantified after manual removal from the bulk soil and careful washing.
4. Total C & N – Quantified on dried and ground plant material (including grain) by high temperature combustion (Trumac CN, Leco Corporation, St Joseph, MI)
5. Total P – Dried and ground plant material was first digested for 24 h in *aqua regia* (3:1 concentrated HCl:HNO₃) before dilution, filtration, and quantification colourimetrically by malachite green (Ohno and Zibilske, 1991) using a microplate reader (Synergy MX multimode plate reader, Biotek Instruments Inc., Winooski, VT).
6. Root morphology – A subsample of washed roots was preserved in 70% ethanol and refrigerated. For analysis, this subsample was scanned on an adapted flatbed scanner (LA2400, Epson, Suwa, Japan) and analysed by WinRhizo image analysis software to quantify root length, circumference, and diameter classes (Regent Instruments Inc., Quebec, Canada). The scanned subsample was then dried at 60°C for a period of >72 h, and root morphology data adjusted to total root biomass on a dry weight basis to obtain total root length data, etc.
7. Isotopic analysis – A sub-sample of plant material was finely ground and weighed into tin capsules before analysis by EA-IRMS (GSL Elemental Analyser coupled to a 20-22 Isotope Ratio Mass Spectrometer, Sercon Ltd., Crewe, UK).



Soil and amendment chemical analyses

1. Extracts to assess soluble / available nutrients
 - a. Soluble chemistry of amendments – Fresh amendment was shaken for 1 h in ultrapure (18.2 M Ω) distilled water (1:20 w/v sample:solution) before centrifugation and filtration through a Whatman 42 filter. Filtrate is then stored frozen until further analysis. Analytical data were corrected for moisture and are reported on a dry weight basis.
 - b. Available C and N in soils – Fresh soil was shaken for 30 minutes in 0.5 M K₂SO₄ (1:5 w/v sample:solution) before centrifugation and filtration. Filtrate was then stored frozen until further analysis. Analytical data were corrected for moisture and are reported on a dry weight basis.
 - c. Available P in soils – Air-dried soil was shaken for 1 h in 0.5 M NaHCO₃ buffered to a pH of 8.5 (1:20 w/v sample:solution) before centrifugation and filtration. Filtrate was then stored frozen until further analysis.
2. pH and electrical conductivity (EC) – Soils and solid amendments were shaken for 30 minutes in ultrapure (18.2 M Ω) distilled water (1:5 w/v sample:solution) before the slurry was analysed by standard pH electrode. Liquid amendments were analysed directly on the concentrated solution as provided by the manufacturer.
3. Moisture content – Fresh soils were dried at 105°C overnight and moisture content was determined as mass loss. Solid organic amendments were dried at 60°C for >72 h due to the potential for the loss of volatiles from these samples at higher drying temperatures.
4. Total C & N – Quantified on dried and ground soil and amendments by high temperature combustion (Trumac CN, Leco Corporation, St Joseph, MI). Samples suspected to contain carbonate-C were fizz-tested using 4 M HCl, and where effervescence was observed these were pre-treated with 5-6% H₂SO₃ on a heated block until effervescence ceased (Baldock et al., 2013b). Samples were then heated to dryness before analysis for C by dry combustion as above. Inorganic C was calculated as the difference between the C concentration of the untreated and treated samples.
5. Mid infra-red (MIR) analysis – MIR was used to obtain an overall picture of the chemical structure of solid amendments. Approximately 100 mg of sample was placed on a steel sample holder and the surface was leveled to ensure a uniform area for spectral analysis. Diffuse reflectance MIR spectra were acquired using a Nicolet 6700 FTIR spectrometer (ThermoFisher Scientific Inc., Waltham, MA, USA) equipped with a KBr beam-splitter, a DTGS detector and an AutoDiff Automated diffuse reflectance accessory (Pike Technologies, Madison, WI, USA). Spectra were acquired over 8000–400 cm⁻¹ with a resolution of 8 cm⁻¹. The background signal intensity was quantified by collecting 240 scans on a silicon carbide disk before analysing each set of 60 soil samples and used to correct the signal obtained for the amendment samples. In total, 60 scans were acquired and averaged to produce a reflectance spectrum for each individual sample, and the Omnic software (Version 8.0; ThermoFisher Scientific Inc.) was used to convert the acquired reflectance spectra into absorbance spectra. The acquired MIR spectra were truncated to 6000–600 cm⁻¹, baseline-corrected using a baseline-offset transformation and then mean-centred before conducting onward statistical analysis (Baldock et al., 2013a). Though we attempted to also obtain spectra on both air dried and freeze dried subsamples of the liquid biological, this was unsuccessful due to the waxy or extremely hygroscopic nature of the dried samples.
6. Nuclear magnetic resonance (NMR) analysis – NMR was used to quantify the chemistry of organic C within the solid amendments, in order to provide information on both the possible source of the organic C (e.g. plant material, microbial, or coal-derived), and its likely stability upon application to soil. Solid-state ¹³C NMR analyses were completed on a 200 Avance spectrometer equipped with a 4.7 T wide-bore superconducting magnet operating at a resonance frequency of 50.33 MHz (Bruker Corporation, Billerica, MA). Weighed samples (150-600 mg) were packed into 7 mm diameter zirconia rotors with Kel-F end caps and spun at 5 kHz. All analyses were completed using full rotors. Chemical shift values were calibrated to the methyl resonance of hexamethylbenzene at 17.36 ppm and a 50 Hz Lorentzian line broadening was applied to all spectra (Baldock et al., 2013b). As for the MIR

analysis, analysis of freeze dried liquid amendments proved impractical due to the waxy or extremely hygroscopic nature of the dried samples.

7. Total elemental analysis – to quantify major (Al, Ca, Fe, K, Mg, Na, S, P) and minor (As, B, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Zn) elements, dried and ground or liquid amendments were first digested in a concentrated acid mixture ($\text{HNO}_3:\text{HClO}_4$, 4:1) in a heated block (Farrell et al., 2013b). The filtered solutions were then analysed by ICP-OES (5100 Synchronous Vertical Dual View ICP-MS, Agilent Technologies, Santa Clara, CA).
8. Particle size analysis (PSA) – Air dried soil samples were sieved to 2 mm to remove large pieces of vegetation and stones. Sieved samples were size fractionated into 0.2-2 mm (coarse sand), 0.02-0.2 mm (fine sand) and <0.02 mm fractions. The <0.02 mm fraction was then separated into 0.02-0.002 mm (silt) and <2 μm (clay) fractions by density fractionation and quantification by hydrometer (Rayment and Lyons, 2011).
9. Available P – P in the 0.5 M NaHCO_3 soil extracts was quantified colourimetrically by malachite green (Ohno and Zibilske, 1991) using a microplate reader (Synergy MX multimode plate reader, Biotek Instruments Inc., Winooski, VT).
10. Extractable C and N
 - a. Total dissolved C (TDC) in extracts and liquid amendments was quantified by high temperature combustion analysis (Thermalox TC/N analyser, Analytical Sciences Ltd, Tewksbury, UK). Dissolved inorganic C (DIC) was quantified on the same instrument in IC mode, and dissolved organic C (DOC) was estimated by difference. For soil samples, any IC was removed prior to analysis by the addition of HCl and shaking before analysis as for TDC.
 - b. Nitrate (NO_3^-) in extracts and liquid amendments was quantified by colourimetry (Miranda et al., 2001) on a microplate reader (Synergy MX multimode plate reader, Biotek Instruments Inc., Winooski, VT).
 - c. Ammonium (NH_4^+) in extracts and liquid amendments was quantified by colourimetry (Mulvaney, 1996) on a microplate reader (Synergy MX multimode plate reader, Biotek Instruments Inc., Winooski, VT).
 - d. Free amino acids (FAAs) in extracts and liquid amendments were quantified by fluorimetry (Jones et al., 2002) on a microplate reader (Synergy MX multimode plate reader, Biotek Instruments Inc., Winooski, VT). These represent the most commonly measured organic nitrogen pool that is considered available to plants and soil microorganisms (Farrell et al., 2014).
 - e. Total dissolved N (TDN) in extracts and liquid amendments was quantified by high temperature combustion analysis (Thermalox TC/N analyser, Analytical Sciences Ltd, Tewksbury, UK). Dissolved organic N (DON) was estimated by subtraction of NO_3^- and NH_4^+ concentrations from the TDN concentration.
11. Isotopic analysis – A sub-sample of soil was finely ground and weighed into tin capsules before analysis by EA-IRMS (GSL Elemental Analyser coupled to a 20-22 Isotope Ratio Mass Spectrometer, Sercon Ltd., Crewe, UK).

Soil biological analyses

1. Microbial biomass C and N (MBC/N) – Estimations of the pools of C and N held in the soil microbial biomass were made using chloroform (CHCl_3) to lyse microbial cell walls and release their contents. These are important data as they not only provide information on the amount of C and N bound in this dynamic pool, but they also provide an indication of the size of the microbial biomass itself, and allow for us to estimate the impact of treatments on the size (and thus presumed health) of the microbial community. Lysing of microbial cells took place in soil samples placed in a dessicator within which a beaker of CHCl_3 was placed, and a negative pressure was drawn to create an atmosphere of CHCl_3 . After >24 h of exposure, the CHCl_3 was removed and the atmosphere flushed to remove any excess CHCl_3 from the samples, before extraction with 0.5 M K_2SO_4 as above. The extracts are then analysed by high temperature combustion (Thermalox TC/N analyser, Analytical Sciences Ltd, Tewksbury, UK). Microbial biomass C/N was then estimated by subtraction of the concentrations of C and



N found in the non-fumigated K₂SO₄ extracts (sections 10a and e above) followed by the application of a correction factor of 0.35 for C and 0.5 for N to account for extraction efficiencies (Voroney et al., 2008).

2. Next generation sequencing (NGS) – In order to understand the impact of biological inputs on the soil microbial community structure, NGS was carried out on appropriate samples. Whilst this analysis cannot be related directly back to function, broad inferences can be drawn from changes in microbial groups associated with known processes e.g. nitrification, and in a short-term project such as this, such microbial shifts may point to possible changes in soil health and crop performance further on. Genomic DNA was extracted from 0.25 g of the frozen soil samples using the MoBio PowerSoil® DNA Isolation Kit (Geneworks, Australia) that involved bead beating and column purification, according to the manufacturer's guidelines. Extracted DNA was quantified (Qubit; Thermo Fisher Scientific, Australia), the resultant DNA was adjusted to 1 ng µL⁻¹ using molecular grade water (SIGMA) prior to storage at -20°C. Bacterial 16S ribosomal ribonucleic acid (rRNA) genes were amplified from the extracted DNA samples using universal core bacterial primers 515F and 806R (Caporaso et al., 2010; Mori et al., 2014), modified with Golay barcodes (Caporaso et al., 2012) fused to Ion Torrent adapters, using amplification conditions described previously (Gleeson et al., 2016; Weerasekara et al., 2016). Following amplification, all polymerase chain reaction (PCR) products were checked for size and specificity by electrophoresis on 1.5% w/v agarose gel, the PCR products were then quantified (Qubit; ThermoFisher Scientific, Australia). The PCR products were then pooled (each site) to achieve a sample that contained all the treatments at the same concentration. The pooled samples were then purified using AMPure XP to remove any small DNA products (primer dimers). Sequencing was performed on the Ion Torrent Personal Genome Machine (Life technologies, USA) using Ion 318 chips and Ion PGM Sequencing 400 kit (Gleeson et al., 2016; Whiteley et al., 2012). Following sequencing, individual sequence reads were filtered using PGM software to remove low quality and polyclonal sequences. All the PGM quality filtered data were exported as FastQ files and split into *.fasta and *.qual files and analysed using the QIIME pipeline (Caporaso et al., 2010). Briefly, sequences were initially screened for quality control whereby sequences were only retained provided they met the following criteria (minimum quality score Q = 20; minimum/maximum length = 130/350; no ambiguous base calls; homopolymers length ≤ 8; barcode miss match = 1; removal of reverse primers; and no mismatches allowed in the forward and reverse primer sequences). All chimeric sequences were identified using USEARCH61 (Edgar et al., 2011) and removed. Unique sequences were then assigned operational taxonomic units (OTUs) by aligning them against the Greengenes 13.5 (GG) reference database with clustering at 97% identity level using the UCLUST algorithm and PyNASt. Singletons were removed and taxonomy was assigned to the representative sequence of each OTU. Following quality filtering and QIIME analysis a total of 30000 sequences per sample. Alpha rarefaction was performed using the phylogenetic diversity, Chao1, the Shannon Index and Observed Species metrics. The sequence data were subsampled to 10000 sequences per sample to ensure fair comparisons between the samples (Gihring et al., 2012).

Screening studies

Given the large number of amendments collated for this project, before expensive field or mechanistic studies could be conducted, laboratory and glasshouse screening of many amendments took place in order to understand the variability between and within product classes, and to assess plant responses.

Laboratory characterisation

Biological inputs come in many forms, and may be designed for seed, soil, or foliar application. Given the soils focus of this research, we targeted biological inputs that can be applied to soils and that had recommended rates provided for use in broad acre agriculture. A total of 69 biological inputs were received and tested, comprising 21 liquids and 48 solids. All amendments were analysed by the most appropriate methods as described in the *Soil and amendment chemical analyses* section above. In some cases (particularly some colourimetric assays), we were unable to provide quantification of certain analytes in some samples due to interference.



Screening pot experiment

This experiment was established with wheat (Trojan) in 2.7 L pots, with 3 kg (wet weight) soil, maintained at 30% water holding capacity (equivalent to 2.47 kg dry weight). The soil was a chromosol, sourced from a paddock near Freeling, SA.

Four seeds were sown in the pot, coupled with an application of amendment at either the manufacturer's specified rate, or where none was given, a rate drawn from literature and application rates of similar amendments. The treatments were either applied in the seedbed with the seed, or surface applied, depending upon manufacturer directions. Where none were given, application was as per similar treatments.

No fertiliser treatment was added to any of the biological treatments, given the relative fertility of the soil utilised in this study, the fact that it was taken from the more fertile 0-10 cm surface layer, and the acknowledgement that the disturbance involved in its handling, homogenisation, and manipulation of the soil that would likely release bound nutrients to the plant available pool, akin to a tillage event. Control treatments included a zero fertiliser control, and an addition of DAP at rates of 50, 100, 150, and 200 kg ha⁻¹. District practice for this area and soil type is 100 kg DAP ha⁻¹ (G. Butler, pers comm). Seedlings were thinned to two per pot at the three-leaf stage, and the remaining seedlings were grown to maturity before harvest at 118 d.

The rationale for the 30% water holding capacity moisture regime was founded upon the acknowledgement that due to the artificial nature and lack of connectedness to the broader soil hydrology, pot experiments cannot easily be maintained with simulated growing season rainfall. Consequently, we have drawn on decisions made for drought treatments in previous studies (Farooq et al., 2013; Singh and Singh, 2006). Choosing a drought treatment meant that the plants were under moderate water stress, and as a result, treatment effects from amendments may have been more apparent.

To gauge changes in water use by the plants as affected by their treatments, watering records were kept as the pots were maintained at constant mass with reverse osmosis (RO) water. Plant growth rate was estimated by taking leaf and stem measurements at regular intervals, and health was evaluated by using SPAD estimates of leaf chlorophyll content. The pot experiment was established in late February 2015 (

Figure 3-Figure 5), and was harvested in June. At harvest (Figure 6), above- and below-ground plant parts were separated, with above-ground biomass determined before being separated into leaf / stem and head. The grain was then manually threshed and its dry weight recorded. Root biomass was also determined. This approach resulted in measures of total biomass, grain and above- and below-ground biomass. Root:shoot ratios were calculated to illustrate differences in plant allocation of biomass as a result of the treatments.

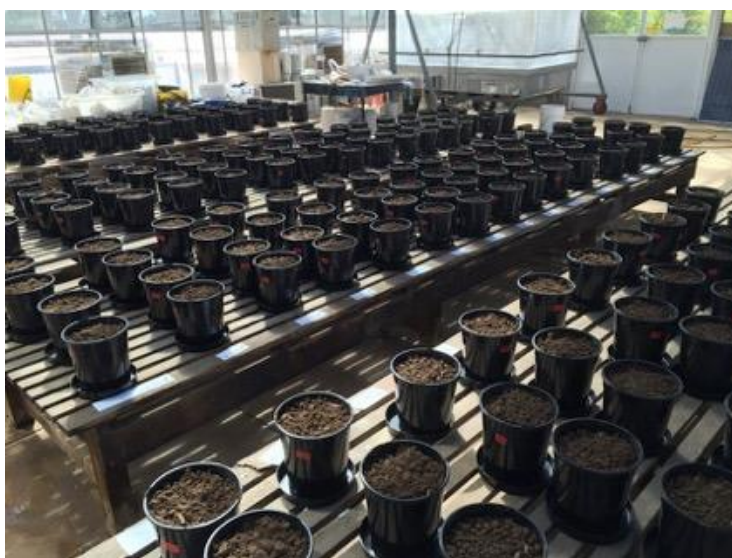


Figure 3: The 240 pots of the screening pot experiment situated in the research glasshouses at CSIRO's Waite Campus. The experiment was fully randomised and blocked to allow for environmental gradients to be accounted for.



Figure 4: Pipetting a liquid biological amendment into the seedbed



Figure 5: A pot after surface application of a guano-based product

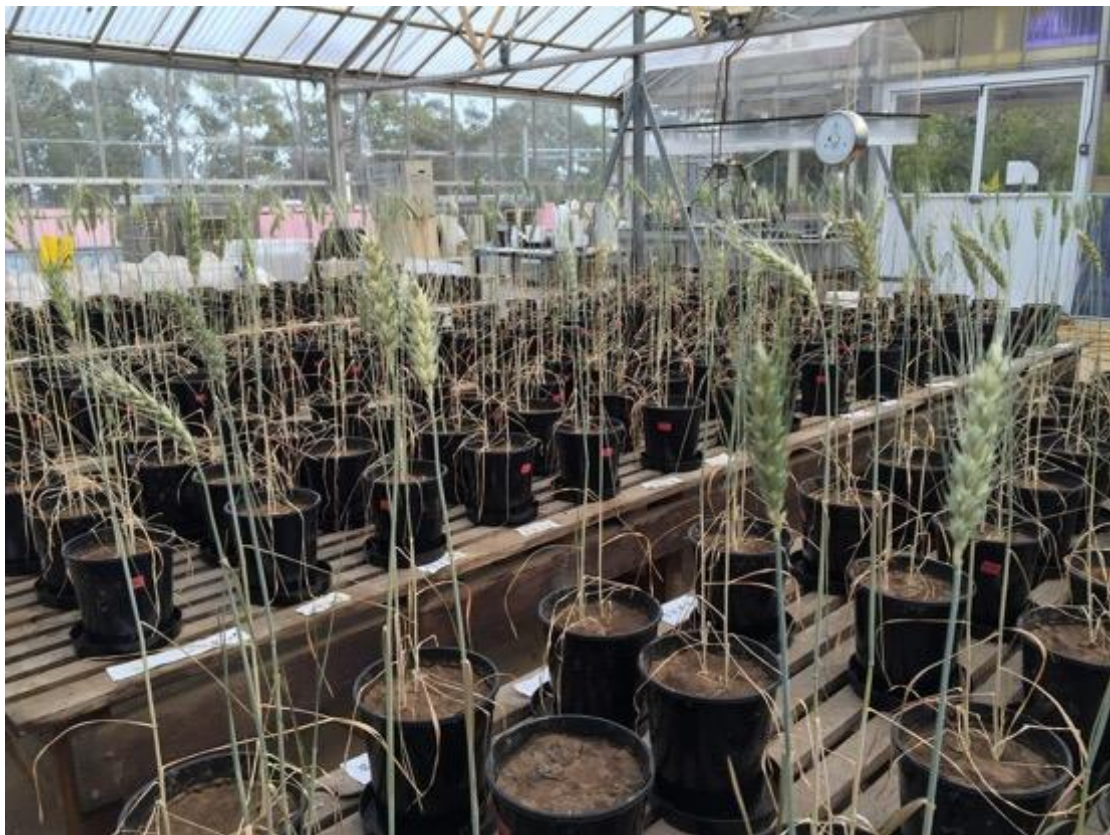


Figure 6: The pot experiment nearing readiness for harvest

Field experimentation

Field set up and analysis

Eight field experiments were established for two consecutive growing seasons across the three GRDC grain-growing regions, and their locations are illustrated in Figure 7. The field sites were situated on a diverse range of soils in order to gain greater understanding of the specificity or broader applicability of individual amendments. Site details are provided in Table 1. Further details are provided in the *Location* section below, and a full information matrix is supplied as an attachment to this report. The Jamestown site ran for one season only (2016-17) as a replacement for the Freeling site that was lost to the Pinery bushfire in November 2015. The Paskeville site ran for two years but the plots were moved in the 2016-17 season due to issues at the trial site.

Table 1: Information on the eight field sites. MAP = mean annual precipitation (mm)

Region	State	Nearest town	Soil type	Soil pH	MAP
W	WA	Buntine	Tenosol	Acid	300
S	SA	Jamestown	Chromosol	Acid	463
S	SA	Paskeville	Calcarosol	Calcareous	393
S	SA	Langhorne Creek	Tenosol	Neutral	379
S	Vic	Inverleigh	Dermosol	Acid	543
N	NSW	Rankins Springs	Calcarosol	Calcareous	377
N	NSW	Parkes	Vertosol	Neutral	609
N	Qld	Mt Tyson	Vertosol	Calcareous	601

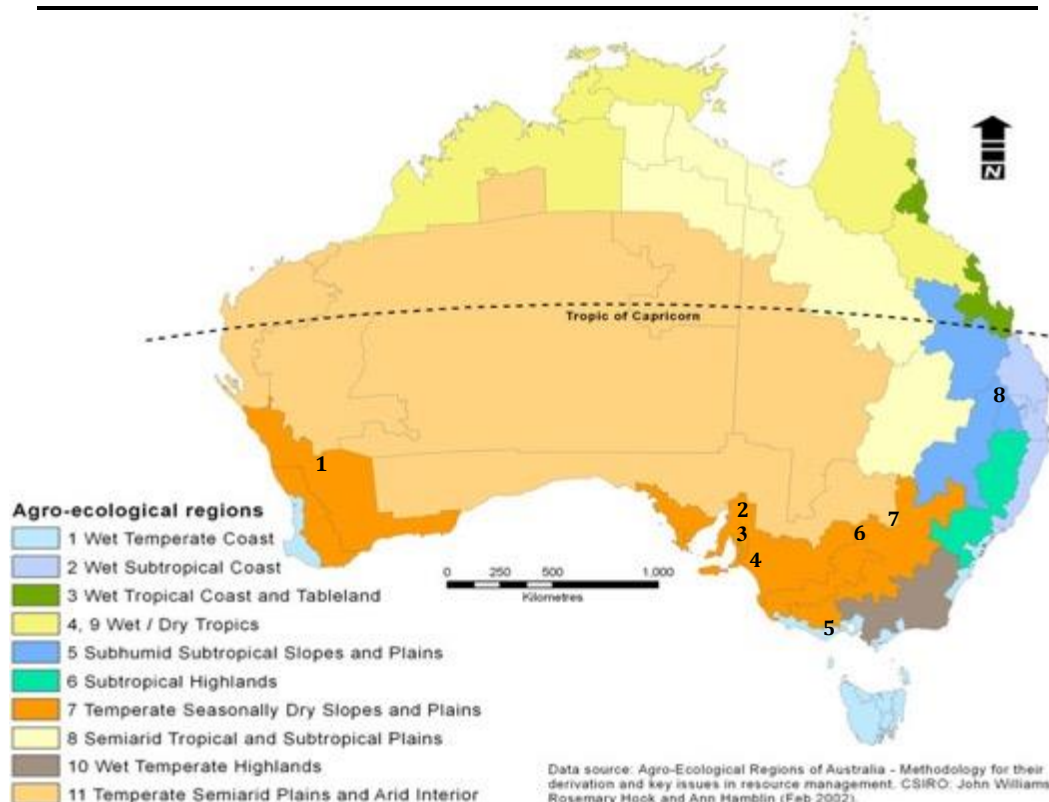


Figure 7: Location of the eight field experiments: 1) Buntine, WA; 2) Jamestown, SA; 3) Paskeville, SA; 4) Langhorne Creek, SA; 5) Inverleigh, Vic; 6) Rankins Springs, NSW; 7) Parkes, NSW; 8) Mt Tyson, Qld.

Each field site had 88 plots, ca. 2 m x 12 m in size, and was established, maintained, and harvested by the grower groups. Plots consisted of 22 treatments (Table 2) replicated four times in a blocked design to allow for environmental and edaphic gradients to be considered. Of these 22 treatments, eight consisted of a range of conventional treatments based upon district practise. These control

treatments allowed for yield-response curves to be generated, and thus provide a more accurate benchmark of where the results from a biological amendment lie relative to conventional inputs.

The remaining 14 treatments consist of biological farming inputs. These were chosen on the basis of grower group input, guided by our laboratory and glasshouse derived data. Though from a scientific perspective, having all eight field experiments identical in treatments would provide most power, clearly this would have resulted in a number of irrelevant treatments in specific regions (e.g. a product produced by a small company in Qld is unlikely to be used in WA, and a product designed to ameliorate acidity would have little likely effect on a calcareous soil). However, several widely available products were included to be tested at all sites. Total nutrient addition rates arising from the treatments are given in Table 3. Every biological input was applied at the producer's recommended rate, and was applied on top of a 'base' conventional fertiliser representing 50% of local district practice. Treatments were applied as per suppliers' instructions, being banded, surface spread, or liquid injected as appropriate and as possible with local field machinery.

Soil samples were taken from each plot at ca. one month after sowing, to capture initial changes in soil biology and chemistry as a result of the experimental treatments. The soils were analysed for water-soluble nutrients (N and P) across all sites in year 1 to provide a cost-effective survey of any major changes to soluble nutrient pools. In the second year, more in-depth analyses were conducted to quantify exchangeable nitrogen and carbon, plant-available phosphorus, microbial biomass nitrogen and carbon, as well as pH and electrical conductivity following methods outlined in *Analytical methods*. Treatments identified of particular interest were subject to in-depth microbial analysis to ascertain whether the inputs have altered microbial community structure and function in relation to soil chemistry and eventual crop yield.

Table 2: Treatments applied at each of the eight field experiments. Treatments consist of eight rates of district practice, four core biological inputs common to all eight sites, a further six biological inputs chosen from a short-list of widely available products, and a final four locally available treatments. District practice conventional fertiliser rates: WA – 65 kg ha⁻¹ Agstar Extra, 25 kg ha⁻¹ MOP, 30 l ha⁻¹ Flexi-N; SA – 100 kg ha⁻¹ DAP; Vic – 80 kg ha⁻¹ DAP; NSW Rankins Springs – 50 kg ha⁻¹ MAP; NSW Parkes – 80 kg ha⁻¹ MAP; Qld – 80 kg ha⁻¹ N, 40 kg ha⁻¹ Granulock Z

Tr	Buntine, WA	Paskeville, SA	Jamestown, SA	Langhorne Creek, SA	Inverleigh, Vic	Rankins Springs, NSW	Parkes, NSW	Mt Tyson, Qld
1	0	0	0	0	0	0	0	0
2	25	25	25	25	25	25	25	25
3	50	50	50	50	50	50	50	50
4	75	75	75	75	75	75	75	75
5	100	100	100	100	100	100	100	100
6	125	125	125	125	125	125	125	125
7	150	150	150	150	150	150	150	150
8	200	200	200	200	200	200	200	200
9	Humate	Humate	Humate	Humate	Humate	Humate	Humate	Humate
10	Biostimulant	Biostimulant	Biostimulant	Biostimulant	Biostimulant	Biostimulant	Biostimulant	Biostimulant
11	Microbial	Microbial	Microbial	Microbial	Microbial	Microbial	Microbial	Microbial
12	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.
13	Humate	Humate	Humate	Humate	Humate	Humate	Humate	Humate
14	Biostimulant	Biostimulant	Biostimulant	Biostimulant	Humate	Biostimulant	Biostimulant	Biostimulant
15	Biostimulant	Microbial	Microbial	Microbial	Biostimulant	Biostimulant	Biostimulant	Biostimulant
16	Microbial	Microbial	Microbial	Microbial	Biostimulant	Microbial	Microbial	Biostimulant
17	Microbial	Alt. Fert.	Alt. Fert.	Alt. Fert.	Microbial	Microbial	Microbial	Microbial
18	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.	Alt. Fert.
19	Compost	Alt. Fert.	Alt. Fert.	Alt. Fert.	Manure	Manure	Manure	Manure
20	Compost	Biochar	Biochar	Biochar	Biosolids	Manure	Manure	Compost
21	Compost	Compost	Compost	Compost	Compost	Compost	Compost	Manure
22	Alt. Fert.	Compost	Compost	Compost	Compost	Compost	Compost	Compost

At harvest, grain yield was quantified on a fresh weight basis on the plot header. Moisture content and nutrient analysis was conducted on subsamples of grain from each plot as per the *Plant analyses* section, with the exception of the three SA trials in year two, when only composite grain samples from all four replicates of each treatment were provided from each site by the grower group's harvesting contractor. In order to provide a means of normalising crop responses across the two years and eight sites, water limited yield potential (WLYP) was estimated using APSIM as outlined below.



Table 3: Total nutrients applied with each field treatment. AR = application rate, TC = total C, TN = total N. All values in kg ha⁻¹ except for the following columns for the liquid amendments: AR (L ha⁻¹), NH₄-N, NO₃-N, K, S, P, Ca, Cu, Fe, Mg, Mn, Zn (g ha⁻¹). Empty cells = analysis not able to be conducted.

Liquids														
	AR	TC	TN	NH ₄ -N	NO ₃ -N	K	S	P	Ca	Cu	Fe	Mg	Mn	Zn
A1	4.7	514.54	888.14	257.36	213.21	1.25	0.24	0.01	0.00	0.13	0.11	0.00	0.11	2.20
A3	10	17.89	6.59			1.88	0.32	0.03	6.52	0.00	0.02	0.43	0.00	0.00
B1	0.25	0.56	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B2	5	4.17	0.53	0.05	0.43	0.05	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00
B3	3	346.99	9.42	0.10	0.31	0.66	0.12	0.29	0.26	0.01	0.01	0.06	0.00	0.02
B4	50	1541.95	2.05	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B5	2.5	51.46	2.96	0.48	0.15	0.14	0.07	0.00	0.02	0.00	0.03	0.01	0.01	0.01
B6	15	12.84	6.16	4.64	0.00	0.09	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00
B7	10	3132.53	5.30	0.16	0.68	0.04	0.87	0.02	0.52	0.00	0.16	0.02	0.00	0.00
B8	5	0.11	0.08			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M4	0.5	0.29	0.01	0.01	0.00									
M5	0.5	0.28	0.07	0.04	0.00									
Solids														
	AR	TC	TN	NH ₄ -N	NO ₃ -N	K	S	P	Ca	Cu	Fe	Mg	Mn	Zn
A2	50	0.04	1.67	0.01	0.00	0.01	0.00	4.23	0.01	0.00	0.01	3.45	0.00	0.00
A4	1500	4.84	0.72	0.05	0.06	7.03	1.90	103.05	306.11	0.09	27.17	3.57	1.19	0.30
A5	50	2.40	0.20	0.00	0.00	8.72	0.77	3.08	6.01	0.04	0.62	1.78	0.10	0.11
H1	10	1.21	0.03	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.14	0.01	0.00	0.00
H2	5	1.58	0.04	0.00	0.01	0.37	0.01	0.00	0.04	0.00	0.05	0.00	0.00	0.00
H3	1500	111.53	27.03	0.08	6.70	33.64	55.19	19.92	139.83	1.32	31.44	6.22	0.63	1.66
H4	250	51.01	0.58	0.00	0.00	0.11	0.14	0.01	0.33	0.00	0.45	0.13	0.01	0.00
M1	0.004													
M2	2	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00
M3	1.75	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00
O1	3000	324.16	32.76	0.10	3.74	18.79	13.41	21.55	164.94	0.26	39.10	14.36	0.74	1.01
O2	3000	505.74	30.52	0.07	0.00	14.13	17.43	15.27	96.78	0.18	16.85	5.14	0.45	0.76
O3	3000	267.77	16.94	3.00	0.00	13.54	2.84	4.28	7.88	0.02	0.72	2.09	0.10	0.19
O4	50	32.00	0.15	0.00	0.00	0.24	0.01	0.02	0.02	0.00	0.01	0.01	0.00	0.00
O5	3000	494.33	30.40	0.02	0.95	21.61	8.56	6.27	72.25	0.12	22.23	10.58	0.33	0.40
O6	3000	356.20	33.30	0.07	1.07	24.13	9.51	6.57	83.58	0.15	26.37	13.19	0.54	0.44
O7	3000	898.34	77.27	1.86	0.37	67.67	16.87	23.52	46.02	0.17	4.86	14.29	1.40	1.00
O8	3000	776.58	145.78	9.25	0.01	17.77	15.75	64.25	23.50	0.99	24.07	17.02	0.38	1.14
O9	3000	443.41	44.04	1.33	0.06	47.68	11.89	19.27	46.23	0.22	4.28	9.75	0.90	0.73
O10	3000	392.85	18.84	0.03	0.00	14.06	2.40	2.83	23.08	0.02	14.55	4.80	0.16	0.23
O11	3000	1047.84	86.12	1.61	0.41	51.77	13.74	26.90	35.17	0.38	1.95	12.50	1.16	1.14
O12	3000	371.55	43.02	0.01	3.66	37.25	36.54	10.33	110.14	0.04	27.18	14.45	0.49	0.20
O13	3000	583.09	67.09	0.27	5.94	59.02	12.05	13.34	57.35	0.07	89.47	31.85	0.44	0.36
O14	3000	272.47	30.64	0.05	2.71	19.71	7.50	8.30	27.85	0.04	290.02	11.39	0.33	0.28
O15	3000													
O16	3000													

The APSIM model and the simulation of WLYP¹

The agricultural production systems simulator APSIM (Holzworth et al., 2014) is a component-based simulation framework containing a suite of modules which can be flexibly configured to simulate agricultural systems, including crop sequences, rotations and intercropping. APSIM runs on a daily time-step by combining daily weather data, soil data e.g. moisture, type, and nutrient, crop cultivar characteristics and management practices e.g. crop sowing date, residue management, tillage, irrigation, and fertiliser applications. In this study, the APSIM model version 7.8, combined with field experimental practices, soil properties and agronomic practices, was used to estimate water-limited potential yield.

In APSIM, crop phenology is divided into phases, with the duration of each phase being determined by a cultivar-specific thermal time target. Daily thermal time is calculated using three cardinal temperatures (base, optimum and maximum) for each crop, modified by vernalisation and photoperiod if a crop is sensitive to these factors. Potential daily biomass production is calculated based on light interception and radiation use efficiency. This can be reduced by sub-optimal temperatures, water and N deficit. Grain yield is a function of grain number, grain filling rate and assimilate re-translocation. Detailed descriptions of the APSIM model can be found in Holzworth et al. (2014) or Keating et al. (2003), or at the APSIM website: <http://www.apsim.info/apsim/>.

¹ This section on APSIM is based on text supplied by Dr Chao Chen of CSIRO Agriculture & Food, Floreat. The simulation of WLYP performed in APSIM was also conducted by Dr Chen.



Our primary objective here was to assess yield potential across study sites and years to provide a means of normalising experimental yield for the evaluation of the effects of the biological inputs, and not to simulate crop phenological development. As such, the default genetic parameters for crop cultivars grown in the field experiments were adopted from APSIM v7.8 to simulate the phenology of wheat and barley. For the simulations, soil parameter values for the identified soil types at trial sites were sourced from the APSOIL data base (www.apsim.info). The agronomic practices performed in the field experiments e.g. sowing date, plant density, residue and fertiliser were used as model inputs. The maximum rooting depth of wheat and barley in all studied sites were estimated to be 1.5 m. Daily weather data during the experimental period were extracted from adjacent meteorological stations from SILO database (Jeffrey et al., 2001). Nitrogen was applied within the model scenario up to flowering by 50 kg ha⁻¹ whenever soil nitrate in the top 60 cm was less than 50 kg ha⁻¹. Such a N top-up strategy ensured no N stress impact was simulated and rainfall was the only factor limiting crop growth in the simulations.

Mechanistic experiments

Whilst field experimentation is the most appropriate means of assessing the performance of biological inputs under realistic conditions, the focus of this project on testing many products meant that mechanistic studies to understand how the products impacted the soil and crop were not possible. As a result, two mechanistic experiments were conducted in the laboratory and glasshouse in order to probe:

1. How biostimulants and humates impact on capture of N from a previous legume crop, and
2. How organic amendments impact on soil microbial community structure and functions related to N and P release

Impact of biostimulants on the capture of legume-derived N

One mechanism by which biostimulants or inocula may facilitate increased plant growth is through increasing the rate of processes involved in the breakdown and release of plant-available nitrogen from soils and litters. This might be a particularly important mechanism where legumes form part of a crop rotation, with the N fixed by these crops contributing to the N nutrition of the subsequent rotation.

Before nitrogen from sources other than mineral fertiliser can be accessed by plants, it was traditionally thought that it must be mineralised to ammonium or nitrate (Schimel and Bennett, 2004). We now know that plants can also access a wide range of small organic compounds such as amino acids and peptides (Farrell et al., 2013a; Hill et al., 2011). Nonetheless, a limit on the production of plant-available N in soils and from litters is the rate of breakdown of macromolecules to oligomers and monomers, and their subsequent mineralisation to ammonium and nitrate. The purpose of this experiment was to investigate the effects of biostimulants on the yield of wheat and its uptake of N from a legume litter that has been labelled with ¹⁵N.

Hypotheses

We hypothesised that biostimulants will stimulate plant growth and capture of N from crop residues through direct plant biostimulatory mechanisms such as increased root growth, and through changed microbial community composition leading to increased rates of nitrogen dissolution and stimulation of mineralisation and nitrification processes.

Materials and methods

Experimental design

A pot experiment was conducted in which wheat was grown in the presence of biostimulants and ¹⁵N-labelled litter. Given the artificial nature of a pot experiment, and the bias against macrofauna responsible for some of the physical movement of litter from the soil into the surface, litter was fully incorporated into the soil. Litter used was *Medicago littoralis* Rhode tops (consisting stems, leaves and pods; 340 g total dry mass, 3.07 %N, 2.77 atm% ¹⁵N [$\delta^{15}\text{N}$ 6749.1 ‰]) and was ground to <1 mm. It was applied at a rate equivalent to 2.4 t ha⁻¹ (4.82 g pot⁻¹), resulting in an addition of 53.2 mg litter N kg⁻¹ soil or 148 mg litter N per pot (soil bulk density of 1.07 g cm⁻³). Biostimulants were selected from



the project inventory, based primarily upon their stated claims regarding improvements in N uptake or NUE; all were suitable for application to soil, rather than foliar. Soil was taken from the 0-10 layer of the Langhorne Creek experiment due to its sandy nature (facilitating easy collection of root biomass). Samples were taken from the four zero fertiliser plots, no mineral fertiliser was added in the experiment. These steps were taken to ensure that effects of biostimulants on release of N from the litter and uptake by the plants would be maximised.

Table 4: Biostimulants to be used in pot experiment

Treatment	State	Rate (field)	Rate (pot)
1	Liquid	3 L ha ⁻¹	6.03 µL
2	Liquid	0.25 L ha ⁻¹	0.5025 µL
3	Liquid	2.5 L ha ⁻¹	5.025 µL
4	Liquid	10 L ha ⁻¹	20.1 µL
5	Liquid	6 L ha ⁻¹	12.06 µL
6	Solid	50 kg ha ⁻¹	0.10 g
7	Solid	5 kg ha ⁻¹	0.010 g
8	Solid	100 kg ha ⁻¹	0.20 g
9	Solid	50 kg ha ⁻¹	0.10 g
10	Solid	50 kg ha ⁻¹	0.10 g

There were a total of 10 biostimulant treatments, a zero biostimulants control, and a zero biostimulants and zero litter control, replicated six times, resulting in 72 individual pots. Soil and litter were mixed for each individual pot in large ziplock bags after initial homogenisation of the bulk soil by cement mixer. At this initial homogenisation stage, reverse osmosis (RO) water was added to take the soil to 70% WHC, which was maintained gravimetrically throughout the experiment. The soil / litter mix was added to a depth of 12 cm and levelled. Upon this surface, within a five-centimetre diameter in the centre of the pot, the target biostimulants were added at the appropriate rate equivalent to their manufacturer-suggested field application rates (see Table 4). These rates resulted in very small additions of nutrients on a per-hectare basis (Table 5)

Table 5: Total nutrients applied with each treatment. AR = application rate, TC = total C, TN = total N. All values in g ha⁻¹ except for AR where liquid amendments are L ha⁻¹ and solid amendments are kg ha⁻¹. Empty cells = analysis not able to be conducted.

	Form	AR	TC	TN	NH4-N	NO3-N	FAA-N	K	S	P
1	Liquid	3	347.0	9.4	0.10	0.31	0.60	0.66	0.12	0.29
2	Liquid	0.25	0.6	0.0	0.03	0.00	0.00	0.00	0.00	0.00
3	Liquid	2.5	51.5	3.0	0.48	0.15	0.22	0.14	0.07	0.00
4	Liquid	10	464.0	16.8	2.64	1.20	1.23	1.37	0.86	0.00
5	Liquid	6	289.7	140.2	2.95	0.12	1.45	0.38	0.05	0.02
6	Solid	50	6033	162	17.2	23.5	0.0	349	19	7.4
7	Solid	5	2048	39			0.2	378	17	0.1
8	Solid	100	31577	826	92.1	231.4	8.4	7413	137	4.5
9	Solid	50	14257	459	2.3	29.6	0.0	153	430	53.4
10	Solid	50	10202	116	0.4	0.0	0.0	23	27	1.9

Liquids were diluted as appropriate, and solids finely ground. On top of this, another centimetre of soil was added and four wheat² seeds were placed over the area that had received the product in the centre, roughly two centimetres apart from each other. A further two centimetres of soil was then added on top of the seeds. A 10 cm Rhizon sampler was inserted vertically into the centre of the pot between the four seeds and samples were withdrawn from this weekly using vacutainers. Wheat (Mace) was grown to commencement of anthesis. When the first plants reached this stage the experiment was ended to ensure that “winners” were not normalised out by resource limitation in the pot. Each pot was thinned to two plants after two weeks of growth.

² The wheat variety “Mace” was selected due to its lack of preference for either nitrate or ammonium – see O’Sullivan et al., 2016, INI Conference, Melbourne



Laboratory analyses

In order to quantify changes in soluble N chemistry during the experiment, non-destructive Rhizon samplers were used to extract soil solution for analysis of DOC, DON, nitrate, ammonium and FAAs. Samples were taken weekly and analysed as detailed in the *Soil and amendment chemical analyses* section.

At end of experiment, plants were harvested, separated into above- and below-ground parts (roots washed), and biomass of both parts quantified. Above-ground phenology (number of leaves, tillers, heads, etc.) was recorded visually, below-ground root traits were quantified as outlined in *Plant analyses* above. Nitrogen concentration $\delta^{15}\text{N}$ was quantified in both roots and shoots.

Soil samples were also taken at the end of the experiment. These were analysed for extractable N and C pools, as well as total C and N, and $\delta^{15}\text{N}$ as outlined in the *Soil and amendment chemical analyses* section. Soil microbial biomass C and N, as well as the soil microbial community composition, and numbers of microorganisms responsible for key nitrogen cycling processes were analysed as outlined in the *Soil biological analyses* section.

Impact of biological inputs on soil microbial function and structure

In addition to direct plant responses, many biological inputs are marketed with claims that soil 'health' is improved, including changes in microbial functions such as nutrient provision and C sequestration, as well as shifts in community structure. Often these shifts are reported to alleviate particular soil or environmental stresses.

The purpose of this experiment was to use a soil incubation to evaluate the effects of four classes of biological inputs on soil microbial function and community structure. The experiment focussed on solid amendments from four classes (biochars, humates, composts, manures). We used our NMR spectra database of a wide range of these products to select those that represent the chemical organic matter variability across the product class using the Kennard-Stone algorithm (Kennard and Stone, 1969).

Hypotheses

A selection of amendments ranging from those containing highly labile material (e.g. fresh manures) through to more recalcitrant materials (e.g. humates and biochars) were used. On this premise, we hypothesised that:

- 1) Products containing more labile components would elicit rapid, but shorter-lasting effects that were measurable earlier on in the incubation, and that these would favour a more copiotrophic microbial community
- 2) Products dominated by more recalcitrant or stable components would elicit a slower but more sustained response, with a concomitant increase in oligotrophic microbial communities and the functions they are classically understood to perform e.g. dissolution of nutrients and breakdown to smaller molecules.

Materials and methods

Sample selection

Following selection on the basis of organic matter chemistry as measured by NMR (Baldock et al., 2013b) by the Kennard-Stone algorithm (Kennard and Stone, 1969), eight amendments from each of the four classes were selected (Table 6).

Laboratory set-up

Soil from the Langhorne Creek field experiments was incubated in PVC pipe off-cuts to which a fine mesh has been attached to the bottom to allow airflow, allowing 30 g soil to be used for each sample. These off-cuts were placed in 1 L Mason jars equipped septa allowing the extraction of gas for respiration measurements to be conducted. Owing to the need to obtain a time series of physical soil samples for onward microbial and chemical analysis, multiple replicates of each treatment were established to facilitate destructive sampling. Amendments were added at a rate of 600 mg amendment-C per 30 g soil (dwt), equivalent to an addition of 2% C to the soil, which allowed clear

differences between background respiration from the soil alone and the respiration of amendment-C to be observed. The incubation was carried out at 50% WHC (0.17 mL water g⁻¹ soil). Amendments and soils were mixed as per protocols from the Filling the Research Gap project on organic soil amendments (Farrell, 2015).

Table 6: Chemical properties of the 32 amendments and soil used in the incubation study. SEM = Standard error of the mean), A:OA = alkyl / o-alkyl ratio (a measure of the extent of decomposition in an organic material (Baldock and Preston, 1995)), BD = below limit of detection, N/A = not applicable.

Type	%C			%N			C:N			A:OA		
	Individual	Mean ± SEM		Mean ± SEM		Mean ± SEM		Mean ± SEM		Mean ± SEM		
1 Biochar	32.0	62.4	± 7.4	2.06	0.5	± 0.2	15.5	326.4	± 78.6	3.0	1.3	± 0.4
2 Biochar	61.3			0.10			612.0			0.8		
3 Biochar	32.8			1.00			32.9			0.6		
4 Biochar	82.7			0.16			504.8			BD		
5 Biochar	75.4			0.37			206.6			1.6		
6 Biochar	87.5			0.22			391.5			0.1		
7 Biochar	70.4			0.14			500.7			1.6		
8 Biochar	57.5			0.17			347.0			1.0		
9 Humate	12.8	39.3	± 4.5	0.34	0.9	± 0.1	37.3	46.9	± 6.3	4.5	2.5	± 0.6
10 Humate	52.2			0.59			87.8			1.0		
11 Humate	31.0			0.72			43.3			3.3		
12 Humate	43.3			1.39			31.1			0.6		
13 Humate	37.5			0.98			38.2			0.4		
14 Humate	47.5			0.91			52.2			2.3		
15 Humate	40.0			1.06			37.7			4.0		
16 Humate	50.2			1.05			47.8			3.6		
17 Compost	26.5	24.4	± 3.3	4.97	1.8	± 0.5	5.3	22.4	± 9.3	1.2	0.5	± 0.1
18 Compost	43.2			0.50			86.2			0.1		
19 Compost	19.1			1.10			17.4			0.2		
20 Compost	16.1			1.28			12.6			0.5		
21 Compost	25.9			2.30			11.3			0.3		
22 Compost	13.6			1.42			9.5			1.0		
23 Compost	28.8			1.38			20.8			0.4		
24 Compost	21.9			1.34			16.3			0.7		
25 Manure	37.6	27.8	± 3.7	2.34	2.3	± 0.2	16.1	11.9	± 1.2	0.5	0.6	± 0.2
26 Manure	35.4			3.07			11.5			0.8		
27 Manure	33.2			1.99			16.7			0.2		
28 Manure	17.7			2.27			7.8			1.8		
29 Manure	30.7			2.24			13.7			0.3		
30 Manure	20.5			2.35			8.7			0.6		
31 Manure	9.5			1.07			8.9			0.7		
32 Manure	37.5			3.08			12.2			0.2		
33 Soil	1.03	N/A		0.09	N/A		11.7	N/A		0.7	N/A	

The incubation ran for 8 weeks, with seven destructive sampling points (at days 0, 1, 3, 7, 14, 28, 56) and 10 respiration measurements in a constant temperature room set at 22°C. This was not a replicated experiment. Rather, we took a regression and multivariate approach to the data analysis across all 32 treatments and seven time points. By having the four groups of amendment represented equally, we were also able to compare between groups. Three replicates of the unamended soil were also prepared at each time period to allow comparisons to the base soil to be conducted, particularly for the respiration measurements. The total number of samples was 245 samples.

Analyses

In order to gain an overall indication of the rate of heterotrophic microbial activity in the samples, headspace CO₂ measurements were made using samples collected by gas-tight syringe and injected into an infra-red gas analyser (IRGA). In order to account for variations in background atmospheric CO₂ in the incubation jars, a baseline CO₂ measurement was taken at the start of each time interval and subtracted from the final measurement. Rates of respiration, and where possible, partitioning into

fast and slow pools was conducted using curve-fitting regression algorithms in SigmaPlot v.13 (Systat Software Inc., San Jose, CA).

Microbial biomass C and N, and extractable DOC, DON, FAAs, NH_4^+ , NO_3^- and PO_4^{3-} were quantified at each destructively sampled time point following methods outlined in the *Soil and amendment chemical analyses* section. As a crude estimate of net DON dissolution, proteolysis, N and P mineralisation, and nitrification rates, changes in the size of the DON, FAA, NH_4^+ , NO_3^- and PO_4^{3-} pools were quantified between each sampling point.

Statistical analysis

Given the varied nature of much of the data collected in this project, several different statistical analysis approaches have been taken depending both upon the desired outcome from individual experiments, and the suitability of the data for certain statistical tests. Univariate tests to assess the significance of treatment effects and relationships between two or more variables were carried out in SPSS v.18 (IBM Corp., Armonk, NY). Multivariate analysis was used extensively, both to explore overall trends in data through various ordination methods e.g. principal components analysis (PCA), principal coordinates analysis (PCoA), and non-metric multidimensional scaling (nMDS), and for formal hypothesis testing using permutational multivariate analysis of variance (PERMANOVA). All multivariate analyses were carried out in Primer v.7 with the PERMANOVA add-on (PRIMER-E, Auckland, NZ).



Location

	Latitude (decimal degrees)	Longitude (decimal degrees)
Trial Site #1	-30.009887	116.339924
Nearest Town	Buntine, WA	
Trial Site #2	-33.301238	138.599415
Nearest Town	Jamestown, SA	
Trial Site #3	-34.025400	137.869267
Nearest Town	Paskeville, SA	
Trial Site #4	-35.260602	139.034862
Nearest Town	Langhorne Creek, SA	
Trial Site #5	-38.085785	143.936205
Nearest Town	Inverleigh, Vic	
Trial Site #6	34.030894	145.967986
Nearest Town	Rankins Springs, NSW	
Trial Site #7	-33.032525	148.100482
Nearest Town	Parkes, NSW	
Trial Site #8	-27.552670	151.577693
Nearest Town	Mt Tyson, Qld	

Research	Benefiting GRDC Region (can select up to three regions)	Benefiting GRDC Agro-Ecological Zone (see link: http://www.grdc.com.au/About-Us/GRDC-Agroecological-Zones) for guidance about AE-Zone locations	
Experiment Title	National Choose an item. Choose an item.	<input type="checkbox"/> Qld Central <input checked="" type="checkbox"/> NSW NE/Qld SE <input type="checkbox"/> NSW Vic Slopes <input type="checkbox"/> Tas Grain <input checked="" type="checkbox"/> SA Midnorth-Lower Yorke Eyre <input type="checkbox"/> WA Northern <input type="checkbox"/> WA Eastern <input type="checkbox"/> WA Mallee	<input checked="" type="checkbox"/> NSW Central <input type="checkbox"/> NSW NW/Qld SW <input checked="" type="checkbox"/> Vic High Rainfall <input checked="" type="checkbox"/> SA Vic Mallee <input type="checkbox"/> SA Vic Bordertown-Wimmera <input checked="" type="checkbox"/> WA Central <input type="checkbox"/> WA Sandplain

Results

Chemical characterisation of biological inputs

We received 69 biological inputs for chemical characterisation, consisting of 21 liquid amendments and 48 solid amendments. These were broken down into five classes:

1. Alternative fertilisers (8)
2. Active microbials (6)
3. Humates (16)
4. Biostimulants (13)
5. Organic amendments (in turn, consisting of)
 - a. Composts (10)
 - b. Manures (14)
 - c. Biochars (1)
 - d. Biosolids (1)

Focusing primarily on metrics relevant to agronomy, these amendments were analysed by a range of techniques in order to understand variability both across the whole inventory and also within each product category. Solids and liquids were separated in the analysis due to the difficulty of relating data on a per mass basis (solids) to data derived on a per volume basis (liquids).

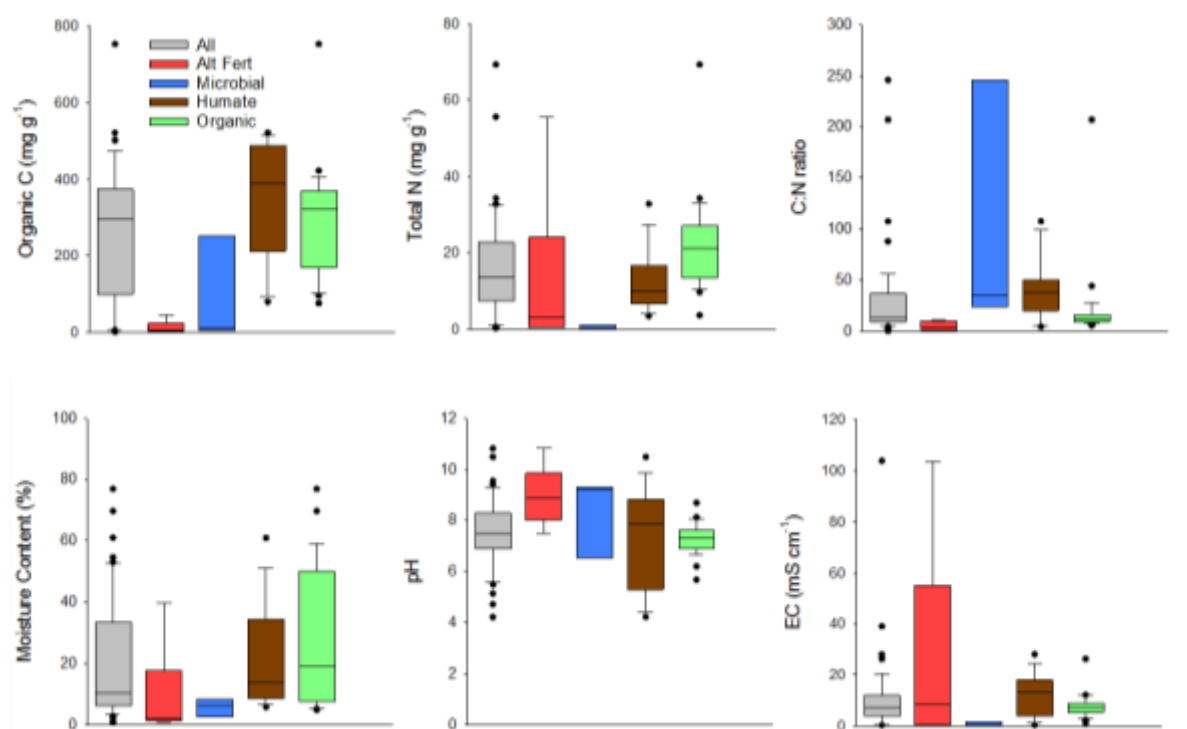


Figure 8: Box and whisker plots of Organic C, Total N, C:N ratio, Moisture Content, pH and Electrical Conductivity (EC) of the solid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles.

Starting with the solids, moisture contents ranged from 0.8% for an alternative fertiliser derived from fly-ash to 76.9% for a fresh manure (Figure 8). In fact, across the bulky organic amendments, there was a very wide range of moisture contents, ranging from 4.7-76.9%, though there was also considerable variance across the alternative fertilisers and humates also. Organic C, total nitrogen

(TN), and thus C:N ratio were also highly variable across all four product classes with the exception of OC in the microbial inocula and N in the alternative fertilisers (Figure 8). The solid alternative fertilisers contained only an average of 11.8 mg g⁻¹ OC which was lower than most of the other biological inputs tested, whilst the average TN value was 12.7 mg g⁻¹, leading to very narrow C:N ratios for the alternative fertilisers relative to the other amendments. With the exception of the biochar (C:N 207), C:N ratios of the organic amendments ranged from 5.3-43.8, with an average value of 13.2. The pH of the biological inputs tested ranged from moderately acid (4.21) to highly alkali (10.81). humates spanned the widest range (4.21-10.48) with an average of 7.15, reflecting a diversity in how these amendments are prepared. Electrical conductivity (EC, a measure of salinity) ranged from near zero to 28.0 for all but the two ash-derived alternative fertilisers (Figure 8).

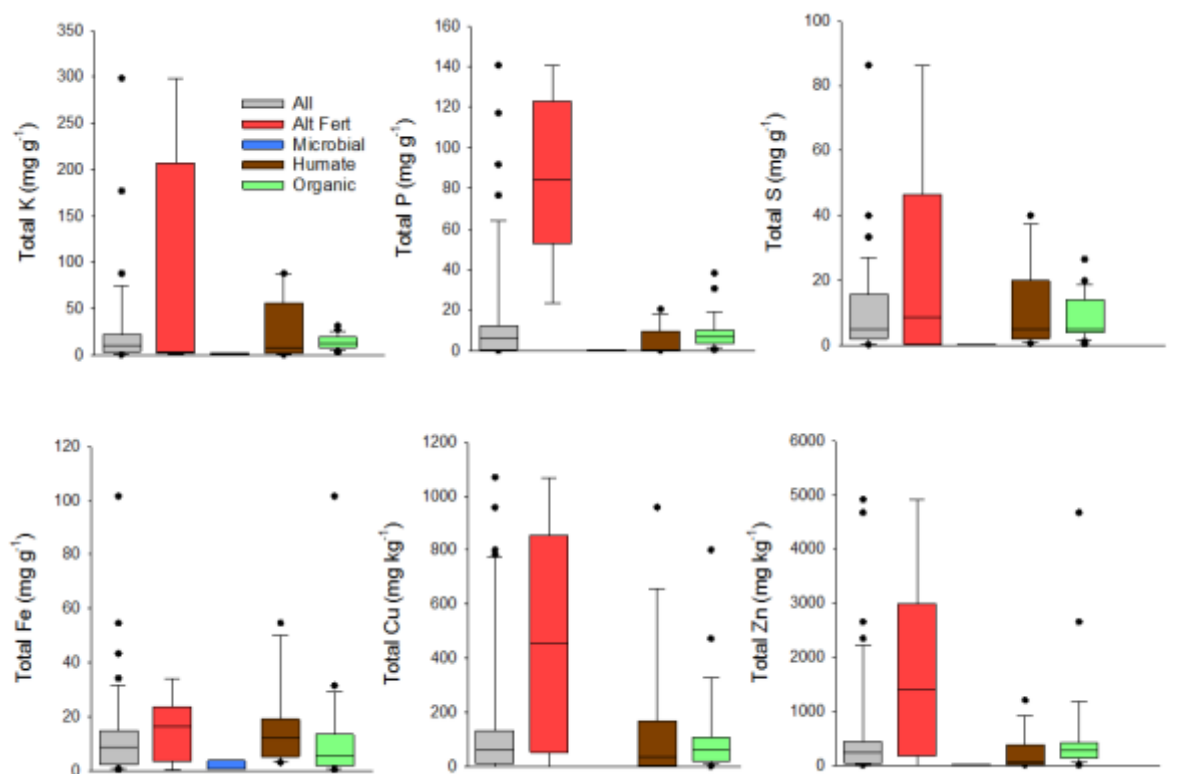


Figure 9: Box and whisker plots of Total, K, P, S, Fe, Cu, and Zn of the solid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles.

The alternative fertilisers also exhibited the widest range in major and minor nutrients for all but Fe (Figure 9), and were elevated particularly in P concentration relative to most of the other amendments. The wide range in nutrient concentrations across the alternative fertilisers possibly reflects the fact that these products are specifically manufactured as fertilisers, and each is targeted to address certain limiting nutrients, hence the wide range in Cu and Zn concentrations in particular. Both the outlying data-points for P, S, and Fe belong to piggery manures, and it is important to note that whilst these manures are particularly nutrient dense, the high concentrations of Cu and Zn may present a concern from a potential soil toxicity aspect in the longer term.

Compared to total elemental measurements (Figure 8), water soluble chemistry was even more diverse within the amendment types, with both DOC and DON spanning five orders of magnitude (Figure 10). As with many of the other analytes quantified above, the microbial inocula had consistently low concentrations of soluble available N (FAA-N, NH₄⁺, NO₃⁻). Of particular note is the wide range of FAA-N – up to 1603 mg kg⁻¹, which is highly elevated relative to expected concentrations in the soil (around 0.1-10 mg kg⁻¹). The high levels of soluble N contained in most of the amendments (averages of 132, 730 and 800 mg N kg⁻¹ for FAA-N, NH₄⁺, NO₃⁻ respectively) would

indicate a likely initial flush of easily accessible N upon application to the soil, the size of which will depend on the application rate.

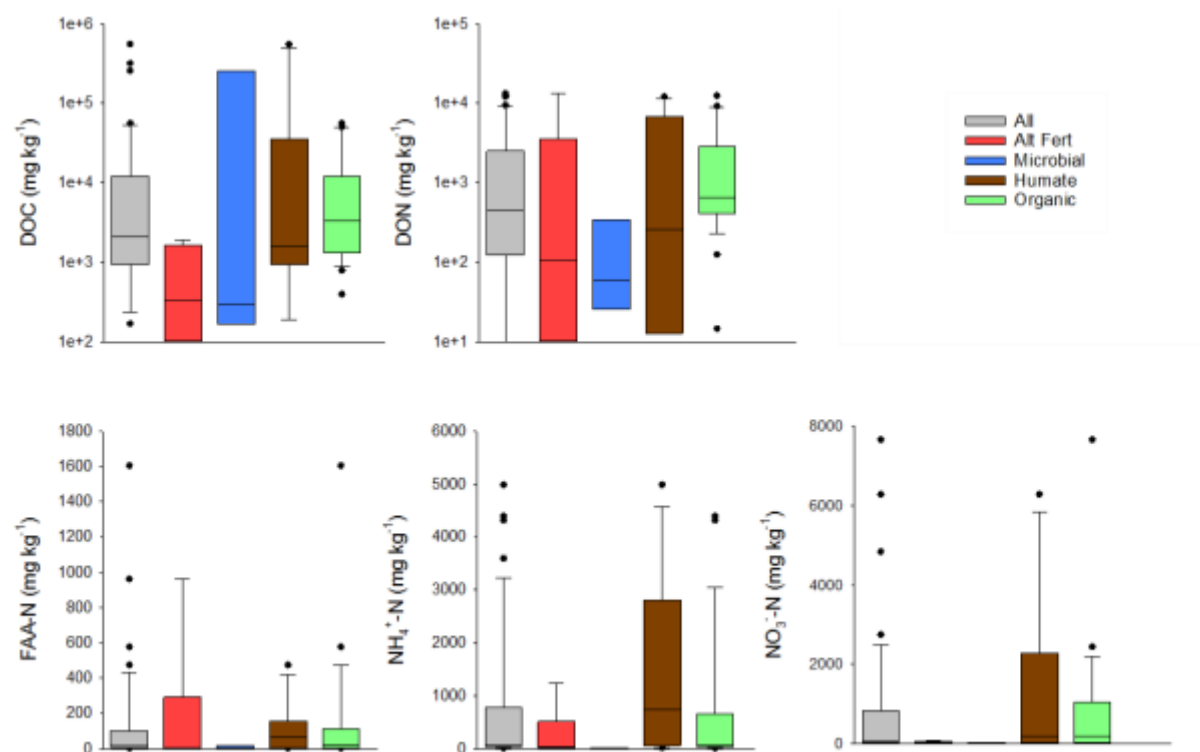


Figure 10: Box and whisker plots of Dissolved organic C, Dissolved organic N, Free amino acid-N, Ammonium-N and Nitrate-N of the solid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles. Note the logarithmic y-axis on the DOC and DON plots.

The overall chemical diversity of the solid biological inputs was examined by MIR spectroscopy coupled with multivariate statistics (Figure 11). This technique investigates all chemical bonds in a sample and can be a useful tool to identify broad chemical (dis)similarity between samples or treatments. However, it is not suitable for all types of amendment, with biochars often proving problematic due either to high infra-red absorbance of some matte black biochars, or high reflectivity of others. Here, the MIR screening has highlighted that with the exception of one compost (containing biochar), all the organic amendments cluster together, with a similar diversity of humates overlapping slightly. The alternative fertilisers sit offset slightly, whilst the microbial inocula separate, probably more due to the matrix they are contained in, rather than the microbes themselves.

Using NMR to more closely examine the chemistry of organic carbon contained within many of the solid amendments (Figure 12), with the odd exception, the humates separate into one group tending to be dominated by aromatic C types (aryl and O-aryl), whilst the organic amendments appear to separate into groups either dominated by carbohydrate- and protein-like C (alkyl, carboxyl) or lipid-like C (O-alkyl, di-O-alkyl). It is interesting to note that these two clusters do not easily separate into either compost or manure groups as might have been expected. Less decomposed plant material is usually dominated by O-alkyl C, whereas in more decomposed materials such as mature composts and soil OC, alkyl C tends to dominate (Baldock and Preston, 1995). As such, it would be expected that materials richer in O-alkyl C would be more readily degraded upon application to soils, possibly releasing nutrients as the C is mineralised by microbial activity. Conversely, organic amendments richer in alkyl or aryl C would be expected to be more recalcitrant and therefore longer lasting upon application to soil.

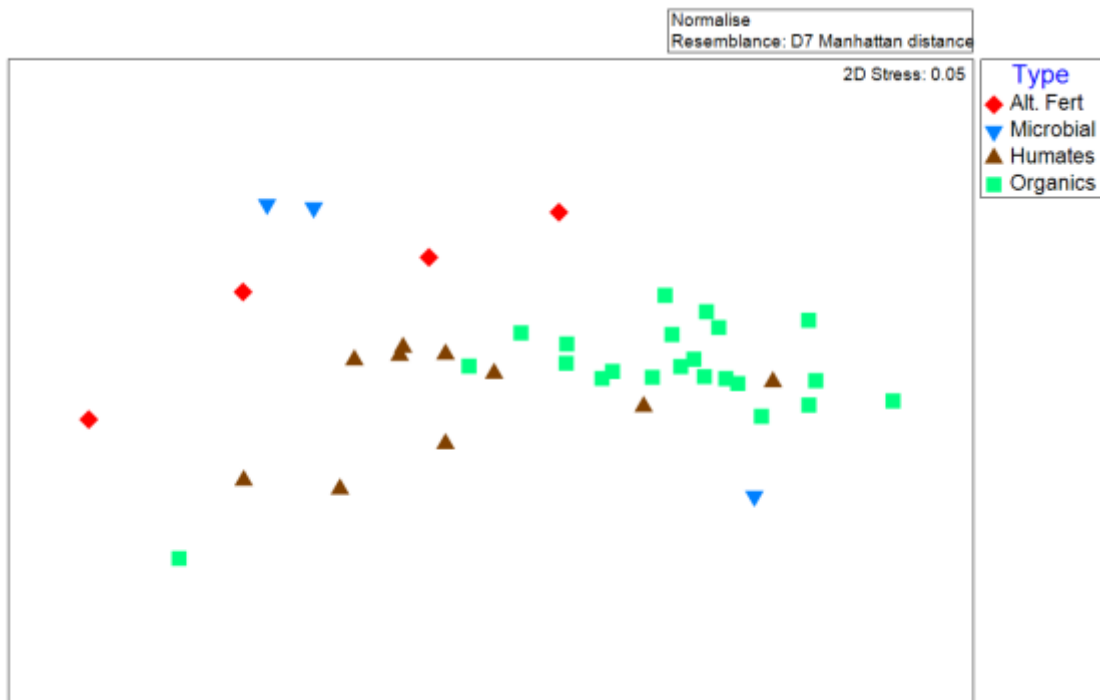


Figure 11: non-Metric Multidimensional Scaling (nMDS) plot of MIR spectra obtained from the solid amendments. Some (notably the biochar) produced poor spectra that weren't included in the analysis. The plot is dimensionless, but data points close together are similar in terms of their MIR spectra and thus chemistry, those which are further apart are dissimilar. The lone organics data-point in the bottom left of the figure is a compost blend containing biochar at a rate of 2% fwt.

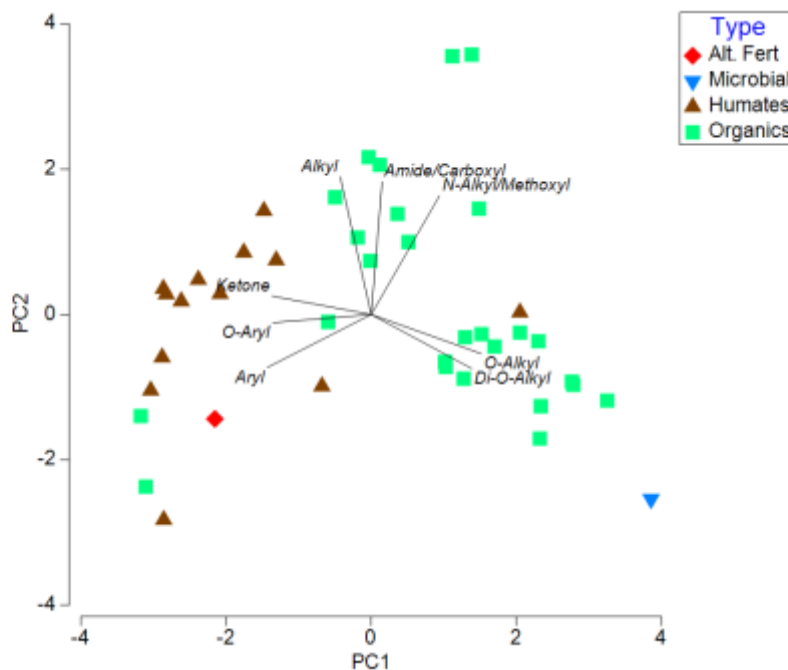


Figure 12: Principal Components Analysis (PCA) of organic matter chemistry as measured by NMR. Low-C amendments were omitted given that this is a measure only of the chemistry of organic matter in amendments.

The liquid amendments were dominated by biostimulants (none of which were available in solid form), with a number of microbial inocula, humates and alternative fertilisers also tested. It should be noted that all the liquids were tested as received, thus for measurements such as pH and EC, these values are for a substrate that would likely be diluted 100-10000x before application to soil. However, given the diversity of amendments and their application rates, it was decided that this was the most appropriate means of obtaining data that was comparable between amendments.

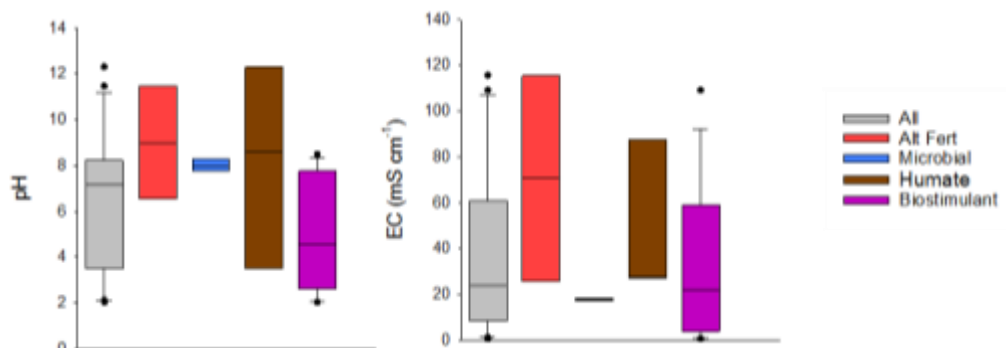


Figure 13: Box and whisker plots of pH and electrical conductivity (EC) of the liquid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles.

Compared to the solid amendments, there was greater diversity in pH, ranging from 2.01-12.3, with the humates having the greatest range. Electrical conductivity covered a similar range to the solid amendments, but with the exception of the microbial inocula, all liquid amendment classes showed a similar range of values (Figure 13).

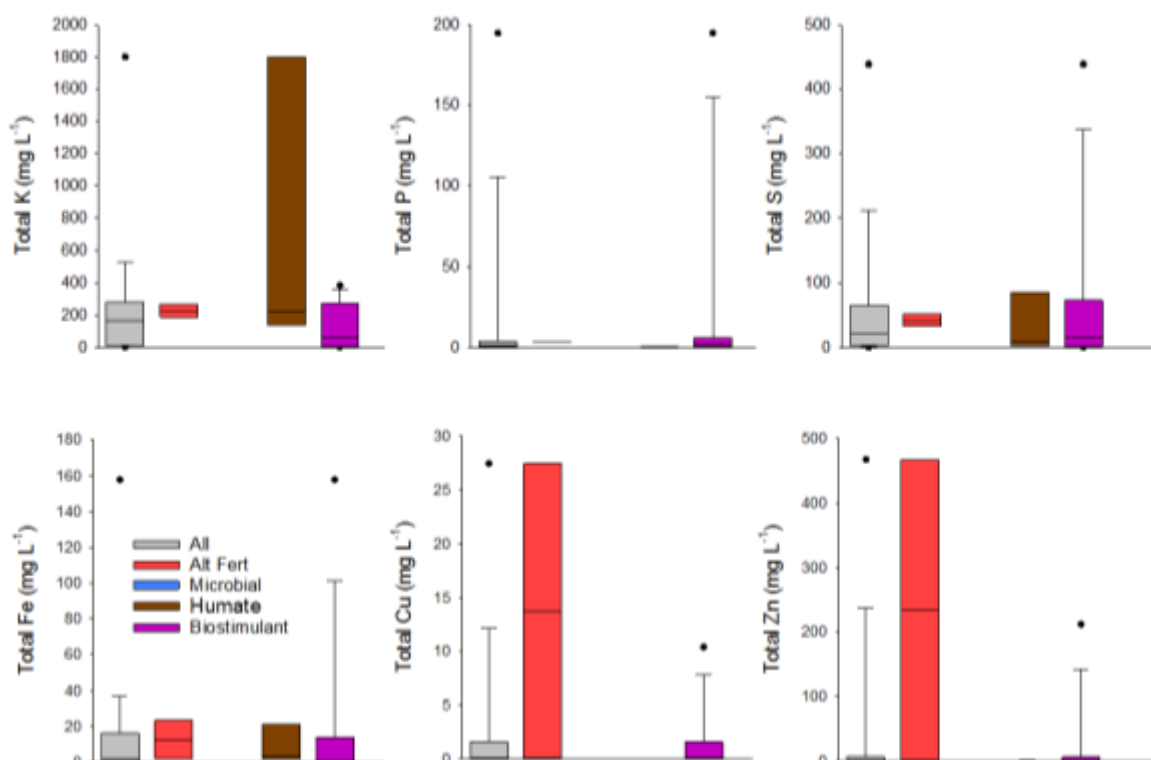


Figure 14: Box and whisker plots of Total K, P, S, Fe, Cu and Zn of the liquid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles.

Variance in the major and minor nutrients (Figure 14) was driven typically by just one amendment from the humates (total K), biostimulants (total P, S, and Fe) or the alternative fertilisers (total Cu and Zn). As with the solid alternative fertilisers, differences in whether micronutrient deficiency was being specifically targeted by the amendment probably dictates the difference between the two liquid alternative fertilisers captured in this dataset.

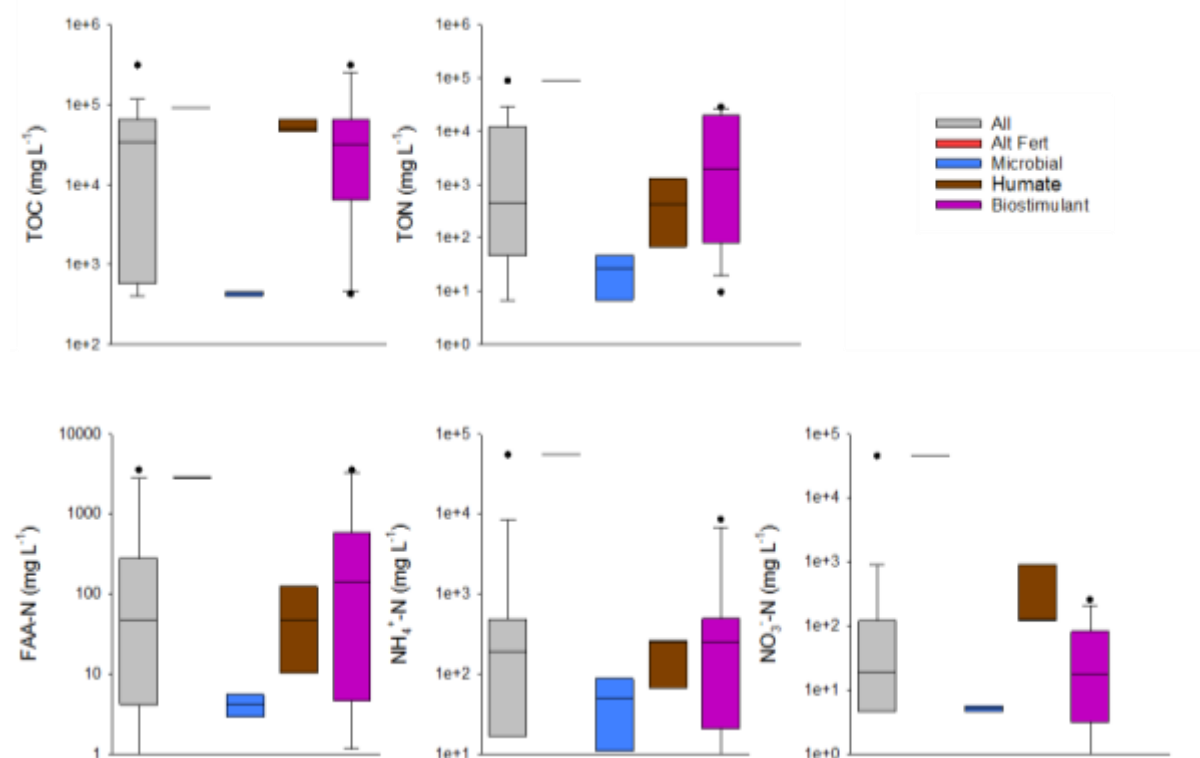


Figure 15: Box and whisker plots of Total Organic C (TOC), Total Organic N (TON), Free Amino Acid – N (FAA-N), Ammonium-N and Nitrate-N of the liquid biological amendments tested in this project, with variance depicted across the entire dataset (grey box), then split by biological input type (coloured boxes). In each dataset, the central line is the median, the bounds of the box are 25th and 75th percentiles, ends of the whiskers are 10th and 90th percentiles, and individual dots are samples beyond these bounds. Where no whiskers are shown the number of data points was below the minimum required to compute percentiles. The single horizontal line for the alternative fertiliser data depicts the only data-point available from the liquid amendments due to chemical interferences in the analyses which make up these data. Note the logarithmic y-axis on all plots.

Total organic C (TOC) and N species (TON, FAA-N, NH₄⁺, NO₃⁻) all varied across five to six orders of magnitude, with the biostimulants generally exhibiting the greatest range in concentrations, and the microbial inocula the smallest (Figure 15). Predictably, the humates were consistently high in TOC (average 5.8 g L⁻¹). As with the solid biological inputs, FAA-N concentrations are again generally highly elevated relative to those found in soils. The single liquid alternative fertiliser that was suitable for these analyses contained the highest dissolved N concentrations of all four N species measured (TON, FAA-N, NH₄⁺, NO₃⁻) and thus differs from most conventional fertilisers where a single source of N e.g. NH₄⁺ in DAP or urea is supplied as the N component of the fertiliser mix.

In summary, our chemical analysis of 69 biological inputs available to Australian grain growers has highlighted substantial variability both across the whole range of biological input types, but also within each category. Alternate fertilisers were particularly variable in their nutrient content, as could be expected given the targeted nature of their manufacture. Biostimulants were also a particularly diverse group of biological inputs, covering a wide range of pH and in nitrogen concentrations. Whilst MIR spectroscopy provided some grouping of solid amendments, it was not suitable for all solid amendments, and instruments designed for analysis of soils that are becoming more commonplace in Australian laboratories since SCaRP and other research are not immediately suited to the analysis of liquids. More targeted NMR spectroscopy identified two chemically differing groups in the organic amendments that did not directly relate to whether the amendment was either manure or compost. It is likely that these differences in organic C chemistry will relate to the behaviour of the amendments

upon application to the soil, with o-alkyl rich organic amendments likely being less stable but possibly contributing to more microbial activity and nutrient cycling.

Plant growth screening of biological inputs

A large pot experiment was conducted over 118 days between February and June 2015 to screen a large number of biological inputs (52 in total) in comparison to a range of conventional fertiliser applications. Plant growth (as length of above-ground parts from soil surface to longest leaf-tip / stem) and health (through SPAD measurements) were quantified over the whole growing period. Water loss through evapo-transpiration was recorded through mass loss at each watering episode. At harvest, above- and below-ground plant parts were separated and their dry mass recorded. Grain yield was also measured and recorded.

The range of conventional DAP fertiliser applications resulted in a typical fertiliser response curve for grain yield (Figure 16), whereby particularly high fertiliser additions (200% of district practice) induced a noticeable drop in yield. Yield at district practice (100 kg ha⁻¹ DAP) was 0.735 ± 0.063 g stem⁻¹ (mean \pm SEM), while yield from the zero fertiliser control treatment was lower (0.589 ± 0.095 g stem⁻¹). There was much variability within the seven biological input classes tested, with the mean for most input classes falling slightly above the yield from the zero DAP control. Investigating the individual treatments within each product indicates that no individual treatment fell well above the limits of what was achieved by conventional fertiliser addition to some level. While there is between-treatment variation within each product category, results indicate that some individual products within the categories of alternative fertilisers, humates, composts, and manures did perform at a level similar to that achieved with conventional fertiliser additions.

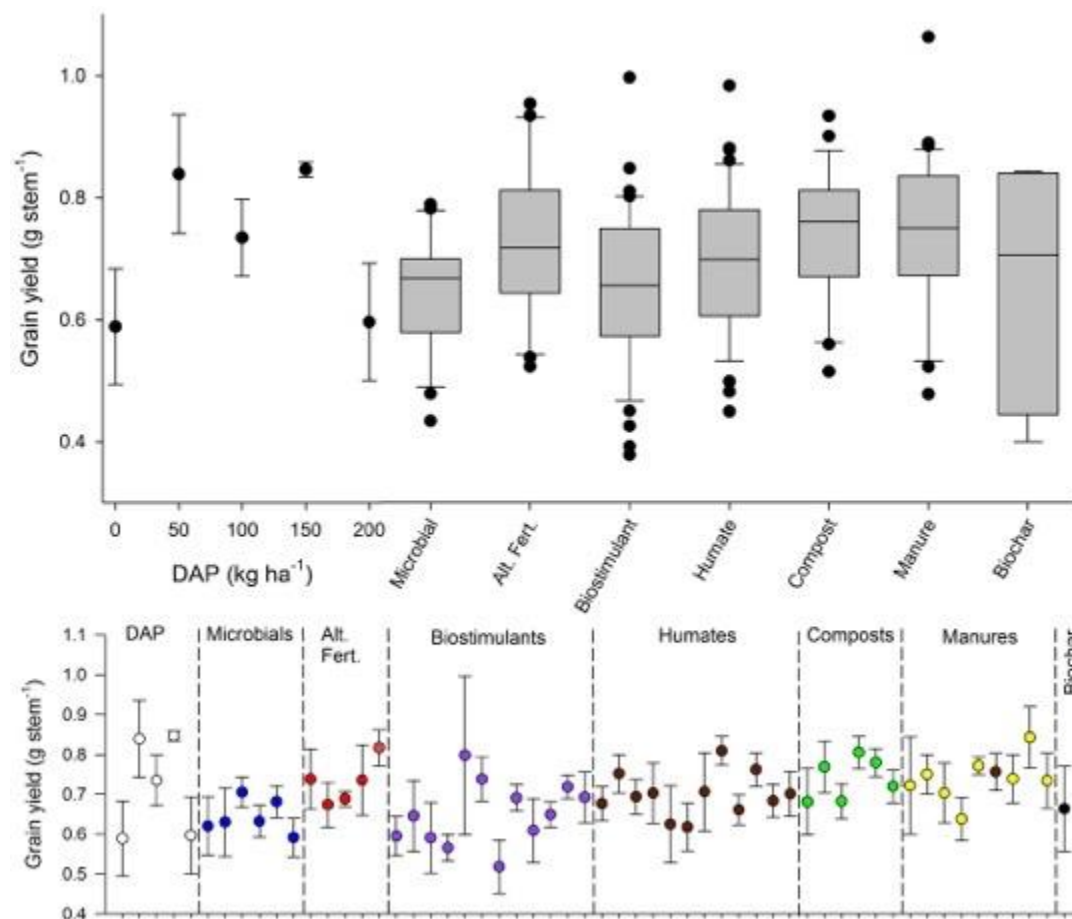


Figure 16: Grain yield (g stem⁻¹) in the screening pot experiment. The top panel shows the five conventional fertiliser applications \pm SEM, and box-whisker plots showing the variation of yield across all product types within each given category. The bottom panel shows grain yield response for each product within the categories \pm SEM.

In contrast to the grain yield data, quite striking increases in total plant biomass were observed with the addition of DAP at all rates, ranging from 1.62 ± 0.12 g stem⁻¹ in the zero control to 2.85 ± 0.21 g stem⁻¹ in the 200 kg ha⁻¹ DAP treatment (Figure 17). As with grain yield, total biomass results were variable within biological input classes, and tended to centre between the zero control and lowest conventional fertiliser treatment (50 kg ha⁻¹ DAP; Figure 17 top panel). Investigating within each input type (Figure 17 bottom panel) suggests that biomass production was much less variable within treatments for most biological inputs, rather, one or more individual treatments were either notably higher (alternative fertilisers, humates, manures), or had greater within-treatment variability (microbial inocula and biostimulants).

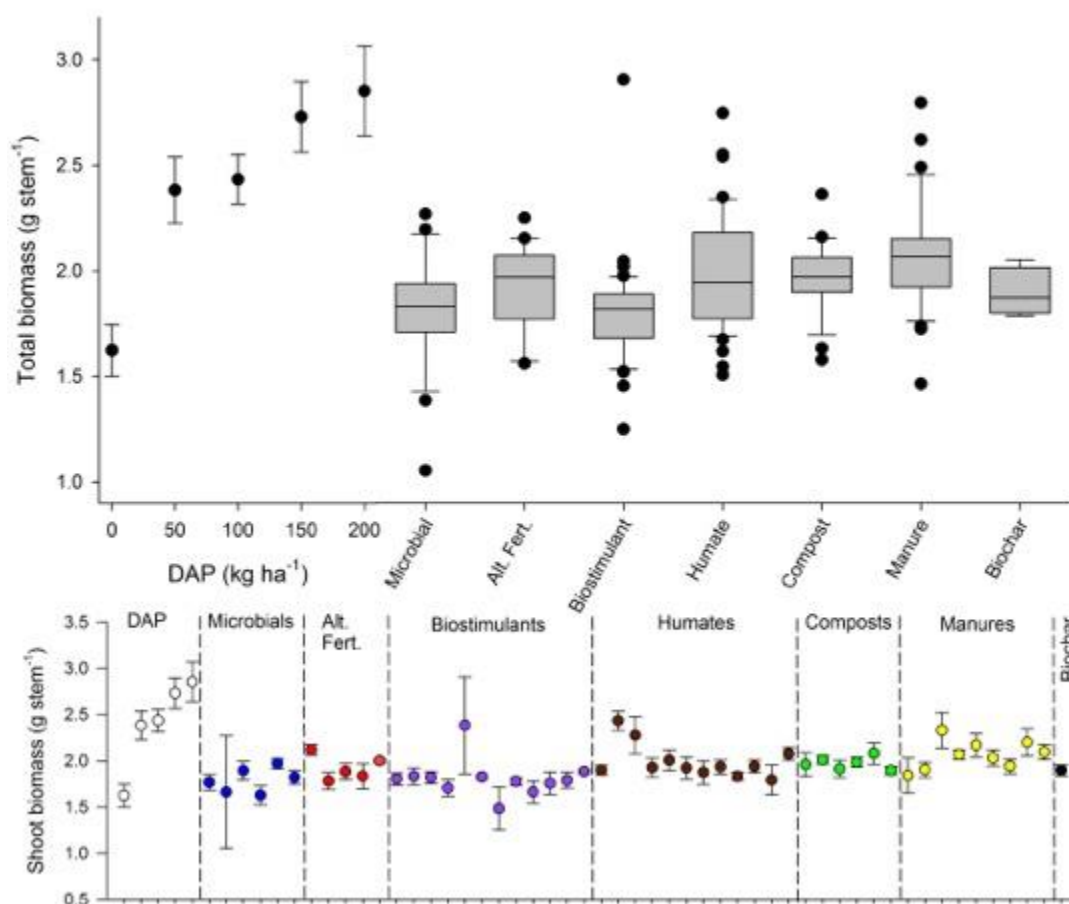


Figure 17: Total biomass production (g stem⁻¹) in the screening pot experiment. The top panel shows the five conventional fertiliser applications \pm SEM, and box-whisker plots showing the variation of yield across all product types within each given category. The bottom panel shows grain yield response for each product within the categories \pm SEM.

Root:shoot ratio, a measure of the allocation of plant resources to above- or below-ground plant parts, tended towards a reduction with increases in conventional fertiliser addition (Figure 18 top panel). This was to be expected, as in providing synthetic fertiliser to the plant, its need to invest energy in soil exploration to source nutrients is reduced. Measurements of root:shoot ratio were highly variable across all treatments and input classes (Figure 18), in part due to the error associated with obtaining accurate root biomass measurements after the destructive process of root washing. Despite this high variability, again, there were a few individual treatments within several of the product classes that stood out as having notably higher or lower root:shoot ratios than the general mean for each class, which fell generally close to the higher fertiliser addition rates in most cases.

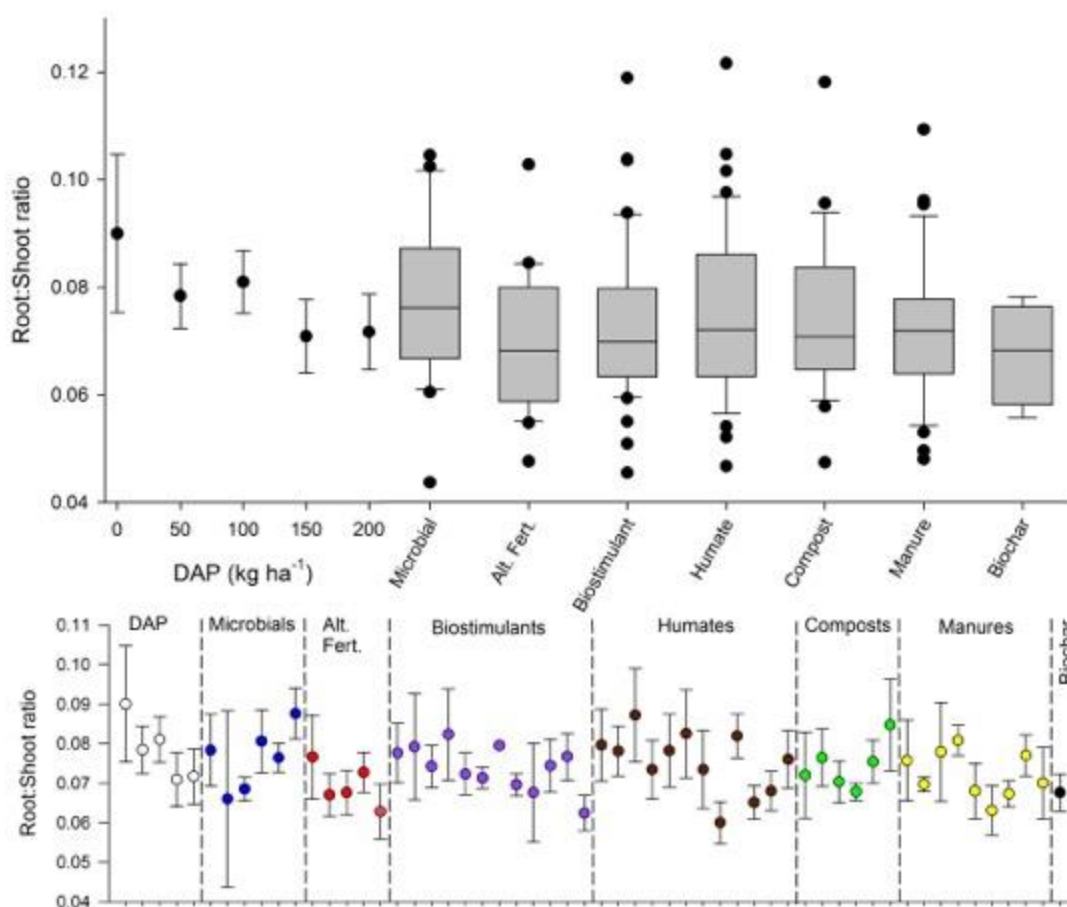


Figure 18: Root:shoot ratio in the screening pot experiment. The top panel shows the five conventional fertiliser applications \pm SEM, and box-whisker plots showing the variation of yield across all product types within each given category. The bottom panel shows grain yield response for each product within the categories \pm SEM.

In a water-constrained growing environment such as that simulated by the watering regime used in the present experiment, and potentially typical of much of the Australian dry-land grains systems, water losses through evapo-transpiration are also important. In the conventional fertiliser treatments, water use increased significantly where DAP was applied (Figure 19). This closely follows total biomass production (Figure 17), and is largely due to increased transpiration from the larger plants that grew with greater nutrient availability. When considering the input classes as a whole (Figure 19 top panel), water use generally fell between the zero control and the 50 kg ha⁻¹ DAP treatment. Though when considering the treatments at an individual level (Figure 19 bottom panel), most again centred around the mean for all treatments (2.44 L pot⁻¹), both the compost and manures input classes had some notable exceptions where water use was much reduced. In comparing these data with those of yield (Figure 16) and total biomass production (Figure 17), it is clear that these savings in water use were not associated with reductions in yield or biomass production.

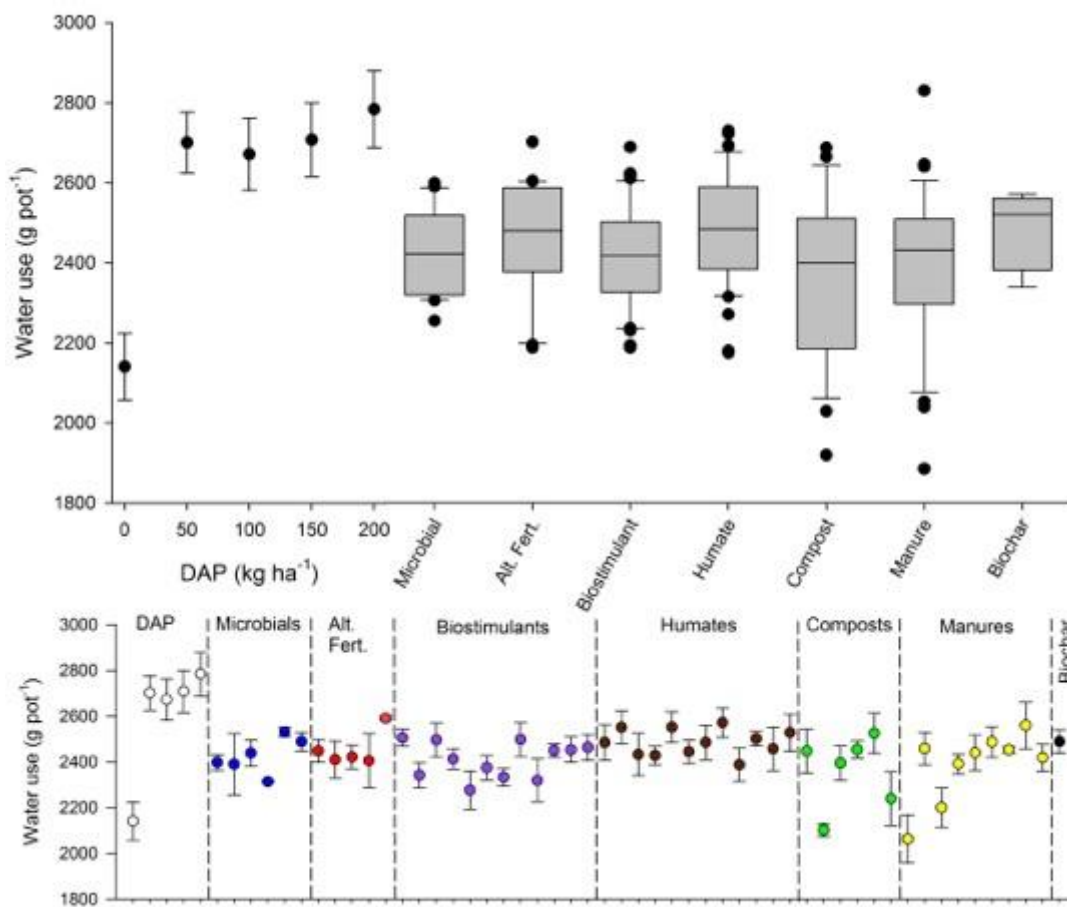


Figure 19: Water use (g pot⁻¹) in the screening pot experiment. The top panel shows the five conventional fertiliser applications \pm SEM, and box-whisker plots showing the variation of yield across all product types within each given category. The bottom panel shows grain yield response for each product within the categories \pm SEM.

For both plant growth and health (Figure 20 and Figure 21), very little variation was observed between any of the treatments in any of the product classes. Typically, the unfertilised control usually returned the shortest plant or lowest SPAD value at each time point. For most of the treatments, there was a period of slow growth between days 36 and 51 of the experiment, during which most plants were at the stem elongation phase of their growth. Most of the treatments also exhibited a slight dip in SPAD readings on day 66, before rebounding on the final date SPAD data was collected (day 79), by which point the majority of plants were at the “flowering to grain fill” growth stage, and watering was soon after ceased.

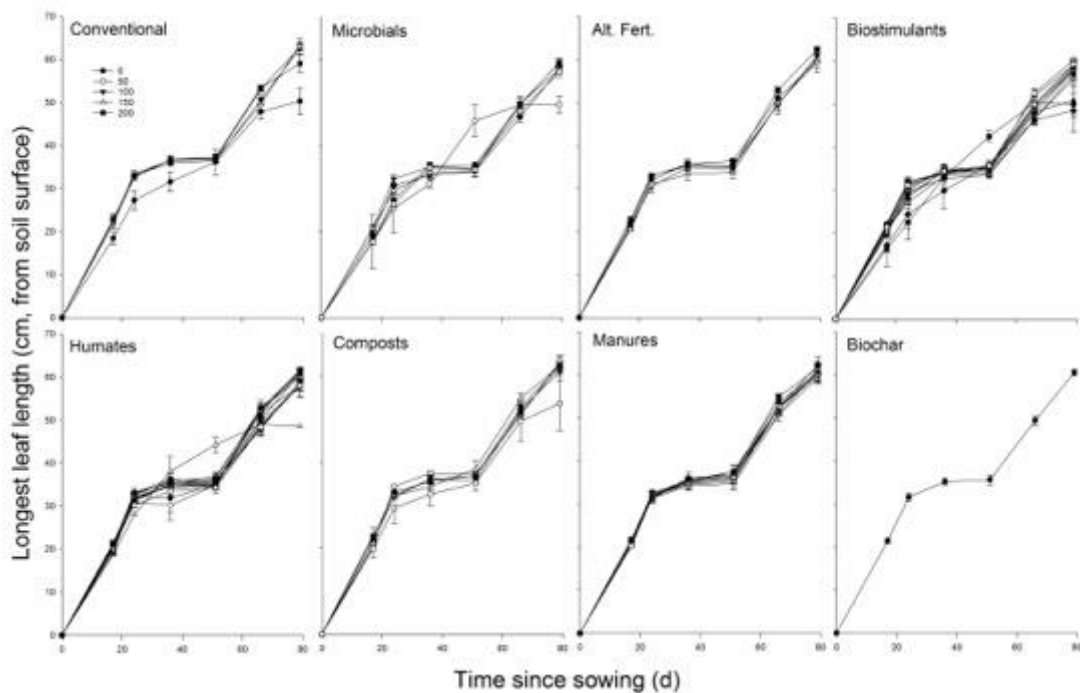


Figure 20: Plant growth as height from soil surface to tip of longest leaf / stem. Values are means \pm SEM for each product / treatment, split by product class. Note the legend only refers to the conventional fertiliser treatments in the top-left panel.

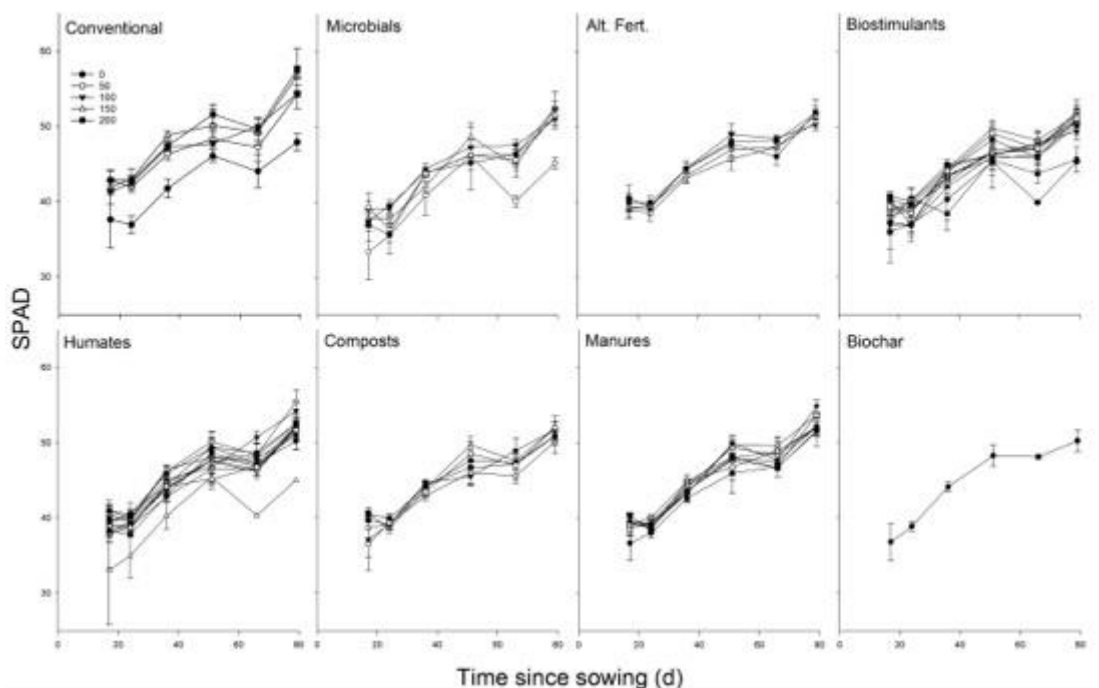


Figure 21: Plant health as measured by SPAD values of leaf green-ness. Values are means \pm SEM for each product / treatment, split by product class. Note the legend only refers to the conventional fertiliser treatments in the top-left panel.

In summary, despite the wide chemical variability observed in the analysis of the various biological amendments tested in this screening experiment, no single product or class consistently induced positive outcomes for any of the plant growth metrics evaluated in this screening pot experiment. We

do note that pot experiments can mask plant responses, but given the number and diversity of treatments to be tested, there is no other feasible means of evaluating plant response. A common criticism of pot experiments is that the pot itself becomes a normalising limiting factor. In order to address this, the measurements taken during plant growth (height and chlorophyll as estimated by SPAD) indicate limited impacts of any amendment (apart from conventional fertiliser) on growth or health of the plants at any stage in their development.

Testing biological amendments in field conditions

Site characteristics

The eight field sites where the plot scale experiments were situated varied widely in both chemistry (Table 7) and physical texture (Figure 22). Soil textures ranged from sand at Langhorne Creek and loamy sand at Buntine through to clay at Parkes and Mt Tyson. Organic C was lowest in the Buntine tenosol ($5.9 \pm 0.6 \text{ mg g}^{-1}$) and over three times as high in the Mt Tyson vertosol ($19.7 \pm 0.6 \text{ mg g}^{-1}$). Most sites had a circum-neutral pH, with the exception of the calcarosol at Paskeville (pH 8.5 ± 0.1) and the chromosol at Jamestown (pH 5.5 ± 0.1). Electrical conductivity (EC) was generally low (sub $200 \mu\text{S cm}^{-1}$) at all sites, and MBC/N generally followed the OC trend, in that sites with lower OC values also had smaller MBC/N pools. Standing soluble nitrogen pools were generally $<10 \text{ mg kg}^{-1}$, with the exception of ammonium in the Jamestown soil. Available P was highest at Rankins Springs, seven times the concentration at Paskeville and Parkes, which had the lowest available P concentration

Table 7: Chemical characteristics of the soils at the eight field sites. Values are means \pm SEM.

Site	TOC (mg g^{-1})	TN (mg g^{-1})	C:N ratio	pH	EC ($\mu\text{S cm}^{-1}$)	MBC (mg kg^{-1})	MBN (mg kg^{-1})	$\text{NO}_3^- \text{N}$ (mg kg^{-1})	$\text{NH}_4^+ \text{N}$ (mg kg^{-1})	DON (mg kg^{-1})	$\text{PO}_4^{3-} \text{P}$ (mg kg^{-1})
Buntine	5.90 ± 0.59	0.395 ± 0.046	15.0 ± 0.4	7.34 ± 0.16	6.0 ± 0.9	208 ± 30	40.5 ± 5.2	1.95 ± 0.06	0.40 ± 0.07	5.38 ± 1.10	15.6 ± 3.5
Jamestown	8.62 ± 0.41	0.881 ± 0.020	9.8 ± 0.2	5.54 ± 0.09	12.6 ± 0.9	210 ± 6	35.3 ± 4.7	7.86 ± 1.30	12.83 ± 3.44	8.31 ± 3.81	24.2 ± 1.6
Paskeville	14.95 ± 0.47	1.203 ± 0.028	12.4 ± 0.2	8.50 ± 0.09	110.7 ± 12.7	330 ± 69	45.5 ± 6.2	0.84 ± 0.22	2.66 ± 0.92	4.26 ± 1.07	8.4 ± 2.5
Langhorne Creek	10.25 ± 0.69	0.876 ± 0.053	11.7 ± 0.3	6.40 ± 0.13	55.6 ± 14.9	243 ± 30	17.4 ± 9.7	10.10 ± 3.05	4.25 ± 2.85	4.17 ± 1.43	29.3 ± 3.2
Inverleigh	10.43 ± 0.30	0.833 ± 0.025	12.5 ± 0.2	7.20 ± 0.10	11.2 ± 0.1	279 ± 28	35.7 ± 3.7	4.14 ± 0.27	0.81 ± 0.08	3.40 ± 0.27	36.5 ± 5.2
Rankins Springs	10.59 ± 0.69	0.906 ± 0.060	11.7 ± 0.1	6.58 ± 0.11	45.2 ± 6.2	190 ± 41	12.9 ± 4.3	1.14 ± 0.26	0.67 ± 0.14	2.99 ± 0.85	42.1 ± 5.1
Parkes	15.62 ± 0.29	1.212 ± 0.026	12.9 ± 0.1	6.63 ± 0.12	5.8 ± 0.7	256 ± 52	28.4 ± 6.4	1.71 ± 0.18	0.82 ± 0.16	2.72 ± 0.26	8.4 ± 3.1
Mt Tyson	19.68 ± 0.59	1.089 ± 0.049	18.1 ± 0.3	7.74 ± 0.07	123.7 ± 18.0	304 ± 40	31.7 ± 13.4	4.06 ± 0.69	1.48 ± 0.13	1.49 ± 0.68	29.3 ± 4.0

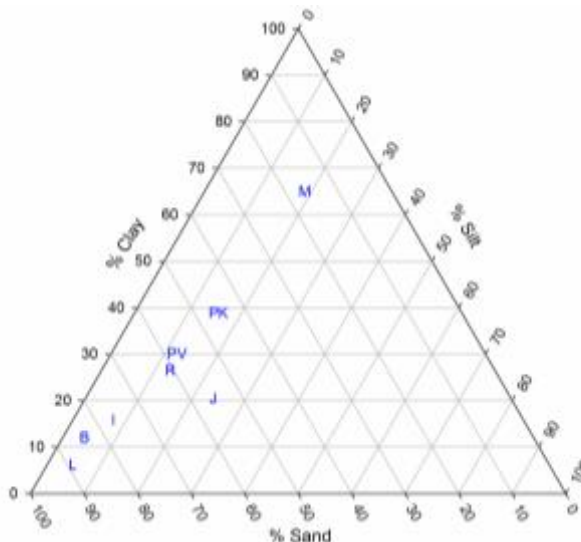


Figure 22: Ternary plot showing soil texture for the eight field sites. B = Buntine, J = Jamestown, PV = Paskeville, L = Langhorne Creek, I = Inverleigh, R = Rankins Springs, PK = Parkes, M = Mt Tyson.

Response to conventional fertilisers

Crop response to conventional fertilisers differed both between site and year (Figure 23). In the 2015 season, which was much drier at all sites except Buntine in WA and Mt Tyson in Qld, several sites (Buntine, Langhorne Creek, Rankins Springs) gave reduced yields at high (200% district practice) conventional fertiliser rates, whilst the others showed either no response or a slight increase (Parkes). Yields ranged from just $1.02 \pm 0.05 \text{ t ha}^{-1}$ in the 25% district practice treatment at Paskeville to $5.97 \pm 0.25 \text{ t ha}^{-1}$ in 2015. In 2016, which was a much wetter season for most of the sites, yields ranged from $1.59 \pm 0.24 \text{ t ha}^{-1}$ in the zero fertiliser control at Buntine through to $7.90 \pm 0.21 \text{ t ha}^{-1}$ for the 75%

district practice treatment at Inverleigh, a >100% increase over the previous year in for the same treatment.

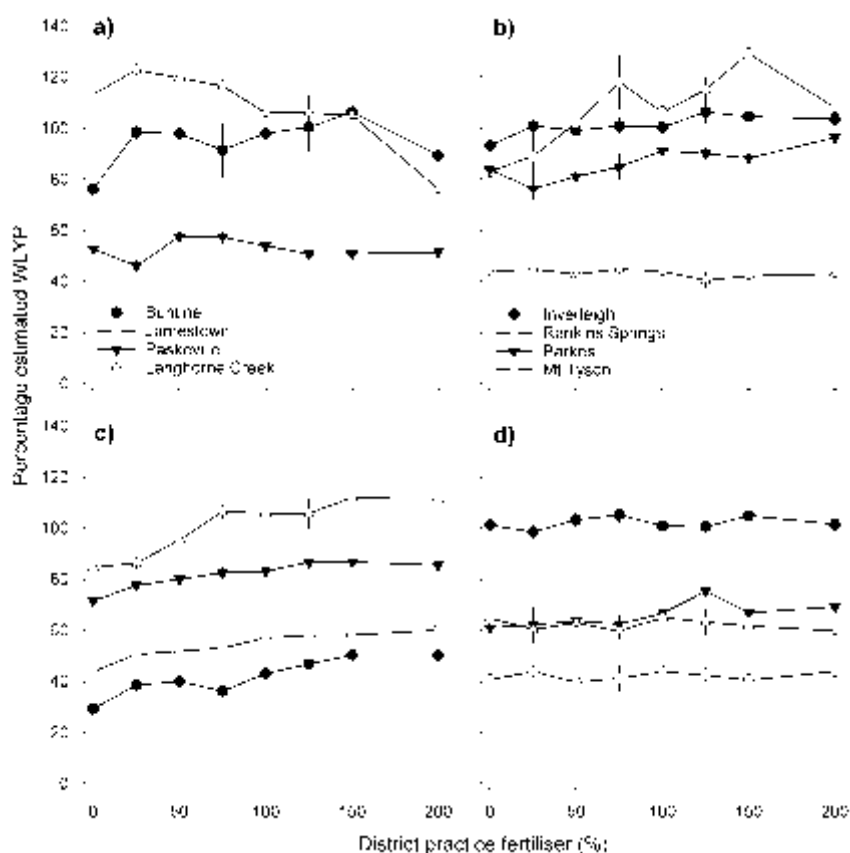


Figure 23: Conventional fertiliser response of grain yield as a percentage of water limited yield potential as estimated by APSIM. Panels a and b are 2015, c and d are 2016. Legends are shared between a and c, and b and d respectively. Values are means ± SEM.

Relative to the predicted WLYP simulated in APSIM, conventional fertilizer treatments typically fell close to or exceeded this value in 2015 with the exception of the Paskeville and Mt Tyson sites (Figure 23). In 2016, fewer sites neared or attained these simulated yield targets, likely due to a significant portion of in-season rainfall (one of the main parameters in the WLYP estimation) occurring in high intensity events that meant that a proportion of this water was likely lost to run-off. Nonetheless, in several of the under-performing sites in the 2016 season, there was clear responsiveness to increased conventional fertiliser application.

Interestingly, in most cases, increased conventional fertiliser application did not lead to changed grain N or P concentrations (data not shown). In the case of N, it was elevated in the 200% district practice treatment relative to the control at Langhorne Creek in 2015, and Mt Tyson in 2015 and 2016, but reduced in the higher fertilised treatments at Parkes in 2016. Grain P was non-responsive at all sites in both years, with the exception of Inverleigh, where increases with increased fertiliser were observed in both seasons.

Response of crop and soil to biological inputs

Despite the many biological inputs tested across eight sites in two years, only four significant ($P \leq 0.05$) effects on grain yield were observed relative to the 50% district practice fertiliser that was applied underneath all treatments. Two of these, a biostimulant (B4) and a humate (H2), both at the Paskeville site in 2015 (Figure 24) actually resulted in significant reductions in yield relative to the control. In 2016 however, one organic amendment (a poultry litter, O11) tested at the two NSW sites resulted in statistically significant increases in yield of 66% and 42% at Rankins Springs and Parkes respectively. These increases came with no penalty to either grain N (related to protein) or P concentrations.

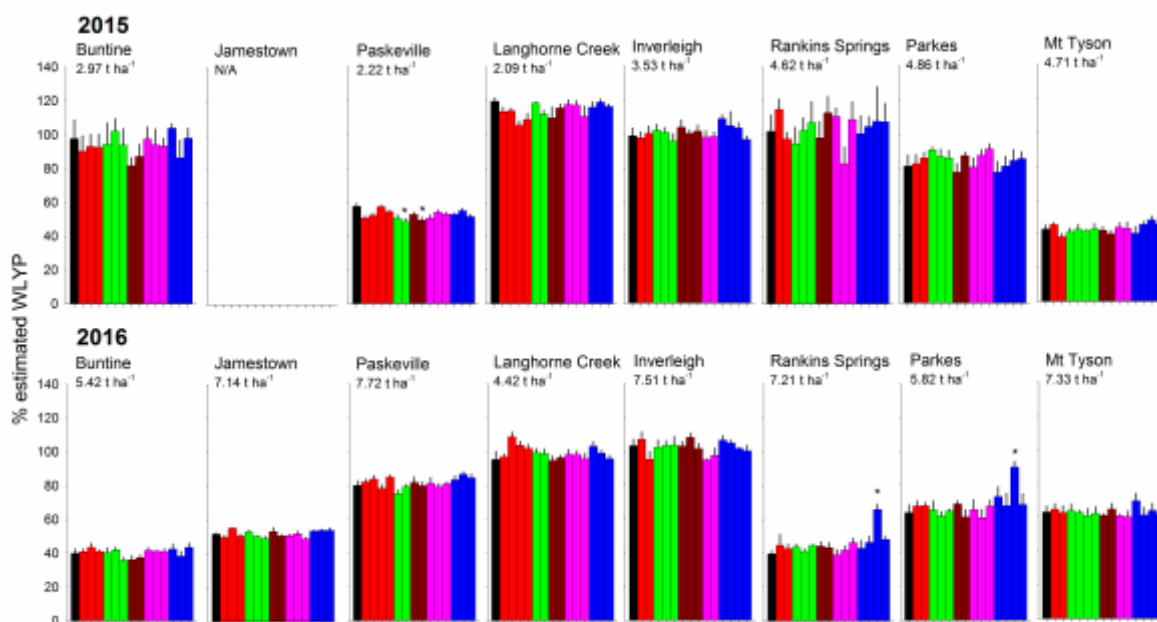


Figure 24: Yield response of grains as a percentage of estimated water limited yield potential simulated in APSIM. Values are the simulated WLYP. Black = control (50% district practice), red = alternative fertilisers, green = biostimulants, brown = humates, pink = microbial inocula, blue = organic amendments. Values are means \pm SEM, asterisks denote significant ($P \leq 0.05$) differences between the treatment and the control.

In recognition that short term (1-2 year) field experiments may be unable to demonstrate differences between treatments, especially given such contrasting rainfall seasons, we quantified nutrient content in the grain, and soil chemical and microbial properties in order investigate whether biological inputs were having more subtle effects that may relate to improved soil fertility, yield, or grain quality in the longer term.

Multivariate analysis to visualise differences across the whole datasets, using principal coordinates analysis (PCoA) based upon a Euclidean distance matrix for grain yield and chemistry and soil biogeochemistry (Figure 25), and a Bray-Curtis similarity matrix for soil microbial community structure data (Figure 26). These analysis allow the visualisation of how similar or different the sites were, and also an indication of the factors driving those differences in each dataset. For all three datasets, permutational multivariate analysis of variance (PERMANOVA) revealed significant differences between all sites ($P < 0.01$). This analysis considers all variables within a dataset together and thus in the context of environmental data such as grain or soil chemistry may mask trends in individual variables, but is useful to provide an indication of the picture a whole data matrix is showing. When used in high dimensional datasets such as microbial community analysis, it is the only meaningful way of analysing the whole dataset.

For grain characteristics, three main clusters are observed (Figure 25 left panel), with Inverleigh tending towards higher yield and C concentration, and the other four sites separating along an axis of N and P concentrations, with Mt Tyson being associated with the highest nutrient concentrations, and Rankins Springs the lowest. There was less defined clustering by site for the soil chemistry (Figure 25 right panel), but nonetheless, a strongly significant ($P \leq 0.0001$) PERMANOVA result for site differences indicates that each site had discrete chemical properties. Here, the x-axis of the figure tends towards a low \rightarrow high available nutrient gradient, whilst the y-axis is aligned with a high \rightarrow low pH, EC, and microbial biomass gradient. Thus, Paskeville separates from all but the Mt Tyson site on the basis of pH, EC and microbial biomass, and shows variance within on the basis of nutrient availability. Jamestown and Rankins Springs separate primarily on the basis of nutrient availability, with Rankins Springs having particularly tight clustering.

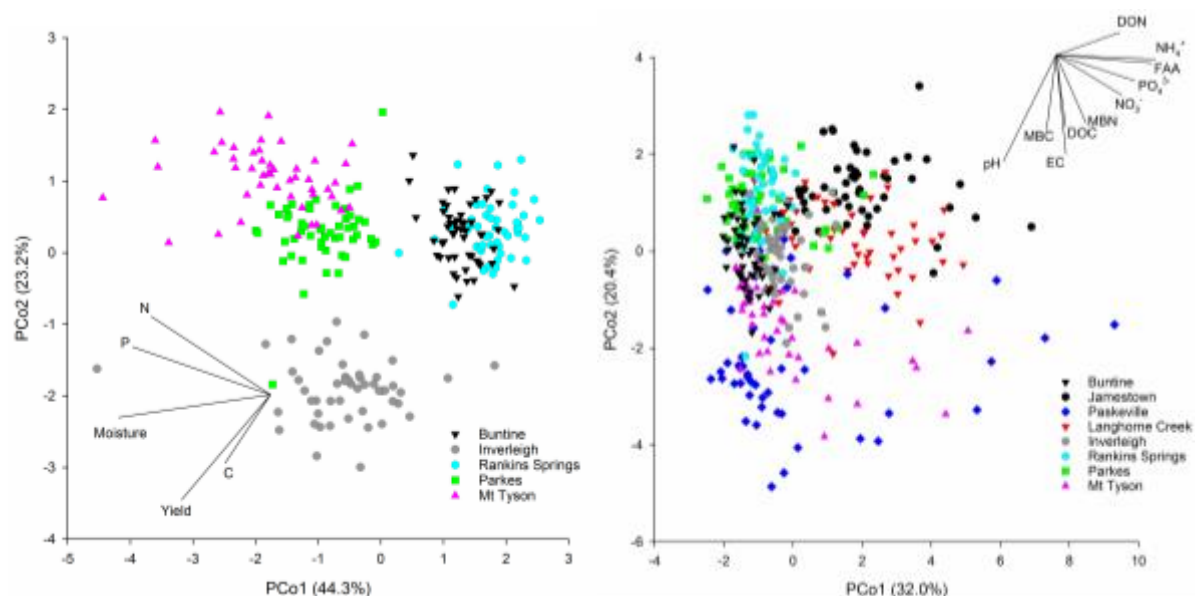


Figure 25: Principal Coordinates Analysis (PCoA) of grain yield and chemistry data (left panel) and soil chemistry data (right panel). Data points closer together indicate similarity, data points further apart indicate dissimilarity. Vectors show direction and strength of relationship of variables with the xy axes of the plot e.g. data points close to the bottom-left of the right-hand plot are associated with higher pH but lower DON concentrations.

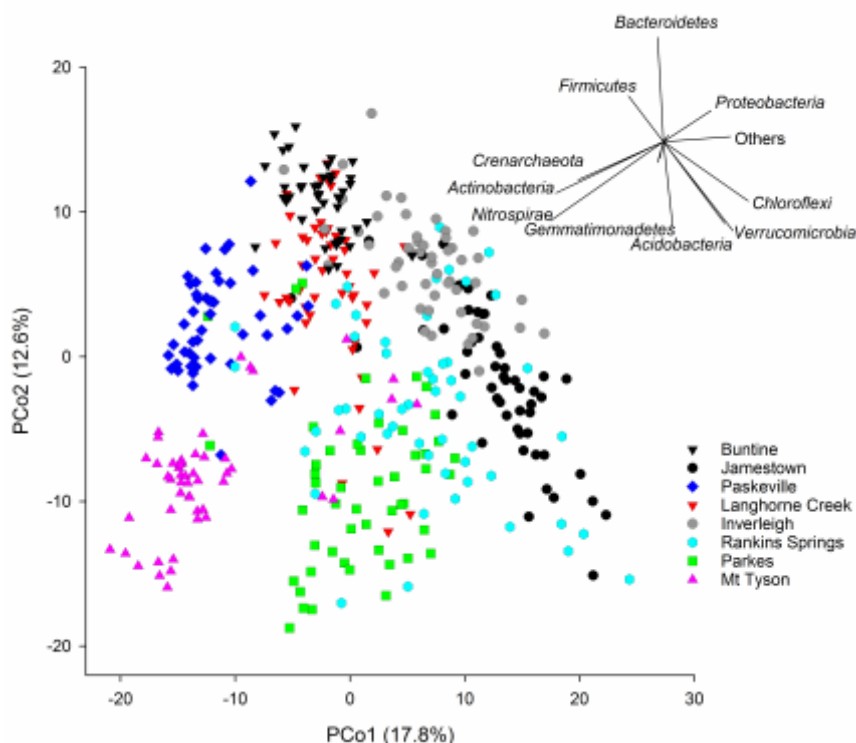


Figure 26: Principal Coordinates Analysis (PCoA) bacterial and archaeal community structure across the eight field sites. Data points closer together indicate similarity, data points further apart indicate dissimilarity. Vectors show direction and strength of relationship of variables with the xy axes of the plot e.g. data points close to the bottom-left of the right-hand plot are associated with higher *Gemmatimonadetes* populations but lower *Proteobacteria* populations.

Sites were also significantly different from a microbial community perspective ($P \leq 0.0001$, Figure 26), with Mt Tyson and Paskeville again forming more discrete clusters, and Rankins Springs and Jamestown showing more variance. The vectors in this figure are the bacterial and archaeal phyla responsible for 95% of the species identified by the molecular microbial analysis, and the proportional make-up of these is shown in more detail in Figure 27. Proteobacteria and Actinobacteria were the main phyla at most of the sites, jointly accounting for between 40-50% of the total species. These phyla play a major role in soil processes and services and are commonly reported in agricultural systems across the world (Janssen, 2006; Zeng et al., 2016). Actinobacteria are known for their ability to degrade complex compounds and recalcitrant materials such as starch, cellulose and lignins (Jenkins et al., 2009). Proteobacteria represent the largest and most metabolically and ecologically diverse phylum which are known to be particularly adept at responding to a variety of C and N compounds entering soils and include metabolic specialist such as nitrogen fixers, nitrifiers, methanotrophs (Fierer et al., 2007). Also, members of both phyla have been identified as plant growth promoting and disease suppressing bacteria that facilitate nutrient acquisition and provide protection against soil-borne fungal plant pathogens such as *Rhizoctonia* root rot on wheat (Barnett et al., 2017; El-Tarabily and Sivasithamparam, 2006). Of the other major phyla, Firmicutes appeared most variable, making up fewer than 5% of the total at Parkes yet over 20% at Langhorne Creek. Firmicutes are metabolically versatile, capable of degrading a variety of complex organic materials and some members are able to act as biocontrol agents against plant pathogens (Hartmann et al., 2015). Their distribution is largely influenced by the soil properties such as pH, clay content and carbon availability (Fierer and Jackson, 2006; Kuramae et al., 2012) and this might explain why they are highly abundant in the sandy soils of Langhorne Creek but less prevalent in the clayey soils at Parkes.

Overall, Mt Tyson separated from the other sites on the basis of increased proportions of the Crenarchaeota, Actinobacteria and Nitrospirae, whilst Buntine and Langhorne Creek had proportionally more Firmicutes, and fewer Acidobacteria, Chloroflexi and Verrucomicrobia. Further analysis of the sequence data from the Mt Tyson site at a higher resolution revealed that Nitrospiraceae was mostly composed of sequences related to the chemolithoautotrophic aerobic nitrite-oxidizing bacteria *Nitrospira* (Pester et al., 2014). Also, Crenarchaeota was dominated by one taxa closely affiliated to *Candidatus Nitrososphaera*, a well known nitrifying archaea that is the main contributor to ammonia oxidation in soils across the world (Gubry-Rangin et al., 2010; Leininger et al., 2006; Yao et al., 2013; Zhang et al., 2012). In contrast, the sandy semi-arid soils of Buntine and Langhorne Creek are dominated by Firmicutes that are able to form spores making them more resistant to disturbance and desiccation (Taketani et al., 2017).

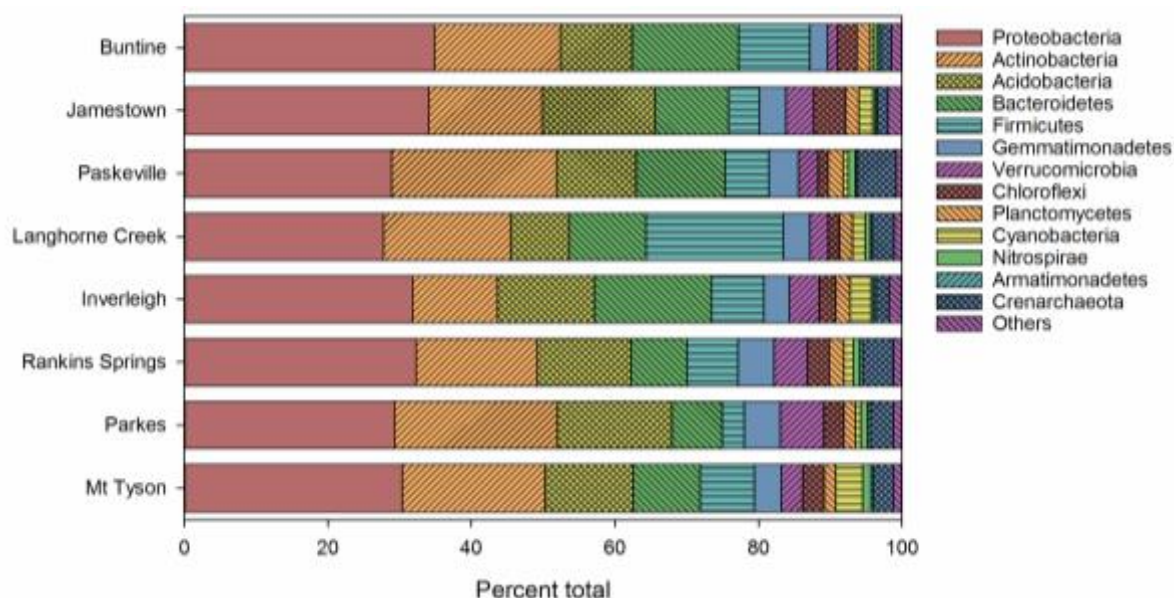


Figure 27: Distribution of microbial community amongst the phyla responsible for >95% of the total bacterial and archaeal abundance.

Given that each site significantly differed overall in terms of grain yield and nutrient concentration, soil biogeochemistry, and soil microbial community structure, analysis of biological input treatment effects

was split by site, leading to eight individual analyses for each data matrix, with the exception of the grain yield and chemistry where the three SA sites could not be included due to the lack of discrete grain sample from each experimental plot. Results of this series of PERMANOVA tests are laid out in Table 8. No significant differences between treatments at the site level were found for the grain yield and chemistry dataset, but at Buntine, Paskeville, Langhorne Creek, Parkes, and Mt Tyson statistically significant treatment effects were observed within either the soil biogeochemistry or microbial community structure matrices, however, differences from the 50% district practice control were only observed at Paskeville, Parkes, and Mt Tyson. It is notable that for the Mt Tyson site, despite very limited impacts of either conventional or biological input treatments on grain yield or chemistry in either year, and a substantial underperformance relative to the simulated WLYP (Figure 23: Conventional fertiliser response of grain yield as a percentage of water limited yield potential as estimated by APSIM. Panels a and b are 2015, c and d are 2016. Legends are shared between a and c, and b and d respectively. Values are means \pm SEM. Figure 24), every treatment for which microbial community structure was successfully generated differed from the 50% district practice control (Table 8).

To probe the detail of which soil biogeochemical variables were significantly affected by the experimental treatments, univariate ANOVAs were conducted. Very few significant effects of any treatment were observed, with only four variables out of the ten examined being significantly affected at any of the sites, with only three significantly differing from the control ($P < 0.05$; Table 9). Of these, one of the three composts used at the Buntine site (O3) reduced the standing FAA-N pool by a factor of two from 0.368 ± 0.047 mg N kg⁻¹ to 0.170 ± 0.058 mg N kg⁻¹. At the Parkes site, the same poultry litter (O11) responsible for the significant increase in grain yield in the 2016 season (Figure 24) resulted in a nearly five-fold increase in NO₃⁻-N concentration (50% district practice = 1.69 ± 0.31 mg N kg⁻¹, O11 = 8.10 ± 0.92 mg N kg⁻¹), despite the fact that at only 145 mg N kg⁻¹ extractable NO₃⁻-N, its initial NO₃⁻-N concentration was ca. 25% of the average soluble NO₃⁻-N concentrations across all the organic amendments applied in the field. Indeed, relative to the other three organic amendments applied at Parkes, this poultry litter had the lowest NO₃⁻-N concentration, though notably it contained 20 times the amount of soluble NH₄⁺-N, 100 times the amount of FAA-N, and nearly twice the amount of DON contained in the other organic amendments. This indicates that rapid nitrification of this available N occurred after application to the soil. However, it is also worth noting that the Rankins Springs site received the same treatments and although yield was significantly increased for this treatment at that site, no concomitant significant increases in NO₃⁻-N concentrations were observed. Finally, at Mt Tyson, a composted feedlot manure (O14) nearly doubled EC values from 100 ± 11 μ S cm⁻¹ to 191 ± 31 μ S cm⁻¹, though the manure's EC of 8.11 mS cm⁻¹ was average for the organic amendments analysed in this project.

When investigating treatment effects relative to the 50% district practice control in the microbial community data, only three sites (Paskeville, Parkes and Mt Tyson; Table 8) had treatments that were significantly different ($P \leq 0.05$) relative to the control. Multivariate SIMPER analysis was used to identify the phyla that most differed between each significantly different biological input treatment and the 50% district practice control, and these are identified in Table 10. Firmicutes and Bacteroidetes appeared to be the most abundant phyla responding to the different fertiliser and biological input treatments and this could be due to fact they are fast-growing copiotrophs that thrive in nutrient rich environments where they outcompete other slow-growing phyla (Fierer et al., 2007). Many genera representatives of these two phyla have been found in different organic materials such as manure or compost (Ryckebøer et al., 2003).

Table 8: PERMANOVA analysis of grain chemistry / yield, soil chemistry, and soil microbial community structure datasets for each site. Only treatments significantly different to the 50% district practice control are listed. Numerical values refer to conventional fertiliser treatments (as percent district practice); alphanumerical codes refer to individual (numbers) treatments within each class (letters): A = alternative fertiliser, B = biostimulant, M = microbial inoculum, O = organic amendment. n.d. = not determined.

Site	Data set	PERMANOVA Result	Treatments significantly ($P \leq 0.05$) different to 50% district practice control
Buntine	Grain	$P = 0.8821$	
	Soil chemistry	$P = 0.2829$	
	Microbial	$P \leq 0.0001$	
Jamestown	Grain	n.d.	
	Soil chemistry	$P = 0.0707$	
	Microbial	$P = 0.5204$	
Paskeville	Grain	n.d.	
	Soil chemistry	$P \leq 0.0001$	150, 200
	Microbial	$P = 0.0395$	O5
Langhorne Creek	Grain	n.d.	
	Soil chemistry	$P = 0.0030$	
	Microbial	$P = 0.9453$	
Inverleigh	Grain	$P = 0.4626$	
	Soil chemistry	$P = 0.2812$	
	Microbial	$P = 0.1498$	
Rankins Springs	Grain	$P = 0.2593$	
	Soil chemistry	$P = 0.5986$	
	Microbial	$P = 0.4539$	
Parkes	Grain	$P = 0.0247$	
	Soil chemistry	$P \leq 0.0001$	0, O11
	Microbial	$P \leq 0.0001$	A1, O5, O6
Mt Tyson	Grain	$P = 0.1228$	
	Soil chemistry	$P = 0.0004$	150, 200, O14
	Microbial	$P \leq 0.0001$	0, 100, 150, 200, B1, M1, A1, O13, O14, O15

Table 9: List of variables at each site where significant ($P \leq 0.05$) differences were observed between the treatments. Only organic amendments (O) had a significant impact on any of the variables tested relative to the 50% district practice control. R = variable reduced in concentration relative to the control, N = neutral response relative to the control (but there were differences between other treatments), E = elevated response of the listed variable to the treatment relative to the control.

Site	ANOVA result	Analyte	Significant treatments
Buntine	$P = 0.034$	FAA-N	O3; R
	$P = 0.034$	PO ₄ ³⁻ -P	N
Jamestown	NS		
Paskeville	NS		
Langhorne Creek	NS		
Inverleigh	$P = 0.046$	NO ₃ ⁻ -N	N
Rankins Springs	NS		
Parkes	$P \leq 0.001$	NO ₃ ⁻ -N	O11; E
Mt Tyson	$P \leq 0.001$	EC	O14; E
	$P = 0.003$	PO ₄ ³⁻ -P	N

Table 10: List of bacterial and archaeal phyla accounting for most difference in microbial community structure between treatments significantly ($P \leq 0.05$) different to the 50% district practice control identified by PERMANOVA analysis in Table 8. Treatment codes follow the same key as Table 6. Bold & italic phyla account for > first 30% of the difference, bold phyla account for the next 20% of the difference, with the remaining 20% in normal text.

Site	Treatment	Most different phyla (up to 30% , 50% , 70% dissimilarity)
Paskeville	O5	<i>Fir, Gem, Bac, Cre</i> , Cya, Pla, Nit, Act
	A1	<i>Bac, Aci, Cya, Gem</i> , Pro, Ver
Parkes	O5	<i>Bac, Aci, Cya, Pro</i> , Cre, Nit
	O6	<i>Bac, Aci, Act</i> , Cya, Gem, Pro
Mt Tyson	0	<i>Bac, Pla, Chl, Ver, Fir</i> , Act, Oth, Cya
	100	<i>Bac, Cya, Ver, Chl</i> , Cre, Fir, Pro
	150	<i>Fir, Act, Bac, Cya</i> , Arm, Pla, Ver
	200	<i>Cya, Bac</i> , Aci, Ver
	B1	<i>Bac, Fir, Cre, Ver</i> , Pro, Cya
	M1	<i>Fir, Pro, Aci</i> , Gem, Ver, Act
	A1	<i>Act, Pro, Bac, Cya, Chl</i> , Fir, Gem, Cre
	O13	<i>Pro, Fir, Cya, Bac</i> , Gem, Chl, Cre
	O14	<i>Fir, Bac, Chl, Pla, Arm</i> , Cya, Oth, Cre
	O15	<i>Bac, Pro, Ver, Act</i> , Aci, Nit

Pro = Proteobacteria, Act = Actinobacteria, Aci = Acidobacteria, Bac = Bacteroidetes, Fer = Firmicutes, Gem = Gemmatimonadetes, Ver = Verrucomicrobia, Chl = Chloroflexi, Pla = Planctomycetes, Cya = Cyanobacteria, Nit = Nitrospirae, Arm = Armatimonadetes, Cre = Crenarchaeota, Oth = others (accounting for < 5% of the total bacterial and archaeal phyla across the entire data set).

In summary, across two seasons and eight field sites, each receiving eight conventional and 14 biological input treatments, very few significant differences were observed in yield, grain quality, soil biogeochemistry or soil biology. Only one biological input, a poultry litter, delivered significant increases in yield, and only then after two years of application. With the exception of the Mt Tyson site, very few treatment effects were observed on the microbial community structure, and impacts on soil biogeochemistry were also sporadic.

Impact of biostimulants and humates on wheat capture of legume derived N

In order to probe mechanisms by which biological inputs may improve nutrient uptake from soil sources by wheat, a pot experiment was conducted in which ^{15}N -labelled legume litter was added to soil to simulate N input from a previous legume rotation. Nitrogen from the legume source was then traced into the wheat crop to ascertain N recovery. Ten biological inputs were used (five solid, five liquid), focusing on humates and biostimulants, which are product classes proposed to improve capture of N from the soil. In addition, two control treatments were also used, one in which litter was applied without any biostimulant or humate, and one in which no litter was applied.

Plant responses

Wheat (cv. Mace) was grown until the first pot reached anthesis to cap growth at the maximal point before which nutrient re-allocation to grain occurs during grain filling. No biological input had a significant ($P \leq 0.05$) impact on above-ground biomass production relative to the litter control, and all treatments including the litter control (with the exception of two liquid biostimulants, Liquids A and B) increased above-ground biomass (Figure 28). There were no significant impacts of any treatment or control on root biomass (Figure 28).

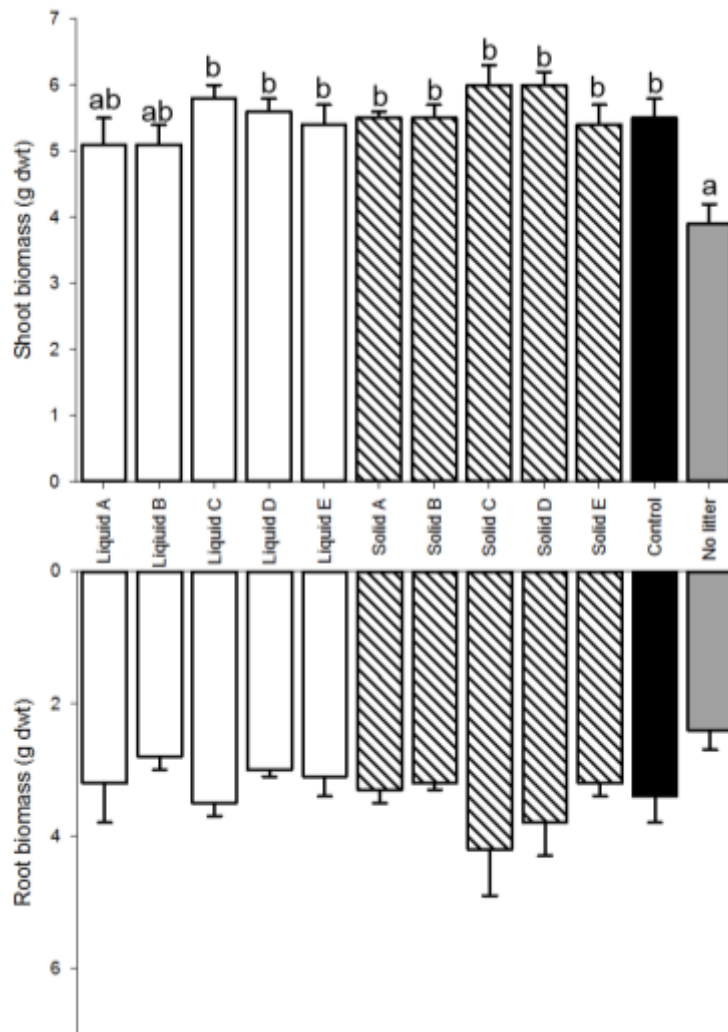


Figure 28: Above- and below- ground plant biomass. Values are means \pm SEM, different letters denote significant treatment effects at the $P \leq 0.05$ level. No significant differences were observed in root biomass.

Total plant N uptake was significantly ($P \leq 0.05$) increased by the presence of litter in all treatments with the exception of Liquids A and B (Figure 29). Importantly, N capture by the plant was significantly ($P \leq 0.05$) reduced in these two treatments relative to the litter control treatment. The implication of this is that these two biostimulants actually reduced the uptake relative to them not being applied in a situation where legume litter had been incorporated into the soil as a source of N. Using the ^{15}N label in the legume litter, we were able to probe whether any of the amendments improved wheat capture of this N resource, as such a finding would be advantageous for growers who incorporate legumes into their crop rotations. As with the total N uptake measurements, we found that the biostimulants Liquids A & B significantly ($P \leq 0.05$) reduced capture of legume derived N (Figure 30) relative to the litter control, clearly demonstrating that these two biological inputs, rather than enhancing N capture, actually reduced it.

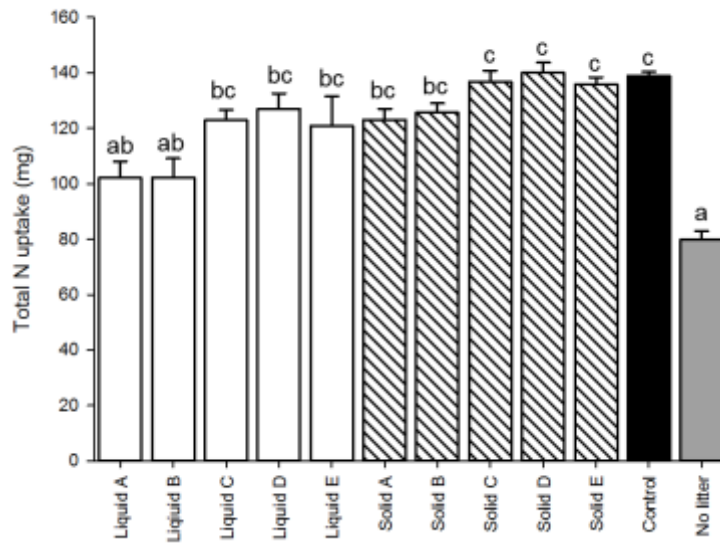


Figure 29: Total plant nitrogen uptake (roots and shoots). Values are means \pm SEM, different letters denote significant treatment effects at the $P \leq 0.05$ level.

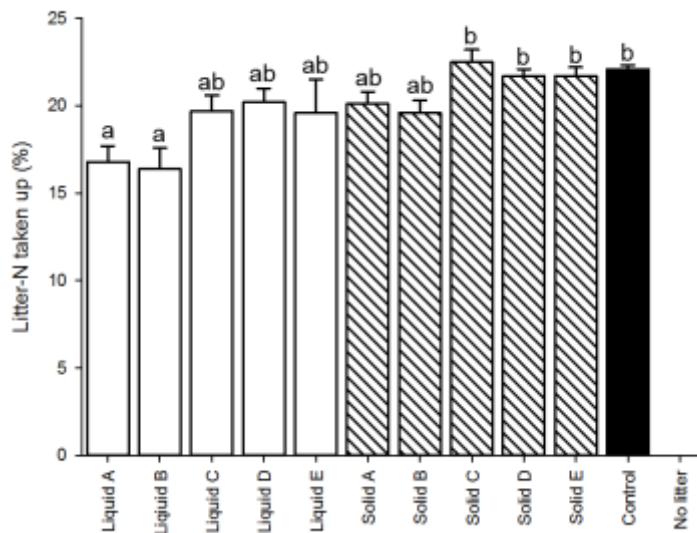


Figure 30: Plant capture of N derived from the labelled legume litter. Values are means \pm SEM, different letters denote significant treatment effects at the $P \leq 0.05$ level.

In addition to biomass and N content measurements, various above- and below- ground traits were quantified including tiller, green and senesced leaf numbers, root length, area, and average diameter, as well as apportionment of roots to several diameter classes. Above- and below- ground trait datasets were analysed separately by PERMANOVA to assess overall treatment effects. No significant effect ($P > 0.05$) was observed for the below-ground trait dataset (data not shown), but there were some significant treatment effects observed for the above-ground traits (Figure 31). All treatments that received litter (including the litter control) had a significant ($P \leq 0.05$) impact on above-ground traits relative to the no litter control. When comparing treatments receiving biostimulants or humates to the litter control, only Liquids A, B, and C had a significant impact, and in line with the biomass and N uptake data, this appeared to be negative relative to the litter control. The canonical analysis of principal components (CAP) ordination of these data indicates that these treatments resulted in above-ground traits more closely resembling those of the no litter control, with the litter and no litter controls primarily separating on the basis of tiller number and root and shoot mass (Figure 31).

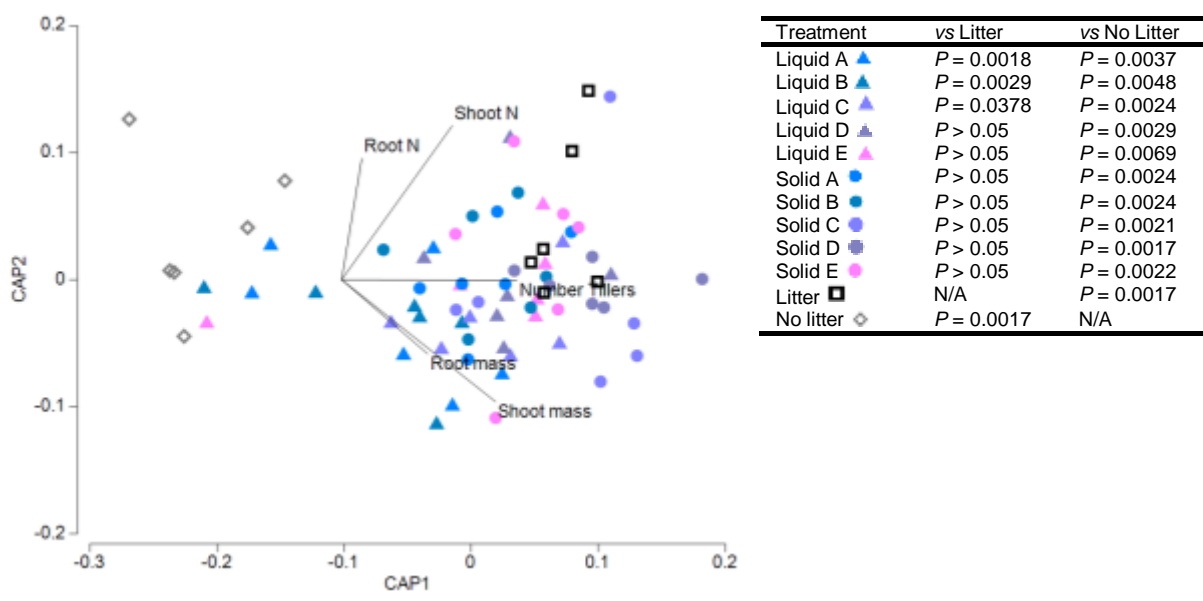


Figure 31: CAP ordination of above-ground plant trait data after significant ($P \leq 0.05$) PERMANOVA result for treatment. Vectors are Pearson correlations against the CAP scores to illustrate how the samples differed. Table lists pairwise results vs litter and no-litter controls for the biological input treatments.

Soil responses

Multiple analytes including total C/N, microbial biomass C/N, extractable N species, pH and EC were tested on soils collected at the time of harvest. No significant ($P \leq 0.05$) treatment effects were observed for the majority of analytes tested, with the exception of microbial biomass N and DON (Figure 32). Even here, no treatment effects were observed between the litter control and samples that had received a biological input, with only Solid B differing from the no litter control for MBN, and solid A differing from the no litter control for DON concentration.

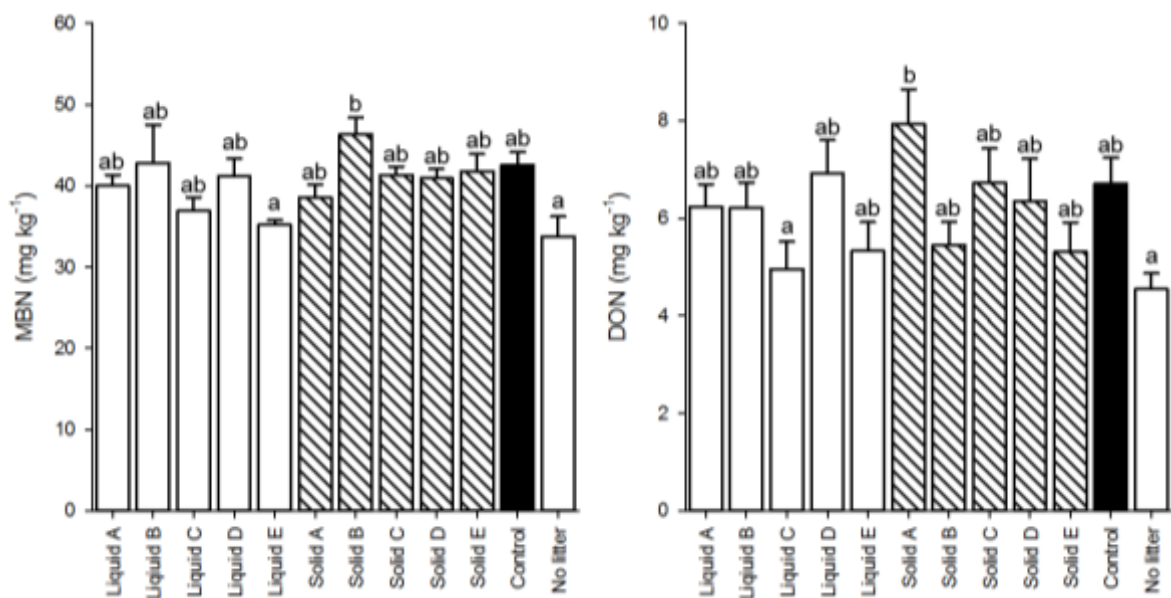


Figure 32: Microbial biomass nitrogen (L) and dissolved organic nitrogen (R) in soils at the end of the experiment. Values are means \pm SEM, different letters denote significant treatment effects at the $P \leq 0.05$ level. No other measured soil variables showed significant treatment effects.

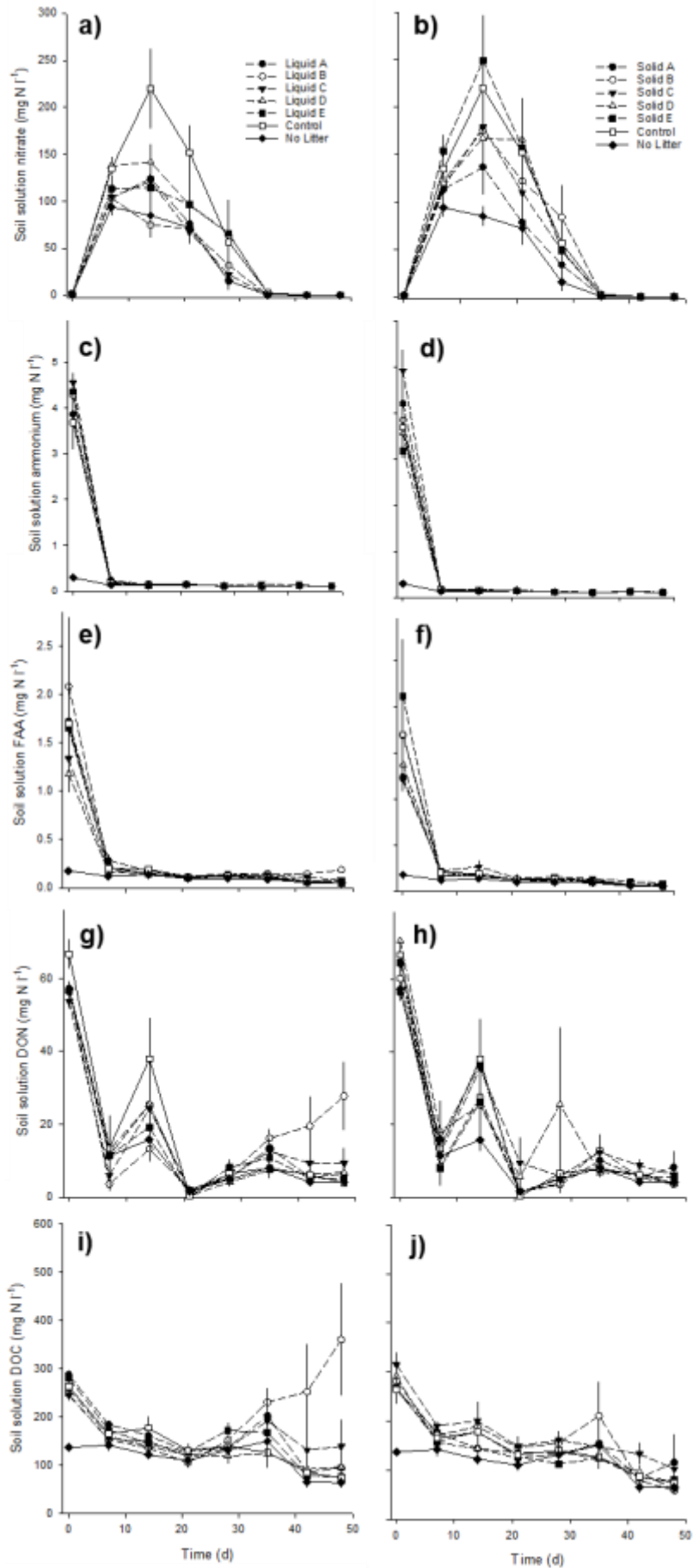


Figure 33: Soil solution chemistry measured over the 48 d growing period. Panels a, c, e, g, and i are data from the liquid amendments. Panels b, d, f, h, j are data from the solid amendments. Both groups of panels also show data from the litter and no-litter controls. Values are means \pm SEM.

The use of Rhizon samplers allowed for non-destructive probing of soil solution, in order for us to monitor changes in nutrient availability over the course of the experiment, and these data are presented in Figure 33. In all treatments, nitrate concentrations rose rapidly to a maximum concentration 1-2 weeks after establishment of the experiment before falling rapidly to baseline concentrations, reflecting rapid nitrification of available N by the soil microbial community before growth of the plants occurred at a rate capable of extracting N from the soil solution equivalent to the rate of nitrification. Ammonium and FAA-N concentrations reduced to near baseline concentrations across all treatments after one week except the no litter control, in which only low concentrations were observed even at time 0. It is likely that most of the ammonium and FAA-N observed at time 0 was sourced from the litter, given that the litter control tracked all the treatments receiving biological inputs.

Dissolved organic N and C concentrations were more variable over the course of the experiment. For the liquid biological inputs, all treatments tracked each other over time, with the exception of the no litter control for DOC, which started off at half the concentration of the treatments that received litter. For the liquid biological inputs, similar trends to the solid amendments were observed, except for liquid B (a biostimulant). Here, concentrations of both DOC and DON started to increase at time 42, and rose again by the end of the experiment at time 48, being significantly higher than both the litter and no litter controls (Figure 33g and i). Given that this treatment tracked the 11 others up until time 42, these observations are not likely to be as a result of a direct addition of DOC or N from the amendment itself. Rather, these observations indicate that this treatment altered either exudation of DOC/N from the plant roots, or microbial processes within the soil. However, as shown in Figure 32, no increase in MBN was observed, and in fact, plant N uptake both in total and from the litter was reduced.

In summary, despite the design of the experiment being tailored towards being able to observe even small differences in soil chemistry, plant growth, and plant N uptake, we found very little evidence to support our hypothesis that biostimulants and humates increase in N availability, uptake or growth of wheat.

Impact of biological inputs on soil microbial activity

In order to develop an understanding of how the chemistry of biological inputs relates to their impact upon biological processes involved in nutrient release and C turnover in soils, we conducted an incubation experiment using a selection of biological inputs that had been chemically characterised. In order to maximise the diversity of organic matter chemistry included in the experiment, we exploited a selection of biochars, manures and composts that also extended to previous projects investigating either biochars or composts and manures. Summary chemical details are provided in Table 6 and the diversity of these biological inputs is shown in Figure 34. The biochars and humates were typified by higher C:N ratios and greater aryl C contents, whilst the composts and manures separated primarily on the alkyl:o-alkyl gradient.

This wide diversity in chemistry was reflected in the range of microbial responses to their addition to soil as quantified by soil nutrient and respiration analyses. Total cumulative CO₂-C evolution ranged from 14.1 mg CO₂-C kg⁻¹ soil+amendment C for one of the biochars (treatment 8) through to 232.6 mg CO₂-C kg⁻¹ soil+amendment C for one manure (treatment 32), a range of one third to five times that of the control soils (Figure 35). However, whilst the range of responses across a set of amendments as varied as biochars all the way through to fresh manures may have been expected, it is important to also note variability within the four classes of amendment. Humates showed the least variability in terms of microbial respiration as a result of their addition to soil, with a range of cumulative CO₂-C evolution from 15.0 to 29.4 mg CO₂-C kg⁻¹ soil+amendment C. At the other end of the spectrum, there was over a six-fold range in cumulative CO₂-C evolution from the manure-treated soils, from 36.1 to 232.6 mg CO₂-C kg⁻¹ soil+amendment C. Thus, in terms of organic matter cycling and turnover, it is very clear that knowledge of more than just the type of amendment is required in order to predict whether organic C turnover is likely to be enhanced or reduced, and to what extent.

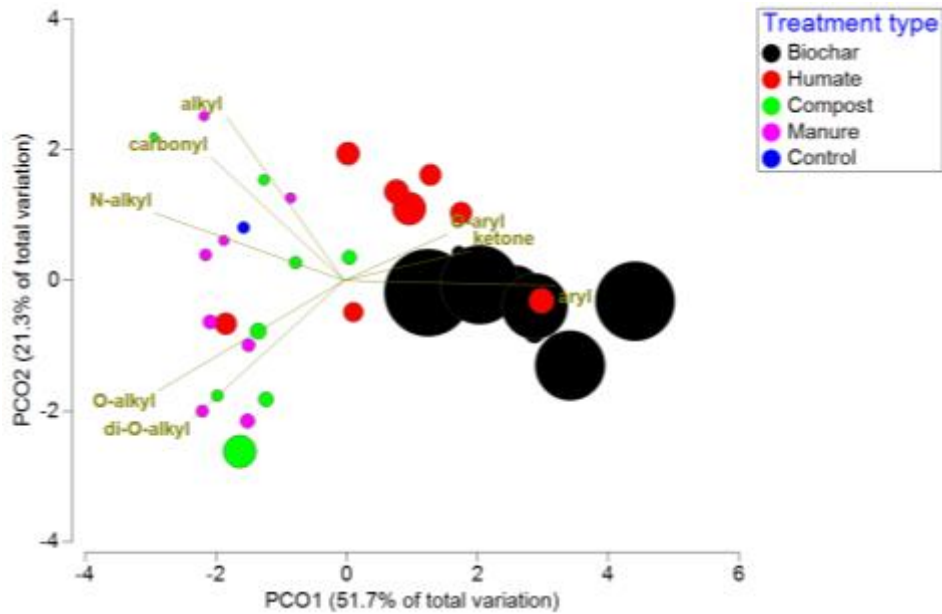


Figure 34: PCoA of organic matter chemistry of the 32 amendments and control soil as quantified by NMR. Bubble size relates to C:N ratio of the sample, with larger bubbles representing larger C:N ratios. Vectors show alignment of axes to chemical groups e.g. biochars (black) being strongly related to aryl (aromatic) C content.

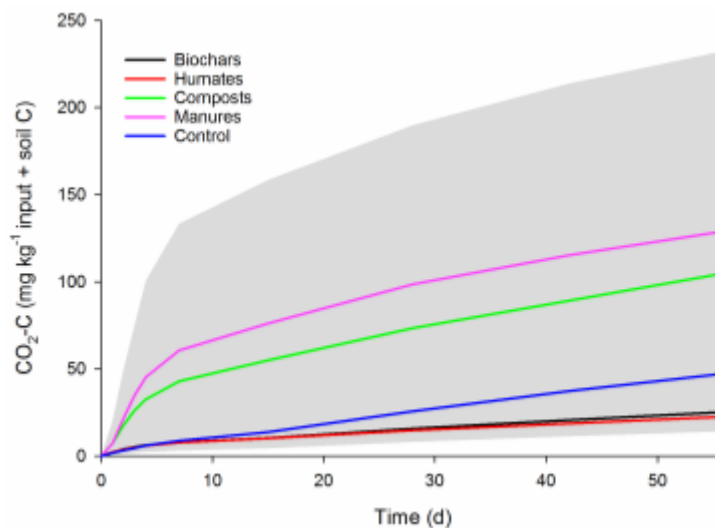


Figure 35: Cumulative respiration data from the incubation. Shaded area covers the range between minimum and maximum observed values across the entire data set. Lines are means ($n = 8$ for biological inputs, $n = 3$ for control soil).

Although $\text{CO}_2\text{-C}$ evolution is a useful metric of overall heterotrophic respiration in a soil, giving a strong indicator of overall microbial activity, in the context of biological inputs applied to grain growing soils, the impact of the amendment on nutrient availability and turnover is of particular relevance. Hence, we designed this experiment to allow for destructive sampling of soils over a short time frame in order to track how amendments affected the available nutrient balance in soil after application. Focus of the experiment was deliberately short (two months) in order to provide high resolution data on changes that occur shortly after application to soil, and which would occur during growth of the crop. Here, manures and composts on average yielded the highest values of available nutrients (Figure 36).

For ammonium, an initial spike up to $180 \text{ mg kg}^{-1} \text{ NH}_4^+\text{-N}$ was seen at day 1, followed by a rapid decline by day 28. It is of note that this highest treatment was actually a humate, rather than a compost or manure as might have been expected given the higher mean concentrations for these two types of amendment after application to the soil (Figure 36). However, this particular humate was

fortified with available N, having a soluble $\text{NH}_4^+\text{-N}$ concentration of over 3.5 g N kg^{-1} , and indeed as Figure 10 demonstrates, humates have a wide range of soluble N concentrations, particularly $\text{NH}_4^+\text{-N}$. Dissolved organic C also spiked at the start of the incubation, with one compost yielding nearly 5 g DOC kg^{-1} , though as with ammonium, concentrations generally decreased towards day 28 (Figure 36), indicating that this initial flush of soluble C was rapidly consumed and respired or stabilised by the soil microbial community.

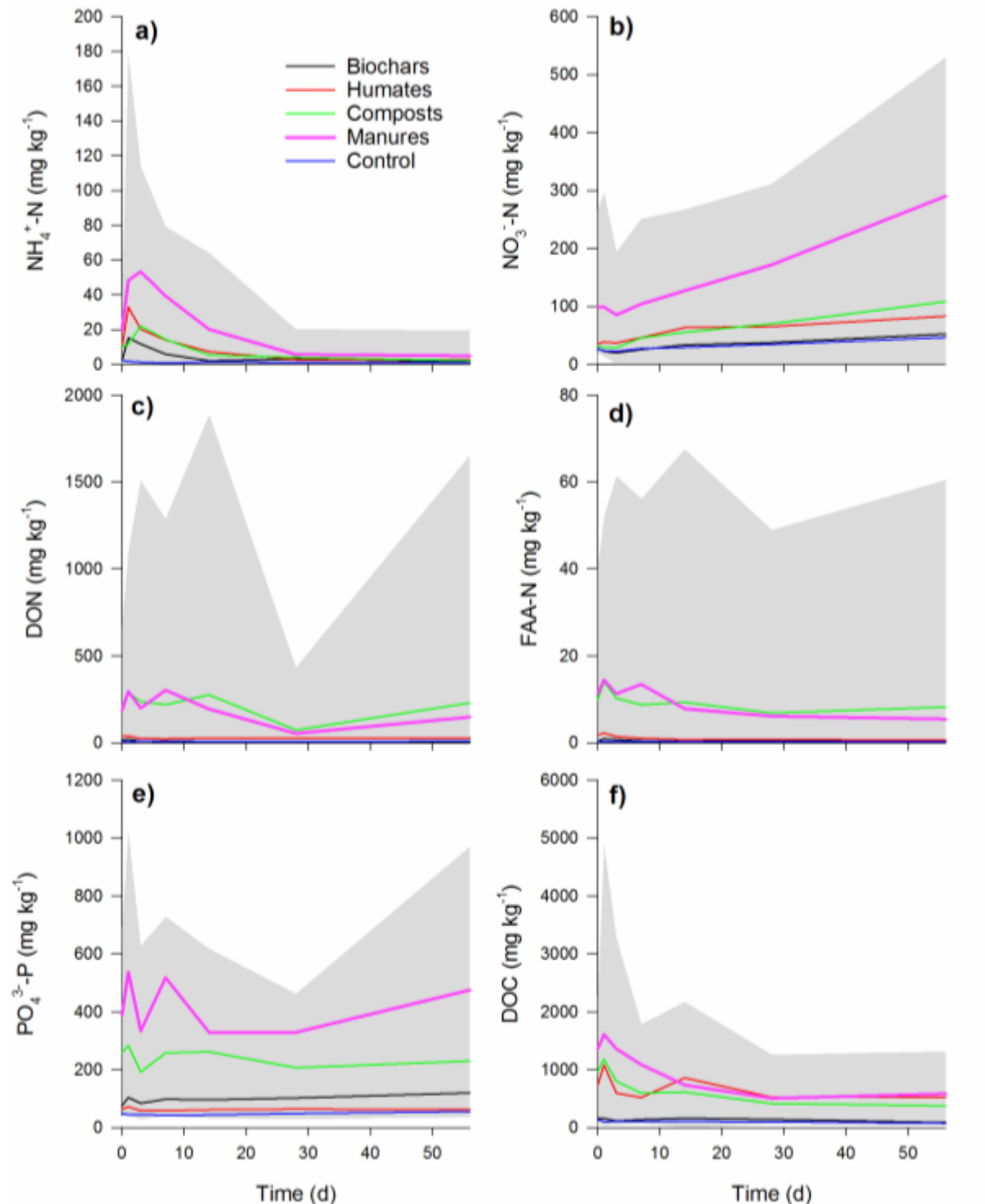


Figure 36: Available N, P and C measured at intervals on samples taken destructively over the course of the incubation. Shaded area covers the range between minimum and maximum observed values across the entire data set. Lines are means ($n = 8$ for biological inputs, $n = 3$ for control soil).

Bulk DON and FAA-N followed similar patterns over the course of the incubation, with the exception of a large dip in DON concentrations of one compost (treatment 17), which had a very high N concentration (4.97% N, over double the N concentration of the next most N-rich compost used in this experiment). This compost consistently yielded the highest DON concentrations throughout the experiment.

In contrast to the other forms of extractable and potentially available N, nitrate N concentrations typically rose over time for all amendment types, and the control soils also. This was expected in the absence of any plant uptake or leaching mechanisms as a result of microbial nitrification of ammonium and organic N. For both nitrate and phosphate, on average, the manures gave the highest concentrations and the greatest increases in available nitrate and phosphate over the course of the experiment. In contrast to the other analytes discussed here, available phosphate was elevated by biochar relative to the control soil by a factor of two as an average across the eight biochars tested in this experiment. However, as with the other amendment types and analytes, this was highly variable across the biochars, with the highest available phosphate concentrations at day 56 varying over a ten-fold range (48-548 mg PO₄³⁻-P kg⁻¹).

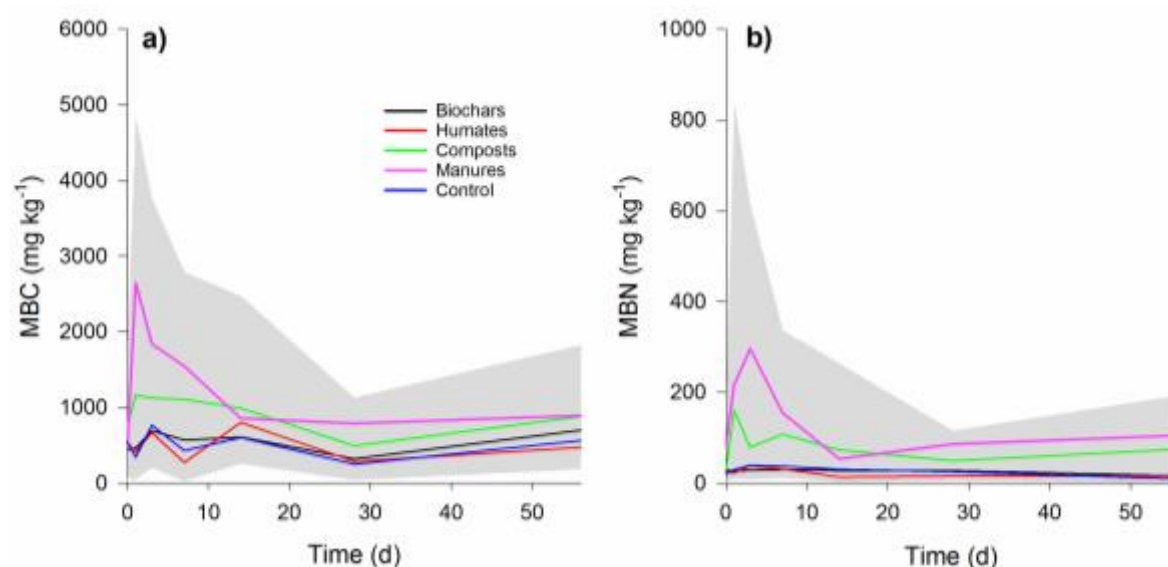


Figure 37: Microbial biomass C and N measured at intervals on samples taken destructively over the course of the incubation. Shaded area covers the range between minimum and maximum observed values across the entire data set. Lines are means ($n = 8$ for biological inputs, $n = 3$ for control soil).

Given that the changes in available C, N and P concentrations discussed in Figure 36 are microbially mediated, we also investigated changes in microbial C and N pools over the 56 days of the incubation (Figure 37). For both analytes, there was a spike particularly in the manure treatments between days one and three before a sharp reduction in MBC/N towards day 14-28. The average MBC/N values were more elevated in the manure sand composts relative to the biochars, humates, and control treatments, particularly for MBN by day 56.

In summary, the wide variety in chemistry of biological inputs observed in the “*Laboratory characterisation*” section was reflected after addition to soil. However, some general observations can be made whereby with the exception of humates for ammonium and DOC, greater concentrations of available nutrients and C were measured across the composts and manures, leading to greater microbial biomass values for both C and N. Despite higher DOC concentrations in the humate treated soils, no increases in microbial biomass C or respiration were observed, indicating limited stimulation of the microbial community.

Discussion of Results

Overview

Our review of the published literature (Abbott et al., 2017) highlighted the widely variable nature of biological inputs that are available, and a significant depth of research that has already been conducted in on many classes of input including biostimulants (Yakhin et al., 2017), humates (Rose et al., 2014), composts and manures (Quilty and Cattle, 2011), biochars (Macdonald et al., 2016), and microbial inocula (Pereg and McMillan, 2015). It also highlighted that in general, most significantly positive impacts either on plant growth or measured soil processes had been observed in laboratory or controlled glasshouse situations, with fewer significant impacts observed when products were tested in the field. Our own laboratory and glasshouse screening studies found that whilst there was considerable chemical variability both within and between biological input classes (Figure 8-Figure 15), when applied at either manufacturer or grower recommended rates, very little effect on wheat growth and yield was observed (Figure 16-Figure 21). This perhaps highlights a difficulty in characterising amendments that are applied at such disparate rates in the field.

Moving to the field, over two years of trials at eight sites in five states and covering all three GRDC grain growing regions, we again found very few positive impacts on grain yield (Figure 24). Further, soil fertility and biology assays revealed only sporadic impacts of biological inputs on the soil. Our two mechanistic experiments also found limited impact of biological inputs at the mechanistic level, with two biostimulants even reducing N capture by wheat (Figure 29), and only nutrient-rich organic amendments improving nutrient availability relative to the control in an incubation experiment (Figure 36). Taken together, these findings indicate that it is unlikely that biological inputs will have positive impacts at the broad scale for Australian grain growers, at least in the short term. Further, the huge variety of amendments available, makes specific recommendations difficult, though product classes do in some cases behave discretely from one another.

Approaches to on-farm testing

In compiling the practical guide to on farm testing (<http://soilquality.org.au/factsheets/biological-farming-inputs-a-practical-guide-to-on-farm-testing>), we presented a clear rationale for the first step in biological input choice to be the identification and understanding of the primary constraints to production. This is important as biological inputs which do not seek to address or moderate the main constraints affecting production are unlikely to have a positive impact. The practical guide provides a brief overview of common stresses impacting grains production within Australia. It then goes on to provide an overview of the classes of biological input available, and provides a means of cross checking modes of action with biological input classes.

Given the wide variety of amendments available to Australian grain growers, but the potential complexity that the testing of a wide number of amendments simultaneously brings, our guide strongly advocated that only one amendment should be examined per on-farm experiment. A brief overview of the potential for variable rate technology (VRT) to be employed in designing on-farm experiments, and the reader was directed towards the wealth of information available from past GRDC projects that aimed to make this technology more accessible for growers.

Acknowledging that VRT is not available to all growers, we focussed our guide on the use of simple strip experiments using on-farm seeders and headers. We placed particular emphasis on the use of replicated experiments where multiple strips of the new practice incorporating a biological input and business as usual (BAU) a control treatment, highlighting why replication is important to confirm whether an observed effect is likely down to the treatment or chance. We also provided a web-based t-test calculator in order to enable growers to examine results themselves: http://soilquality.org.au/calculators/t_test.

As we conclude in our scientific review (Abbott et al., 2017), in many regards, the task of ascertaining which individual biological inputs will work in specific situations is perhaps an almost insurmountable problem from a science project perspective. This is of particular relevance when the constant change of product availability and composition is considered, which would reduce the longevity of any 'definitive' results on the efficacy of product "x". Instead, low cost grower-led on-farm testing of products deemed suitable to potentially address local constraints to production possibly provides a means of more meaningfully examining the suitability of biological inputs in real on-farm scenarios.



Inventory and screening

We demonstrated huge variability both within and between classes of biological input, which perhaps brings into question the value in classifying biological inputs. However, whilst great variability in terms of individual chemical parameters exists, our integrative spectroscopic analyses using MIR (Figure 11) and NMR (Figure 12) demonstrated some clustering overall by amendment type. Further, our deep review of the available literature also revealed commonalities within and differences between product classes in terms of both their expected mode of action and their likely residence times (Figure 2).

Comparison both within and between product classes on the basis of chemistry is further complicated by the wide range of application rates recommended. Some biostimulants are applied at rates as low as $250 \text{ mL}^{-1} \text{ ha}^{-1}$, whereas organic amendments may be applied at rates in the range of $1\text{-}20 \text{ t ha}^{-1}$. In order to at least normalise rates for the biological inputs perceived to be most similar, in keeping with advice from the several grower groups who assisted in this project, we set an application rate of 3 t ha^{-1} for all organic amendments, though even then as this was on a fresh weight basis, and on products ranging in OC concentrations from 7.6% to 75%, this still represents a significant spread of application rates, and this was equally reflected in N (0.4% to 6.9%) and P (0.3 mg g^{-1} to 38 mg g^{-1}) concentrations.

Despite this wide range in chemical properties our controlled screening of biological inputs under moderate drought conditions provided very little evidence that wheat growth or yield was affected. For three organic amendments, we did see a reduction in water loss from the pot relative to conventional fertiliser treatments, but very little in the way of grain yield response was observed. Further, despite the moderate drought stress this experiment was run under, no impacts of biological inputs were observed on plant growth or health.

Responses in the field

By incorporating a conventional fertiliser response curve at each experimental site, we were able to see not only if the site was responsive to conventional fertiliser, but also identify whether any potential increases in yield observed in the biological input treatments bettered what would have been achieved with increased conventional fertiliser application. Though response to fertiliser expressed as percentage WLYP was relatively low in the first year, this was a relatively dry season across most of the sites. In the second and much wetter year, several of the sites were much more responsive to increases in conventional treatments (Figure 23). We normalised our yield data to predicted WLYP given that in dry-land systems this is recognised as often being the main constraint to crop productivity (van Ittersum et al., 2013). Especially for Buntine, Jamestown, Paskeville and Langhorne Creek, increases in traditional fertiliser facilitated increases towards or even slightly above simulated maximum water limited yields, though less impact was seen at the other four sites.

Across the two years and 38 biological inputs investigated, the fact that we only observed one biological input significantly increasing yield (albeit at two sites) highlights that at least for these sites, the majority of the time, biological inputs did not mitigate limitations to growth. Further, only at one site (Parkes) was grain quality significantly impacted, and here differences between individual biological input treatments, rather than between a biological input and the control were observed.

Some impacts of biological inputs on soil chemistry and also soil biology were observed, with soil microbial communities impacted by all treatments at the Mt Tyson site, which interestingly had been the least responsive across the two years in terms of grain yield. This site is a classic Darling Downs vertisol with high clay content (Figure 22), so might have been expected to be less impacted than a sandy soil such as that found at Buntine or Langhorne Creek. This might explain why there was a higher abundance of the ammonia-oxidizing archaea (AOA) *Candidatus Nitrososphaera* (Hatzenpichler, 2012), a member of the nitrite-oxidizing *Nitrospira* (Pester et al., 2014) in the Mt Tyson site that catalyses the first and second step of the nitrification pathway, respectively. These findings are consistent with a previous study that found in general AOA were more abundant in the younger, clayey, more fertile soils of the northern grain regions (Jenkins et al., 2016), while AOB tended to dominate in the highly weathered, ancient and acidic soils of Western Australian (Banning et al., 2015; Jenkins et al., 2016). Since, these taxa play a major role in nitrogen cycling and increasing the availability of nitrates to plants it is interesting to see that there were no significant gains in yield at the Mt Tyson site.

Our results also suggest that some bacterial phyla are significantly influenced by water availability since there was a marked increase in the relative abundance of Firmicutes and fewer Acidobacteria, Chloroflexi and Verrucomicrobia in the semi-arid tenosol soils of Buntine and Langhorne Creek. As mentioned earlier Firmicutes' ability to form spores, makes them more tolerant to desiccation and harsher environmental conditions (Taketani et al., 2017). As copiotrophic r-strategists they have evolved survival strategies such as high growth rates and metabolic versatility to compete for the limited resources (particularly labile carbon) typically found in a less structured soils (Fierer et al., 2007). In contrast, Acidobacteria, Chloroflexi and Verrucomicrobia are described as oligotrophic with their lower growth rates and a preference for growing on relatively recalcitrant forms of C (Fierer et al., 2007; Trivedi et al., 2013).

In conclusion, it is clear that across a wide range of biological inputs, soil types and rainfall seasons, we found limited impacts of biological inputs on grain yield or quality, soil biogeochemistry, or for the most part, soil microbial community structure. The main caveat to these findings is that in carrying out experiments over only two years, longer-term impacts have not been investigated. However, returning the discussion to the timeframe over which different classes of biostimulant are likely active for (e.g. Figure 2), with the exception of humates and organic amendments, most biological inputs would only likely be active for the growth season in which they are applied. Thus, it is questionable whether in those cases longer-term trials would reveal significant impacts.

Mechanistic probing

In addition to the soil chemical and microbial investigations conducted as part of the field experiments, two experiments were carried out in order to probe specific impacts of biological inputs on soil function and nutrient release.

As one of the proposed mechanisms by which biostimulants and humates improve plant productivity is through improving nutrient capture and uptake by the target plant (Yakhin et al., 2017), we examined whether biostimulants or humates improved capture of N by wheat when grown under controlled conditions. Further, we used ¹⁵N-labelled legume residue to test whether the amendments specifically improved the capture of N in a scenario where a legume had been grown in the previous rotation. Whilst there is much discussion about how yield gaps can be closed by improving nutrient use efficiency (van Ittersum et al., 2013), in particular noting apparent opportunities given a global mean of ca. 50% nitrogen use efficiency from fertiliser (Lassaletta et al., 2014), it is important to understand that increases in uptake of nutrients from the soil without replacement risks longer-term soil degradation. This is pertinent given growing recognition of elemental stoichiometry in SOM (Kirkby et al., 2011), and thus if nutrients are being removed from the soil without replacement, loss of SOM is likely. However, in a situation where a grower has included a legume within a rotation sequence, it is clearly advantageous for the following crop to be able to extract as much of that fixed N as possible in order to maximise returns on the investment into the legume (Thönnissen et al., 2000).

Here, we demonstrated unequivocally that when provided ideal conditions for plant growth and microbial activity, N capture by wheat, either from the bulk soil or from a legume litter source was not positively impacted by biostimulants or humates. Indeed, in two cases, the opposite was actually observed, and N capture was reduced by two of the biostimulants tested. Further, relative to the control, no positive impacts on growth were observed, and impacts on above-ground phenology resulted in plants that were intermediate between the control that received litter but no biostimulants / humates, and the no-litter control that had much reduced N availability. Previous studies have highlighted that humates may increase plant NO₃⁻ uptake by increasing root membrane proton pumping (Pinton et al., 1999), and humic substances extracted from composts have been found to both increase and decrease nutrient uptake dependent upon dose rate (Ayuso et al., 1996; Keeling et al., 2003). Biostimulants have also been shown to alter N mineralisation and nitrification (Chen et al., 2003), though in the present study the only significant change in soil solution N availability was the DON pool in one of the biostimulant treatments that reduced wheat N uptake. Despite the conclusions of a recent review into the overall performance of biostimulants in agriculture (Calvo et al., 2014), we found little to support these findings in our study of biostimulants and humates available to Australian grain growers, despite the utilisation of an experimental design that would promote favourable responses if they were present.

To further probe soil microbial responses and to relate biological input chemistry to nutrient release, we conducted an incubation experiment using well characterised biological inputs from a range of



manures, composts, humates and biochars. The dynamics of soluble C, N and P pools were quantified over 56 days, along with microbial biomass and heterotrophic respiration. Though humates increased DOC concentrations, this had no impact on microbial respiration or growth. Though often considered as available C, the bulk of DOC whilst soluble is often found to be resistant to microbial activity (Qualls and Haines, 1992), and Kim Thi Tran et al. (2015) found negligible effects of lignite (from which many humates available within Australia are manufactured (Little et al., 2014; Rose et al., 2014)) on microbial activity or community structure in the short term. Similarly, only limited impacts on N availability were observed (Kim Thi Tran et al., 2015).

In contrast, the more nutrient-rich composts and humates stimulated both microbial activity and the release of N, ultimately in the form of nitrate as available N was nitrified over the 56 days of the incubation. We speculate that this N and P is from the amendments themselves, rather than any stimulatory effect on the release of nutrients from the soil.

Conclusion

This project has taken several approaches to investigating the suitability of a wide range of biological inputs for use in broad acre grain growing within Australia. Our in depth review of published literature and data found that whilst there are certainly positive results observed in scientifically rigorous studies, these can often be very context specific. Secondly, many of the positive results reported either came from laboratory or glasshouse experiments, and usually on higher value (and thus higher input) crops such as vegetables and fruit. We found very limited reporting of consistent results of any class of biological input in the dry-land broad acre context.

Our database of over 60 biological inputs allowed us to chemically characterise a number of amendments available and marketed to Australian grain growers. We found substantial variability both within and between the various product classes. The within-class variation makes broad recommendations difficult. We also found during the course of the project that several products changed, and different products became available. This constantly changing inventory of products, coupled with the variability in chemistry observed indicates that concentrating on properties of individual amendments is probably not a feasible mechanism of assessing suitability of biological inputs. Another complication in interpreting the characteristics of biological inputs is their widely differing rates of application (from $<1 \text{ L ha}^{-1}$ to several t ha^{-1}).

Despite the large variability observed across our inventory of biological inputs, we observed very few effects on wheat growth when >50 biological amendments were compared at their field application rates in a controlled glasshouse experiment. Further, from the 36 amendments investigated in the field across eight sites and two seasons, only one biological input significantly increased wheat yield relative to the conventional fertiliser control. Some more subtle impacts on soil fertility and microbial community structure were observed at three of the sites, but this was generally sporadic. It is also worth noting that as these experiments occurred in contrasting rainfall seasons across most of the sites. The only positive impacts on yield occurred in the second wetter season, and water stress was in general less limiting, but as the biological inputs were re-applied on the same plots in the second year, it is possible that these positive responses occurred as a result of a cumulative effect. However, given the lack of an overall increase in the effect of biological inputs in the second year, it appears that neither climate nor cumulative effects could explain the large increase in yield observed from the one poultry litter treatment that did increase grain yield at two sites.

Within the context of their designs, the two mechanistic experiments indicated that biostimulants and humates did not improve uptake of N by wheat, nor did organic amendments appear to improve N and P availability beyond the release of a portion of the nutrients they contained over the course of an incubation.

When interpreting the results from this work, it is important to consider that the experiments were designed around a minimal intervention strategy in terms of changes to farm practice. Whilst we reduced conventional fertiliser inputs applied in tandem with the biological inputs, we also compared the performance of the treatments against this reduced fertiliser dose, rather than against 100% district practice BAU. So, whilst we feel that the data from the field experiments in particular are a fair test of biological input performance across a wide range of climatic conditions and soil types found within the dry-land cropping zones of Australia, it is possible that different results may have been observed if larger changes to farming practice had taken place, but such research fell outside the scope of the present project.

Given the ever-changing variety of biological inputs available, science-driven projects such as this, as valuable as it has been to provide a snapshot, may not be the most cost effective tool to deliver field relevant information to growers. Rather, on-farm grower-led testing of biological inputs may be a more appropriate way to examine different products. These trials should take on board recommendations within the practical guide to on-farm testing that was produced as part of this project. However, it would also be important to ensure that a centralised database of information (with appropriate metadata including crop rotation, rainfall, soil type, etc.) was maintained. This would also facilitate the opportunity for investigations into the impacts of amendments on soil fertility and health once it has become apparent from crop performance data which amendments appear most promising. Given that even biological inputs applied at high rates such as composts and manures have been shown on the whole to have little impact over two years of field application, emphasis should be placed on longer-term investigations that not only allow for repeated applications, but would also cover differing rainfall years.



Implications

- The literature reviewed in this project highlights that in some circumstances a number of biological inputs may increase resilience or productivity of cropping systems.
- However, much of this research was conducted in higher-value horticulture and market gardening systems.
- Our substantial characterisation effort indicates that it is difficult to provide a “one size fits all” recommendation on classes of amendments to growers.
- We strongly encourage growers to consider their specific constraints on production, and base their preliminary choice of product based upon that likely to provide some way of alleviating that constraint.
- Having made a choice, it is also important that growers conduct a simple but rigorous test of the product on their property. The practical guide and calculators produced in this project can assist in both design and interpretation.
- Across the lab, glasshouse, and field, results from this project indicate that likelihood of increased yield and short term improvements to soil fertility may be low, further emphasising the need to test products first.
- It may be possible that more significant results could have been observed with longer term trials, though we conducted our experiments across two very different rainfall years at six of the eight sites. In the case of organic amendments, in much large ($>1 \text{ t ha}^{-1}$) applications are delivered to the soil, longer term positive outcomes may be more likely than more ephemeral amendments (see Figure 2).



Recommendations

The strongest recommendation we can make after conducting this project is that growers considering the use of biological inputs should be clear about the constraints they face at the local level to increased production, and consider their choice of product based upon a realistic likelihood of it mitigating that constrain in some way. For example, in a neutral or alkaline soil, a product that as one of its modes of action mitigates soil acidity is unlikely to have an impact. Growers should then seek to obtain results from a scenario similar to their own, or conduct on-farm testing in a manner similar to that proposed in the practical guide.

Further research in this field needs to take into consideration the following factors:

- Broad acre dry-land grains production often relies on relatively low per-hectare profits delivered across large areas. As a consequence, economic factors that may make the use of some biological inputs suitable for intensive horticultural systems may not apply in a broad acre scenario. Research should be targeted at amendments that will be economic to apply across large areas of land.
- Given the ever changing variety of products available on the Australian and international markets, intensive scientific research on specific products may not necessarily yield the best outcomes for the industry.
- We recommend that on-farm participatory grower-led experiments are the most appropriate pathway to further develop our understanding of the efficacy of biological inputs in the broad acre dry-land grains context. Provided that these are appropriately designed, a centralised database could be established to collate results and enable any positive trends to be identified. Such a database would require scientific curation, with a set of experimental protocols established to ensure quality and cross-comparability of data. This would also provide a resource of known sites to target soil biogeochemical and fertility research to underpin positive yield results with an understanding of the mechanism behind the crop performance.



Glossary and Acronyms

Alt. Fert.	Alternative fertiliser
ANOVA	Analysis of variance
AOA	Ammonia oxidising archaea
AOB	Ammonia oxidising bacteria
BAU	Business as usual
C	Carbon
CAP	Canonical analysis of principal coordinates
CO ₂	Carbon dioxide
DAP	Di-ammonium phosphate
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DON	Dissolved organic N
dwt	Dry weight
EC	Electrical conductivity
FAA	Free amino acids
fw	Fresh weight
GRDC	Australian Grains Research and Development Corporation
MIR	Mid infra-red
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
N	Nitrogen
NMR	Nuclear magnetic resonance
NO ₃ ⁻	Nitrate
NH ₄ ⁺	Ammonium
nMDS	Non-metric multidimensional scaling
OC	Organic carbon
P	Phosphorus
PA	Precision agriculture
PCA	Principal components analysis
PCoA	Principal co-ordinates analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational analysis of variance
PO ₄ ³⁻	Phosphate
rRNA	Ribosomal ribonucleic acid
SARDI	South Australian Research and Development Institute
SCaRP	Soil Carbon Research Programme
SEM	Standard error of the mean
TDN	Total dissolved nitrogen
TIC	Total inorganic carbon
TN	Total nitrogen
TOC	Total organic carbon
VRT	Variable rate technology



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