IMPROVED EFFICIENCY OF AGRO-CHEMICAL FERTILISERS AS A CONSEQUENCE OF ENHANCED SOIL TREATMENTS:

Investigating the Impact of Converte Bio-Fertiliser and Seed Primer on Soil and Plant Health

This report was part of a Building Partnerships JobsforNSW grant study awarded to Converte Pty Ltd. in partnership with the Western Sydney University and Corkhills Ag based in Boorowa NSW.

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EXECUTIVE SUMMARY

Consumer demands and environmental concerns are driving the rapid increase in the growth and use of bio-fertilisers, alone or in combination with traditional agrochemicals. However, to further increase the uptake and use of bio-fertilisers by growers supporting scientific evidence will be required, including the mechanisms by which particular brands of bio-fertilisers can increase farm productivity and soil health. The aim of this project was to evaluate the impact of Converte's universal natural plantfood (UNP) and seed primer on soil biological health at two different sites in NSW. We examined the impact of bio-fertilisers on soil biological activities (respiration, potential enzyme activities associated with nutrient cycling) and assessed microbial diversity and composition using next-generation sequencing and quantitative polymerase chain reaction (qPCR).

Our analysis demonstrated that microbial activity (as assessed by respiration) was stimulated under bio-fertiliser treatment in both the wheat and pasture systems studied here. This was supported by increases in either, or both bacterial and fungal abundance at each site. Positive impact of bio-fertiliser treatment on the fungal community was particularly strong at the arable site, Boorowa.

Although potential enzyme activities were variable, there was the general observation that activity of enzymes involved in N and P mobilisation were consistently higher in treated plots compared to control plots. While there was no evidence that this translated to improved nutrient content of the crop (grain) at Boorowa, grain yield was consistently higher under treated plots compared to control and visual evidence for a healthier sward under treatment at the pasture site was strong.

Although the alpha diversity (the species pool) largely remained unchanged (with the exception of bacterial species in the 10-20cm soils from Laggan), a clear shift in microbial community structure was observed at both sites under the bio-fertiliser treatments. Generally, universal natural plantfood (whether alone or in combination with seed primer) saw greater changes in the composition of the microbial (bacterial and fungal) community from control soils than seed primer under wheat. While the composition of the microbial community changed with depth, bio-fertiliser treatment effects on the microbial community composition were still seen in deeper soils,

It was evident that bio-fertiliser treatment had a positive impact on microbial activity and abundance and on the crop performance measurements made here. Because both farms also received a high level of synthetic fertiliser and chemical input, crop response to bio-fertiliser was likely to be curtailed somewhat as a consequence. Nevertheless, clear overall positive impacts on several soil health attributes were evident. More data and analyses can support if the observed changes in activity and communities can further explain the mechanisms of Converte products on other soil health attributes and farm productivity. On-going analysis of this data set and further partnerships with experiments established during this collaboration will provide detailed mechanism which promote soil biological health of the farm.

SUMMARY OF FINDINGS

- UNP additions stimulated basal respiration (considered a good predictor of overall biological activity in the soil) by 38% and 41% in Boorowa (wheat) and Laggan (pasture) respectively.
- There was some evidence that UNP and CSP altered the catabolic abilities of microbial communities in deeper soils (10-20cm) at Boorowa (wheat), but treatment had limited impact on the catabolic profiles in the upper (0-10cm) soil layer.
- The response of potential enzymes activities were variable, but typically stimulated under UNP+CSP additions in Boorowa (wheat) and under UNP additions at Laggan (pasture). This was particularly notable for enzymes associated with N and P cycling, suggesting increased N and P mineralisation under these treatments at the respective sites.
- UNP additions increased bacterial gene abundance (as a proxy for bacterial biomass) by 36% and 138% in Boorowa (wheat) and Laggan (pasture) respectively. At Boorowa, UNP+CSP increased bacterial gene copies by 55% providing strong evidence that UNP treatment stimulated bacterial growth in these soils.
- Fungal gene abundance (as a proxy for fungal biomass) was particularly responsive to the bio-fertiliser treatment at Boorowa (wheat) and observed in both the 0-10 cm and 10-20cm soils. There was no evidence that UNP affected fungal abundance at Laggan (pasture).
- There was no effect of bio-fertiliser treatment on the diversity of bacterial or fungal communities but each treatment did significantly alter the composition of bacterial and fungal communities at both Boorowa (wheat) and Laggan (pasture) and treatment effects were evidence at both 0-10 cm and 10-20 cm. On-going analysis will provide taxonomic resolution to these treatment effects.
- UNP significantly increased soil C and N by about 50% at Laggan (pasture). Visual observations of the field showed a greener, denser sward in UNP treated soil (Figure 2) and this likely drove higher plant-derived C-inputs into the soil and stimulated microbial (particularly fungal) growth as evidenced by higher gene copy numbers in these soils.
- Bio-fertiliser treatment increased total grain weight by 20-58% on collected samples at Boorowa. This was statistically significant in CSP treatment only.

Contents

ABREVIATIONS

16S rRNA – 16S subunit ribosomal ribonucleic acid

AG - α-glucosidase

ANOSIM – analysis of similarity

ANOVA – analysis of variance

BG - β-glucosidase

C – carbon

Ca – calcium

CB - cellobiohydrolase

CSP – Converte seed primer

Cu – copper

DNA – deoxyribonucleic acid

Fe – iron

H' – Shannon diversity index

K – potassium

LAP - leucine aminopeptidase

MAP - mono-ammonium phosphate

Mg – magnesium

Mn – manganese

N – nitrogen

NAG - N-acetyl-β-D-glucosaminidase

NGS – next generation sequencing

P – phosphorus

PCA – principal components analysis

PERMANOVA – permutational multivaraite analysis of variance

PHOS – phosphatase

qPCR – quantitative polymerase chain reaction

RDA – redundancy analysis

S – sulphur

Si – silicon

UNP – Converte universal natural plantfood

WHC – water holding capacity

XYL - β-Xylosidase

Zn – zinc

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1. BACKGROUND

With an increasing global demand for food production there is a need to increase agricultural productivity in existing agricultural lands. Under conventional farm management practices, productivity growth in Australian agricultural systems has not been fully realised, in part due to loss in soil biology/health and the effects of climate change on plant growth.

Improving and maintaining soil health is an essential part of increasing agricultural productivity as a healthy soil promotes and sustains functions directly linked to farm productivity (e.g. cycling and retention of nutrients, water storage and disease suppression). Soil health can be impaired or improved with management and have long-term consequences for productivity and profitability. Soil microbial communities are a key component to soil health because they provide key services including nutrient cycling, soil formation and primary production. It has also been increasingly recognised that soil microbial communities play a key role in disease suppression and resilience to climate change. Despite an increased awareness that microbial diversity plays a significant role in soil health and productivity, our understanding of the relationship between soil microbial diversity and soil health is poorly understood.

There is increasing evidence that Bio-fertilisers can enhance crop production across a wide variety of cropping systems. Some bio-fertilisers contain particular micro-organisms known to promote plant growth, while others contain a complex mix of organic and mineral components designed to stimulate the activity of indigenous soil biota. Converte's Universal Natural Plantfood (UNP) is a bio-fertiliser that aims to feed soil microbes and promote soil biological formation, increase macro and micronutrient pools and transfer to plants, and, improve resistance to weather extremes[1]. Such bio-fertilisers have the potential to improve soil health by stimulating the activity of resident microbial communities which could lead to enhanced nutrient cycling and improved physical health of the soil through increases in bacterial and fungal biomass that are important for aggregate formation and water retention. However, our understanding of the mechanisms by which bio-fertilisers alone, or in combination with traditional fertilisers, affects microbial diversity, improves soil health and leads to increased crop productivity, is limited and there is a need for further scientific evidence to support the increased uptake of bio-fertilisers as a sustainable farming approach that leads to increased farm productivity.

2. AIM

The aim of this project was to evaluate the impact of Converte's universal natural plantfood (UNP) and seed primer on soil biological health at two different sites in NSW: a controlled trial plot at Boroowa (wheat crop in 2017) and a working farm at Laggan near Crookwell (under pasture). We examined the impact of bio-fertilisers on soil biological function by measuring basal respiration (a broad measure of microbial activity), substrate-induced respiration (a measure of the catabolic capability of soil microbial communities), the activities of key enzymes involved in carbon, nitrogen and phosphorus cycling (a measure of the potential for nutrient cycling/release, linked to nutrient availability and hence crop performance). Soil biological diversity and community composition were measured using qPCR and cutting-edge next-generation sequencing technology to further understand the relationship between microbial diversity, soil health and farm management.

3. RESEARCH METHODOLOGY

3.1. Field sites

Extensive plot history background was provided in the Milestone 1 report, 30th January 2018[2]. Soils were sampled from Boorowa and Laggan field sites using a 5 cm diameter soil auger. All soils were transported on ice back to the laboratory, sieved (< 2mm) and subsampled for soil physical, chemical and biological analysis. Soils were either air-dried (40˚C until constant weight) for physical and chemical analysis, stored at 4˚C for biological activity, -20˚C for potential enzyme activities or at -80˚C for molecular analysis.

3.1.1. Boorowa

At Boorowa, EGA Kittyhawk wheat was sown across the site at 75 kg ha^{-1} on 01/05/2017. The whole site was fertilised with mono-ammonium phosphate (MAP, 110 kg ha^{-1}) and received later two additions of urea (120 kg ha^{-1} on 28/7/17 and 23/8/17). The paddock was split into 9 plots (Figure 1), but for the purposes of this work we concentrated on Control (T4), Converte seed primer (CSP, T5), Converte Universal natural plantfood (UNP, T7) and Converte UNP + Converte seed primer (T8). Seed primer was added to seeds prior to sowing at 5L/tonne. Converte UNP was applied at 2.5L ha^{-1} on 23/6/17. Total annual rainfall in 2017 was 574 mm, which was slightly below the annual average for the site (609 mm) based on available records (Station number 070220) for the last 120 years[3] (Appendix I).

As reported in Milestone 2 report (May 2018)[4], soils were sampled in December 2017 from the field in Boorowa, NSW on a farming system utilising Converte's soil treatment products, Universal natural plantfood (UNP) and seed primer (CSP; Figure 1). Five replicate soil samples per treatment area were collected at randomly selected locations. Soil cores were taken from 2 depths, 0-10cm and 10-20cm from control, Converte seed primer (CSP), Converte universal, universal natural plantfood (UNP) and UNP+CSP treatments.

Plot T1 (0.66 hectares) Awaken @ 2.3L/ha (2017)	**Plot T2 (0.66 hectares)** Awaken @ 2.3L/ha (2017) + Converte seed primer @ 5L/tonne (2017)	**Plot T3 (0.66 hectares)** Awaken @ 2.3L/ha (2017) + Awaken ST (2017)
Plot T4 (0.66 hectares) Untreated control (2016 & 2017)	**Plot T5 (0.66 hectares)** Converte seed primer @ 5L/tonne (2017)	**Plot T6 (0.66 hectares)** Awaken ST (2017)
Plot T7 (1.33 hectares) Converte UNP @ 2.5L/ha (2016 & 2017)	**Plot T8 (1.33 hectares)** Converte UNP @ 2.5L/ha (2016 & 2017) + Converte seed primer @ 5L/tonne (2017)	**Plot T9(1.33 hectares)** Converte UNP @ 2.5L/ha (2016 & 2017) + Awaken ST (2017)

Figure 1. Arrangement of apportioned foliar treatment and seed primer treatment zones within the trial paddocks at Boorowa. There are a total of nine different treatments with the trial block, but for the purposes of this work we concentrated on Control (T4), Converte seed primer (T5), Converte UNP (T7) and Converte UNP + Converte seed primer (T8).

3.1.2. Laggan

Soils were collected from a farm growing a pasture crop in Laggan, NSW. Soils were sampled from two treatments (Figure 2); an untreated control and a plot where Converte universal natural plantfood (UNP) @ 2.5 L/ha (applied in 2017) was applied in a single application. Both the untreated control and Converte plots were fertilised with the same rate of super phosphate fertiliser. Five replicate soil samples per treatment area were collected at randomly selected locations. Soil cores were taken from 2 depths, 0-10cm and 10-20cm giving a total of 20 samples.

Total annual rainfall in 2017 at Laggan was 796 mm, which was below the annual average for the site (853 mm) based on available records (Station Number 070025) for the last 134 years[5] (Appendix II).

Figure 2. Visual difference between untreated and UNP-treated pasture soils at Laggan at time of soil sampling (December, 2017).

3.2. Soil Chemical properties

Total soil carbon (C) and nitrogen (N) were determined on finely-milled (MM400 mixer mill, Retsch, Haan, Germany) air-dried (40 ˚C) soil by dumas combustion on a TruMac CN analyser (LECO, Australia).

3.3. Soil biological function

3.3.1. Soil Respiration and catabolic diversity

Soil respiration is considered a good predictor of overall biological activity in the soil. Further, the use of a wide range of carbon sources in soil respiration assays can provide a catabolic ability of the microbial community and can provide useful information about the size of the microbial community and its ability to decompose organic matter. Soil respiration and catabolic fingerprinting was assessed using the MicroResp™ respiration system[6] following the manufacturers instructions. Briefly, water was used to determine basal respiration and 15 different carbon sources (Appendix III) were used to assess the substrate induced respiration (SIR) response of the soil microbial community to the addition of a range of carbohydrates, amino acids, carboxylic acids and other carbon sources. The Shannon–Wiener index was used to determine the catabolic diversity of carbon substrate utilisation profiles.

3.3.2. Potential enzyme activities

Potential enzyme activity in soils was determined using fluorimetric labelled substrates as previously described[7]. Six enzymes associated with carbon, nitrogen and phosphorus cycling were assessed (Appendix IV). α-glucosidase (AG) and β-Glucosidase (BG) targeted sugar degradation, Cellobiohydrolase (CB) and β-Xylosidase (XYL) targeted cellulose and hemicellulose degradation respectively. N-acetyl-β-D-glucosaminidase (NAG) targeted chitin degradation. Leucine aminopeptidase (LAP) targeted protein degradation while phosphatase (PHOS) targeted phosphorus mineralisation. Total C enzyme activity was determined as the sum of BG, AG, CB and XYL activity. Total N enzyme activity as determined as the sum of NAG and LAP activity.

3.3.3. Microbial community composition

We determined both the relative abundance, diversity (number of species) and composition of species for the bacterial and fungal communities on DNA extracted from frozen (-80 ˚C) soils. Total genomic DNA was extracted from soil samples using a Powersoil DNA isolation kit (Qiagen, USA) following the manufacturer's instructions. DNA was quantified on a NanoDrop 2000/2000c Spectrophotometer (Thermo Scientific, Wilmington, USA).

3.3.3.1. Relative abundance of Bacterial and Fungal genes

Quantitative polymerase chain reaction (qPCR) was used to quantify the abundance of the bacterial 16S rRNA genes and fungal ITS gene as a proxy for bacterial and fungal abundance in soil a modified method previously described[8]. Full method details, primers and PCR conditions are detailed in Appendix V.

3.3.3.2. Amplicon sequence analysis of bacterial and fungal communities

Microbial diversity and community structure was assessed using next generation sequencing (MiSEQ) using amplicon based sequencing at the Western Sydney University Next Generation Sequencing (NGS) facility[9]. Sequence data were processed using Qiime2 (2017.12 version). Statistical analysis was performed using R software. Full details of analysis are outline outlined in Appendix VI.

3.4. Grain quantity and nutrient content Data analysis

At Boorowa, four to 17 wheat heads were sampled across each replicate field plot. The number of grains per head was counted, grains de-husked, weighed and dried at 40˚C until a constant weight. All grain samples within a replicate treatment plot were bulked together to give 20 samples in total. Each sample was fine-milled and analysed for plant macro and micro nutrient analysis by the Environmental Analysis Laboratory (Southern Cross University, Lismore NSW).

3.5. Data analysis

Data was analysed by either one-way (treatment) or two-way (treatment x depth) analysis of variance (ANOVA) unless otherwise stated. Data that did not fit the assumptions of the test was either log- or arcsin sqrt- transformed prior to analysis. All analysis was performed in SigmaPlot V14 (Systat Software, USA).

4. RESULTS

4.1. BOOROWA

4.1.1. Soil Chemical properties

Soil C and N is shown in Figure 3. Total soil C was significantly greater in 0-10 cm soils (1.63 % ± 0.04) compared to 10-20 cm soil (0.92 % ± 0.04) (Figure 3a, Appendix VII.1). A significant treatment effect was evident (Appendix VII.1, $P < 0.05$) whereby % C was greater in untreated control soils (1.38%) compared to CSP treated soils (1.15%), although this was not statistically different within each soil depth. This trend was also observed for total soil N (Figure 3b), although treatment differences were not statistically significant (Appendix VII.1, $P > 0.05$). However, these differences were small and given the field variations, agronomic significance is likely to be minor and insignificant.

In the 10-20cm soils, soil CN ratios were significantly lower in all treated soils compared to control soils (Figure 3c). Soil CN ratios in control soils were 5.6 %, 13.9 % and 12.3 % higher compared to CSP ($P < 0.05$), UNP ($P < 0.001$) and UNP+CSP ($P < 0.001$) treatments respectively (Appendix VII.1). Additionally, soil CN ratios were 8.9 % and 7.3 % higher in CSP treated soils compared to UNP ($P < 0.001$) and UNP+CSP ($P < 0.01$) treated soils respectively (Figure 3c, Appendix VII.1). This supports the observation of increased soil N in UNP and UNP+CSP treatments (Figure 3b).

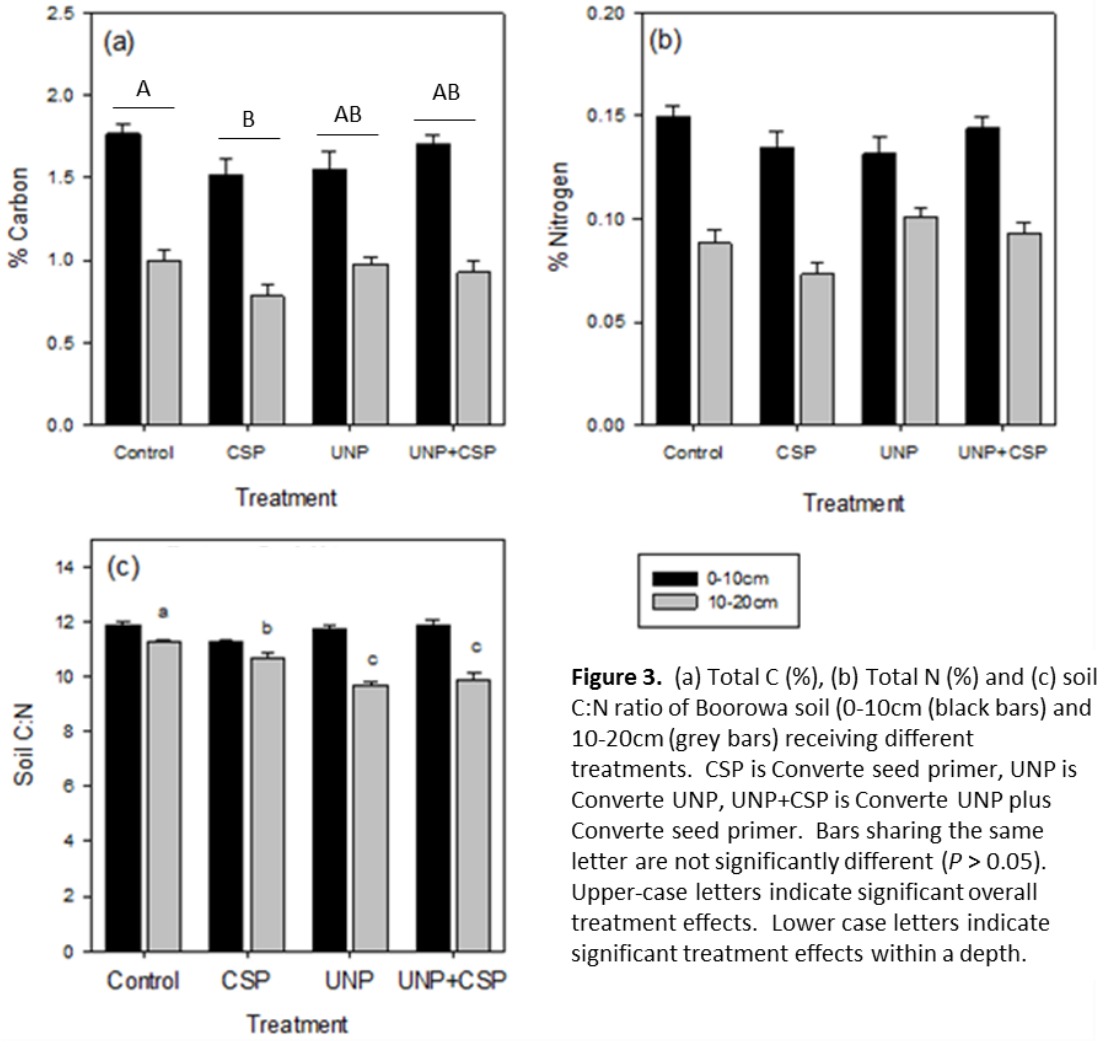

Figure 3. (a) Total C (%), (b) Total N (%) and (c) soil C:N ratio of Boorowa soil (0-10cm (black bars) and 10-20cm (grey bars) receiving different treatments. CSP is Converte seed primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte seed primer. Bars sharing the same letter are not significantly different ($P > 0.05$). Upper-case letters indicate significant overall treatment effects. Lower case letters indicate significant treatment effects within a depth.

4.1.2. Soil Biological Function

4.1.2.1. Soil Respiration and catabolic diversity as a measure of soil function

Basal respiration (which is considered a good predictor of overall biological activity in the soil) was 38% and 57% greater in UNP treated soils compared to control ($P < 0.05$) and UNP+CSP ($P < 0.05$) treated soils, respectively (Figure 4a, Appendix VII.2) and was significantly lower in 10-20 cm soils than 0-10cm soil (Appendix VII.2). In CSP treated soils, the response to glucose addition was 92 % and 76 % lower compared to control and UNP+CSP treated soils respectively (Figure 4b, Appendix VII.2). A similar trend was observed for the total carbohydrates (Figure 4d, Appendix VII.2). The ability of the microbial community to utilise more recalcitrant lignin substrate was lower in CSP and UNP soils compared to control soil (Appendix VII.2). There was no significant effect of treatment on total amino acid (Figure 4e) or total carboxylic acid utilisation (Figure 4f; Appendix VII.3).

Assessing the diversity of C-substrates utilised can provide information about the catabolic diversity (H') of the microbial community in a particular soil. A soil exhibiting a low H' is unlikely to utilise a wide range of C-substrates compared to one exhibiting a high H'. In the soil tested here, H' was similar across all treatments in the 0-10 cm soils, with a small reduction in diversity in CSP treated soils compared to control soils (Figure 5a, Appendix VII.2, $P < 0.01$), although this difference was small and likely not to be agronomically significant. In 10-20cm soils, catabolic diversity was greater in UNP+CSP treated soils compared to that observed in soils treated with CSP ($P < 0.05$) or UNP ($P < 0.05$) alone (Figure 5a, Appendix VII.2), which suggests that the UNP+CSP treated soils have the ability to utilise a wider range of C-sources than in soils treated with CSP or UNP alone. However, treatment differences were not different from control soils, and the small differences detected were likely to be insignificant.

Principal components analysis (PCA) was used to depict the level of similarity between the catabolic profiles of the samples, considering all 15 C-substrates used in the assay. Samples that cluster towards each other in the PCA plot (Figure 5b) are more similar to each other than ones that do not. Analysis of similarity (ANOSIM) was used to statistically test the catabolic profile response. There was a strong difference in the catabolic profiles of the microbial community in 0-10cm and 10-20cm (Figure 5b, ANOSIM $P < 0.001$). Within the 0-10 cm layer the catabolic profile of the CSP treated soils differed significantly from the control soils ($P < 0.01$), suggesting that addition of CSP selected for microbial communities that utilised different C-sources compared to the control. Within the 10-20cm layer CSP+UNP differed in their catabolic profiles from UNP ($P < 0.05$) and from CSP ($P < 0.01$). Further work is required to identify the substrates driving these difference and the microbial communities responding to these substrates in order to better understand the significance of these changes imposed by the Bio-fertiliser treatments.

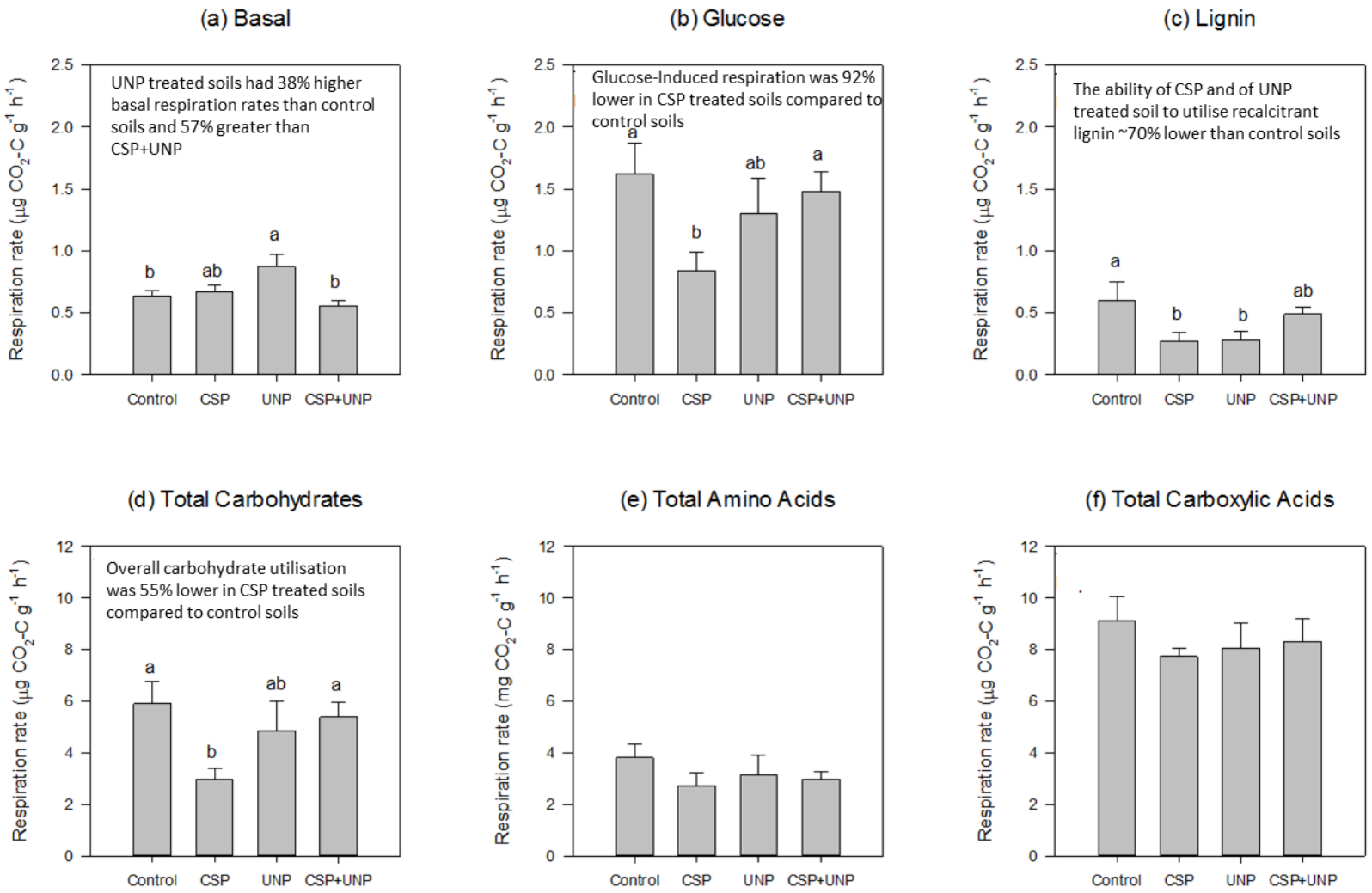

Figure 4. (a) Basal respiration, (b) glucose-induced respiration, (c) lignin-induced respiration, (d) total carbohydrate respiration, (e) total amino acid respiration and (f) total carboxylic acid respiration rates in Laggan soil (0-20 cm) receiving different treatments. Control is untreated control, UNP is Converte UNP. Error bars are ± one standard error (n=5). Bars with different letters are significantly different ($P < 0.05$).

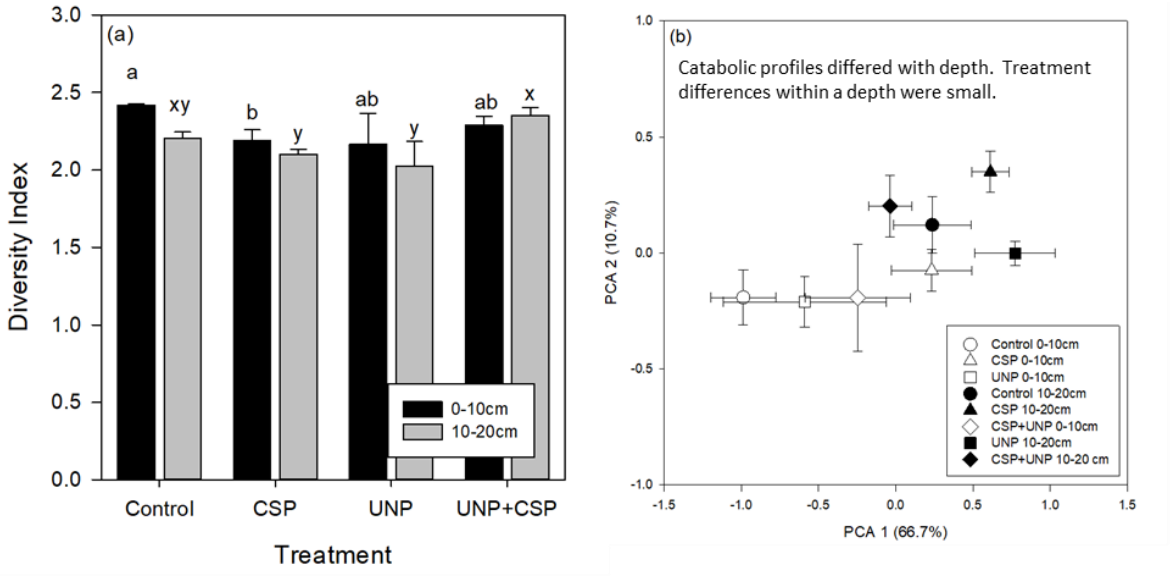

Figure 5. (a) Catabolic diversity (Shannon Diversity index (H') of microbial communities of 0-10 cm (black bars) and 10-20cm (grey bars) soils receiving different treatments at Boorowa. (b) Principal components analysis (PCA) of the substrate induced respiration response to 15 different C-substrates (Appendix I) of microbial communities at Boorowa. Numbers in parenthesis are percentage variation explained by the principal component. The figure can be interpreted as samples that cluster closer together are more similar in their catabolic response than those that are further apart. CSP is Converte seed primer, UNP is Converte Universal natural plantfood. Within a depth class, bars with different letters are significantly different ($P < 0.05$). Error bars are ±one standard error (n =5).

4.1.2.2. Potential enzyme activities as a measure of soil function

Potential soil enzyme activities associated with C-cycling, with the exception of α-glucosidase (AG), were highest in UNP+CSP treated soils and lowest in UNP treated soils (Appendix VIII), as such there was significant treatment effect on total C associated enzymes (Figure 6a, $P < 0.001$, Appendix VII.4) where by total C-associated enzyme activities were 20%, 60% and 170% greater in UNP+CSP compared to control, CSP and UNP treated soils respectively.

Treatment effects on N-associated enzyme activities (N-acetyl-β-D-glucosaminidase and Leucine amino peptidase) were less evident and tended to be lowest in control soils and highest in UNP+CSP treated soils (Appendix IX, Appendix VII.4) which was reflected in significantly higher total N-associated enzyme activities where by in the 0-10 cm soils, total N associated enzyme activities were 38% and 48% greater in UNP+CSP treated soils compared to control and UNP treated soils respectively (Figure 6b, $P < 0.05$, Appendix VII.4). This trend was also reflected to some extent in the 10-20cm soils.

Similarly, for phosphatase, activities were highest in UNP+CSP treated soils at both 0-10cm and 10-20cm depths (Figure 6c). At 0-10cm, phosphatase activities were 37%, 160% and 54% greater in UNP+CSP treated soils compared to control, CSP and UNP treated soils ($P < 0.001$, Appendix VII.4). Further, activities were significantly lower in CSP treated soils compared to control (90% higher) and UNP (68% higher) treated soils. Similarly at 10-20cm depths, activities were 106% and 47% higher in UNP+CSP treated soils compared to control and CSP treated soils respectively (Figure 6c ,$P < 0.001$, Appendix VII.4).

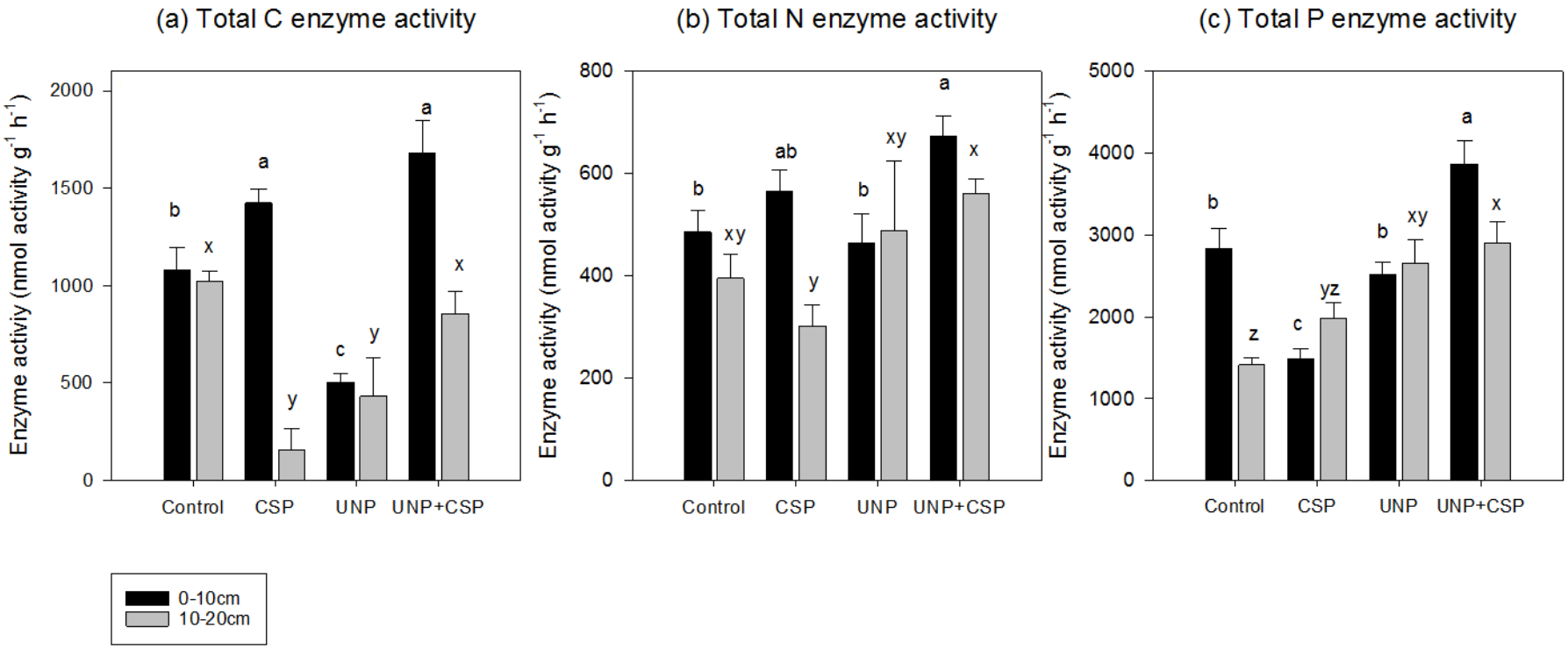

Figure 6. Total potential soil enzyme activities associated with C, N and P cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Boorowa. Total C is β-Glucosidase(BG) + α-glucosidase (AG) + Cellobiohydrolase (CB) + β-Xylosidase (XYL), Total N is N-acetyl-β-D-glucosaminidase (NAG) + Leucine aminopeptidase (LAP), Total P is phosphatase. Control is untreated control, CSP is Converte Seed Primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte Seed Primer. Error bars are ± one standard error (n=5). Within a soil layer, bars sharing the same letter are not significantly different ($P > 0.05$).

4.1.2.3. Bacterial and fungal abundance

Bacterial 16S rRNA and fungal gene abundance data (as assessed by qPCR) was used as a proxy for bacterial and fungal biomass within the soils. Bacterial 16S rRNA gene abundance was 36 % and 55 % higher in UNP and UNP+CSP compared to control soils (Figure 7a), but this was only statistically significant for UNP+CSP treated soils ($P < 0.05$, Appendix VII.5). As expected, bacterial gene copies were significantly lower in 10-20cm soils than 0-10 cm soils (Figure 7a, $P < 0.001$).

Fungal gene copy numbers were highest in UNP+CSP>UNP>CSP>Control and significantly impacted by treatment (Figure 7b, $P < 0.001$, Appendix VII.5) such that fungal copy numbers were 2 orders of magnitude (20 fold) greater in UNP+CSP treated soils compared to control soils, and one order of magnitude (10 fold) greater in UNP+CSP compared to CSP and UNP treated soils (Figure 7b). This striking treatment effect was seen in both the 0-10cm soils and the 10-20cm soils (Figure 7b). Consequently a similar treatment effect was seen on Fungal:Bacterial ratio in these soil at both depths (Figure 7c, Appendix VII.5) whereby fungal:bacterial ratios in UNP+CSP treated soils were 9.9, 9.8 and 9.6 fold higher than in control, CSP and UNP treated soils respectively ($P < 0.001$).

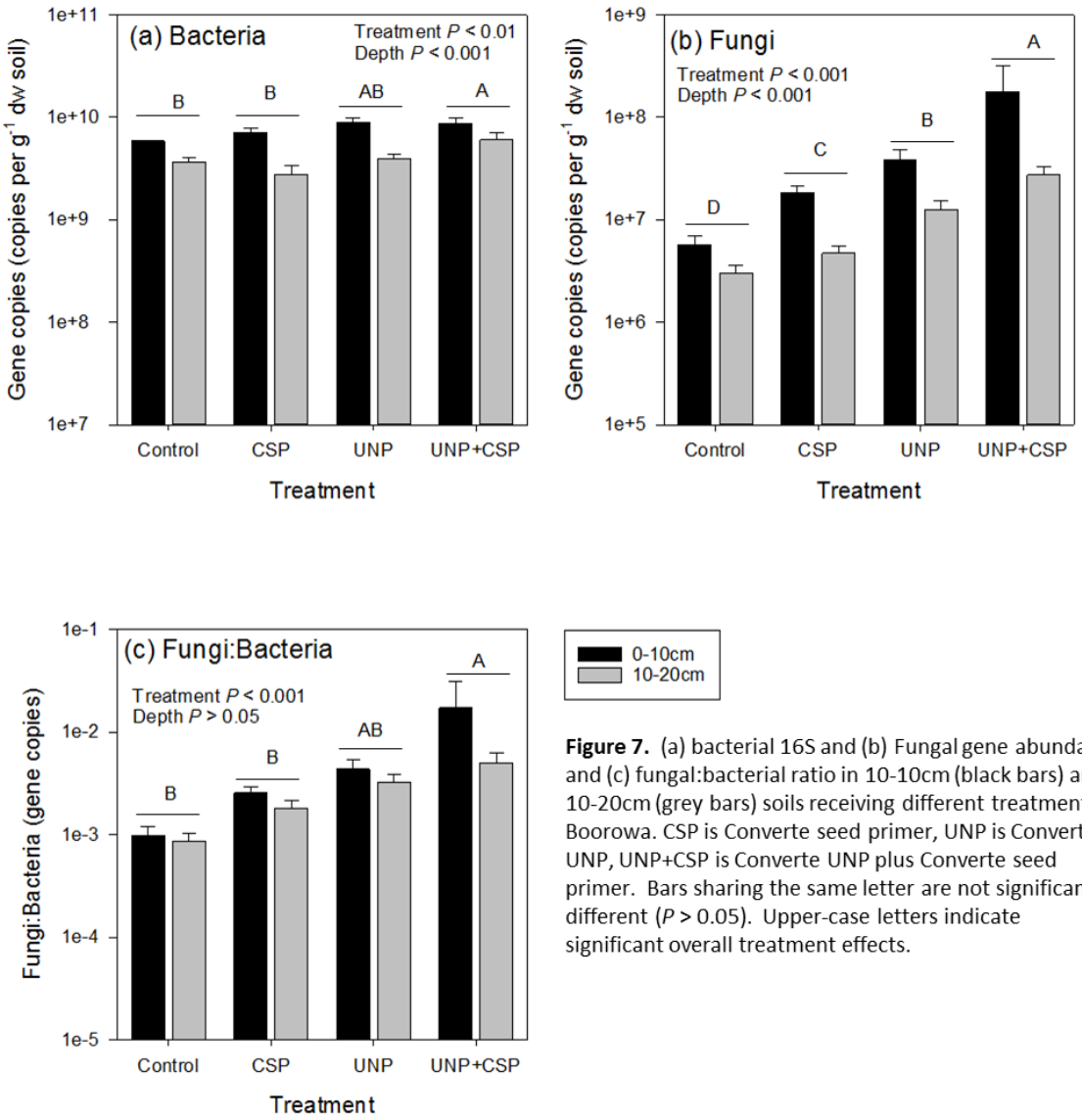

Figure 7. (a) bacterial 16S and (b) Fungal gene abundance and (c) fungal:bacterial ratio in 10-10cm (black bars) and 10-20cm (grey bars) soils receiving different treatments at Boorowa. CSP is Converte seed primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte seed primer. Bars sharing the same letter are not significantly different ($P > 0.05$). Upper-case letters indicate significant overall treatment effects.

4.1.2.4. Microbial Community Composition

Soil microbial communities are highly diverse and assessment of the diversity of soil microbial community can provide a quantitative assessment of 'species' number and variability within a soil. Although a full understanding of the relationship between soil microbial diversity with soil health remains debatable, it is generally considered that soils with a high level of diversity are more stable and resilient to stresses (and thus healthy) than those with a lower level of diversity. Additionally, because different groups of soil microbes perform different functional roles in soil, understanding how different treatments alter the composition of the soil microbial community is important to identify the mechanisms by which Bio-fertilisers promote nutrient cycling, provide resistance to stress (biotic and abiotic) and enhance crop growth. Here we determined the impact of Bio-fertiliser treatment on both bacterial and fungal diversity and on the composition of the bacterial and fungal community.

At Boorowa, treatment did not exert a significant influence on the soil bacterial or fungal species diversity (Table 1), but there were significant treatment effects on the composition of both the bacterial (Figure 8, $P < 0.001$, PERMANOVA) and the fungal (Figure 9, $P = 0.0001$, PERMANOVA) such that the microbial communities associated with each treatment were distinct from each other at both the 0-10cm layer and 10-20 cm layer for both bacteria ($P = 0.00001$, PERMANOVA) and fungi ($P < 0.0001$, PERMANOVA) (Figure 8 and 9 respectively).

Redundancy analysis (RDA) was used to depict the level of similarity between the composition of the microbial community as assessed by amplicon sequence analysis of extracted DNA. The data can be interpreted as samples that cluster towards each other in the RDA plot (Figure 8 and 9) are more similar to each other than ones that do not. The striking treatment differences in bacterial and fungal composition (as depicted by the discrete clustering by treatment in the RDA plots) provides strong evidence that the Bio-fertiliser treatments do influence the soil microbial community. Additionally, it is evident that the different treatment (CSP or UNP alone, or in combination) select for different members of the microbial community. Further data analysis will help to identify which taxa drive these differences and will help to determine the mechanisms by which each of the bio-fertiliser treatments causes these differences.

Table 1. Shannon diversity (H') of bacterial and fungal communities in control and Converte-treated soils at two different depths at Boorowa, as determined by amplicon sequence analysis. CSP is converte seed primer, UNP is Converte natural plantfood. Numbers in parenthesis are ±one standard error

Treatment	Depth	Bacterial H'	Fungal H'
Control	0-10 cm	9.52 (0.19)	5.97 (0.25)
	10-20 cm	9.17 (0.25)	6.39 (0.21)
CSP	0-10cm	9.54 (0.05)	6.36 (0.08)
	10-20cm	8.99 (0.12)	6.31 (0.12)
UNP	0-10cm	9.36 (0.16)	5.97 (0.22)
	10-20cm	9.36 (0.15)	5.87 (0.32)
CSP+UNP	0-10cm	9.60 (0.12)	6.23 (0.12)
	10-20cm	9.16 (0.13)	6.19 (0.15)

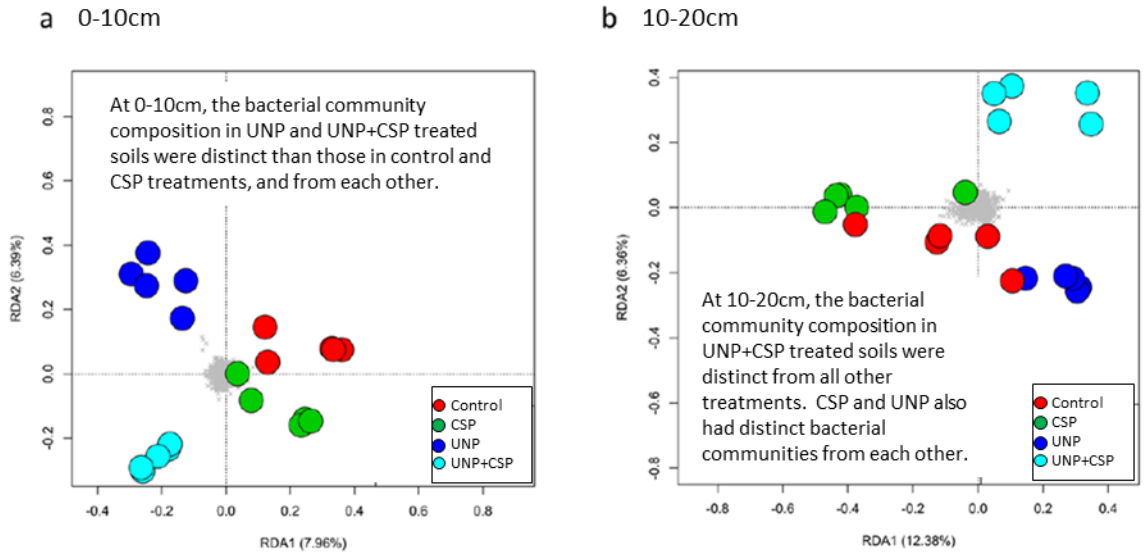

Figure 8. Redundancy analysis (RDA) biplot of the bacterial community composition as assessed by amplicon sequence analysis of DNA extracted from soils under different treatments at Boorowa: a) 0-10 cm and b) 10-20 cm. Numbers in parentheses are percentage variation explained by the axes. CSP is Converte seed primer, UNP is Converte universal natural plantfood UNP. PERMANOVA demonstrated a significant effect of treatment ($P = 0.0001$) and depth ($P = 0.00001$), but no treatment x depth interaction ($P = 0.20$). The data can be interpreted as samples that cluster closer together on the RDA plot are more similar to each other than ones that do not

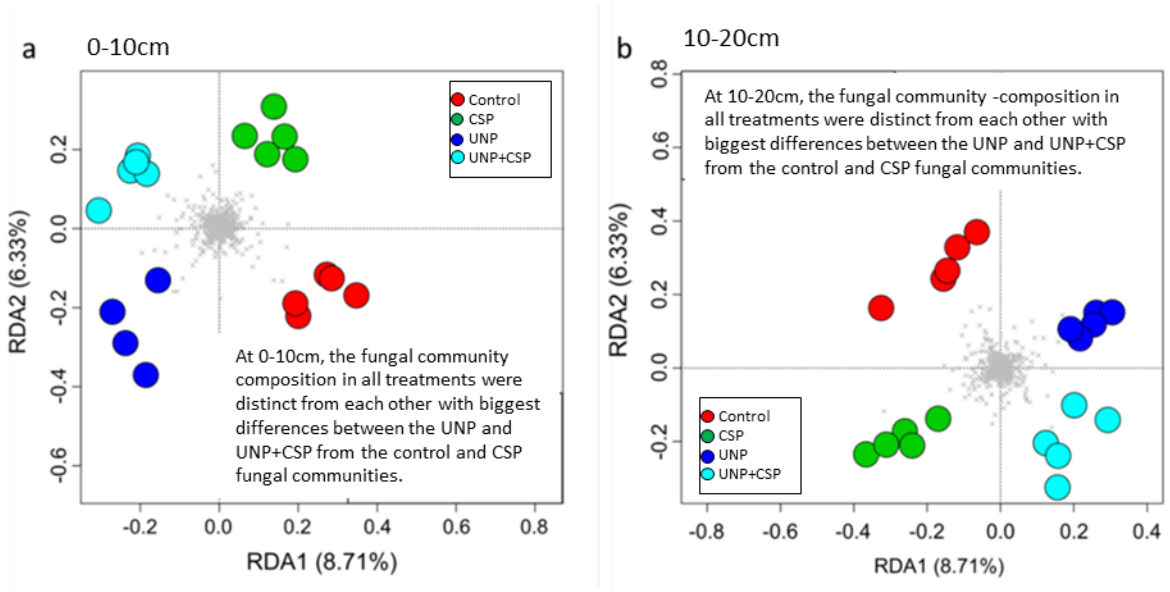

Figure 9. Redundancy analysis (RDA) biplot of the fungal community composition as assessed by amplicon sequence analysis of DNA extracted from soils under different treatments at Boorowa: a) 0-10 cm and b) 10-20 cm. Numbers in parentheses are percentage variation explained by the axes. CSP is Converte seed primer, UNP is Converte universal natural plantfood UNP. PERMANOVA demonstrated a significant effect of treatment ($P < 0.001$) and depth ($P = 0.00001$), but no treatment x depth interaction ($P = 0.058$). The data can be interpreted as samples that cluster closer together on the RDA plot are more similar to each other than ones that do not

4.1.3. Grain quantity and nutrient content

The number of grain and grain weights are presented in Figure 10. The highest grain number per head was found in CSP (32 ± 2.3) treated plots and the lowest (20 ± 3.4) in control plots and 25 UNP and UNP+CSP treated plots (± 4.2 and 2.5 respectively) (Figure 10a). Mean total grain weight per head was higher in all treated plots compared to control plots (Figure 10b) whereby UNP, UNP+ CSP and CSP had 20%, 33% and 58% higher grain weights per head respectively than control plots. This difference was only statistically significant for CSP treated plots and results are in broad agreement with yield data generated from the grain yield monitor at time of harvest (Appendix X) whereby yield ranged from 2.4 to 3.4 tonnes ha^{-1} (John Ridley, per. com.). Spatial variation in other soil parameters across the field, such as high zinc along the western edge must be taken into consideration when interpreting these results. The individual grain weight did not differ significantly between treatments (Figure 10c).

Nutrient content of grain samples collected from Boorowa are presented in Table 2. There was no effect of treatments in any of the parameters measured with two exceptions. Firstly, control plots had 75-88 % higher levels of Zn in control plot compared to treated plots (Table 2a, Appendix VII.6, $P < 0.05$). This was driven by higher soil Zn concentrations in the control plot (data not shown, John Ridley (Converte Pty Ltd.) personal comm.) which is likely to be a consequence of historic activity at the site (close proximity to the road and historical flooding in this plot may be an explanatory factor for this observation). Secondly, grain collected from CSP treatment had 23% higher levels of Mn than in control and UNP+CSP (Table 2a, Appendix VII.6 $P < 0.05$). Manganese is an essential micronutrient important in resistance to many soil-borne, and fungal leaf diseases. Further, Mn-deficiency is reported to be associated with reduced tolerance to stresses including salinity and drought. Thus the apparent higher levels of Mn in grain samples growing on CSP treated soils reported here warrants further investigation on the levels of Mn in other plant parts, in other fields and their consequences for resistance to different stresses.

There was no significant difference in the ratio of selected elements, or the crude protein content of the grains collected from the different treatments (Table 2b).

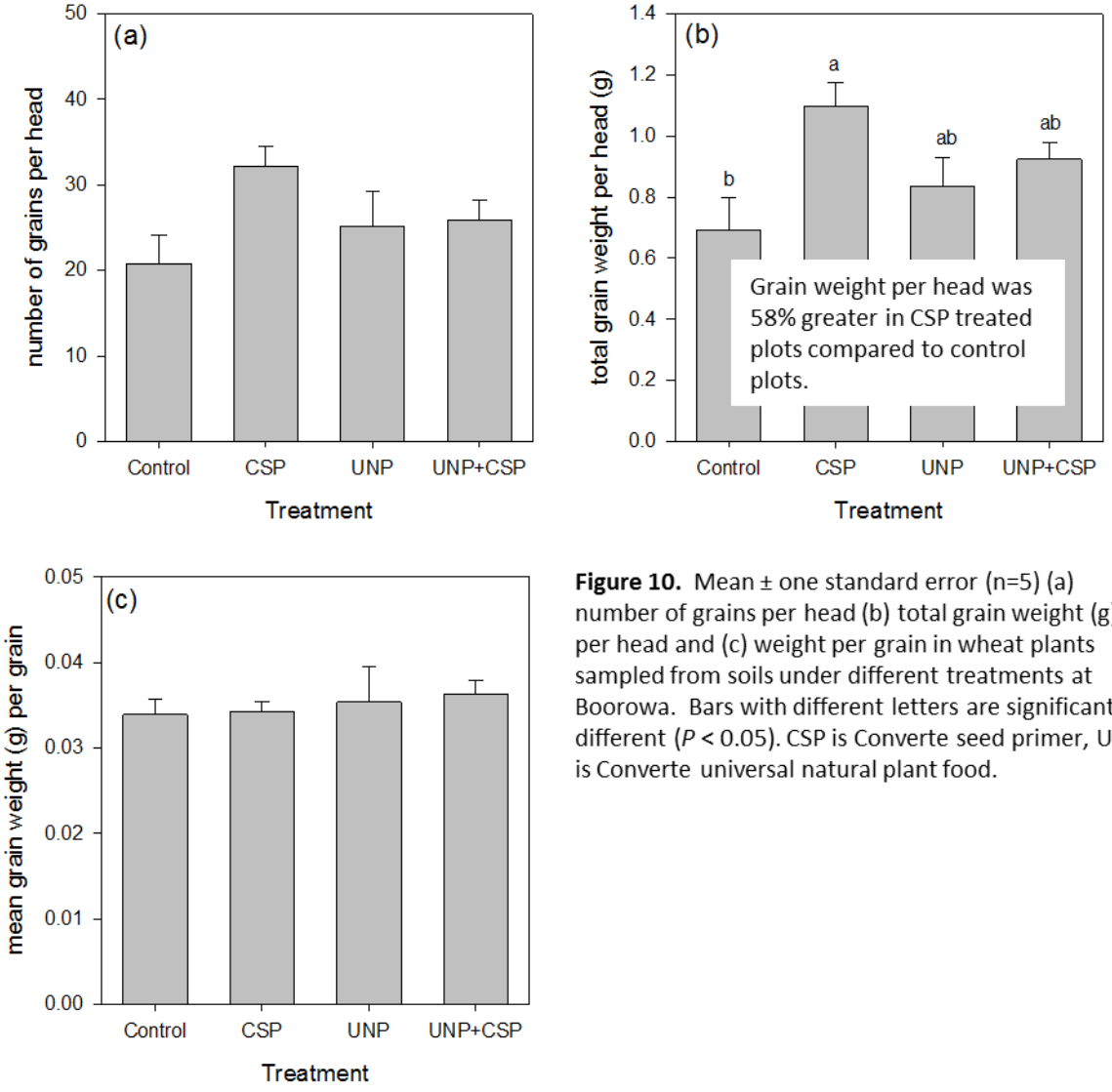

Figure 10. Mean ± one standard error (n=5) (a) number of grains per head (b) total grain weight (g) per head and (c) weight per grain in wheat plants sampled from soils under different treatments at Boorowa. Bars with different letters are significantly different ($P < 0.05$). CSP is Converte seed primer, UNP is Converte universal natural plant food.

Table 2a. Concentration of selected macro and micro nutrients in Boorowa grain samples under different field treatments. Numbers in parentheses are ± one standard error (n = 5). Control is untreated control. CSP is Converte seed primer. UNP in Converte universal natural plantfood. Numbers in the same column sharing the same letter are not statistically different ($P > 0.05$).

	Macronutrient (%)					**Micronutrient (%)**						
						(%)		**(mg kg^{-1})**				
Treatment	**C**	**N**	**P**	**K**	**S**	**Mg**	**Ca**	**Cu**	**Zn**	**Mn**	**Fe**	**Si**
Control	44.00 (0.122)	2.914 (0.073)	0.297 (0.033)	0.381 (0.037)	0.177 (0.002)	0.127 (0.003)	0.062 (0.004)	4.696 (0.285)	43.61 (8.104)[a]	44.851 (4.094)[b]	(43.66 (9.341)	268.86 (4.330)
CSP	44.08 (0.107)	2.742 (0.138)	0.273 (0.022)	0.381 (0.018)	0.166 (0.005)	0.130 (0.005)	0.063 (0.004)	4.444 (0.167)	23.38 (1.456)[b]	55.221 (2.582)[a]	30.01 (1.272)	284.27 (13.11)
UNP	44.14 (0.068)	2.938 (0.137)	0.290 (0.031)	0.372 (0.012)	0.174 (0.004)	0.129 (0.004)	0.063 (0.007)	4.293 (0.077)	24.84 (2.882)[b]	45.949 (1.287)[b]	29.09 (1.112)	234.88 (15.60)
UNP+CSP	44.02 (0.058)	2.766 (0.130)	0.292 (0.045)	0.383 (0.029)	0.172 (0.007)	0.133 (0.009)	0.062 (0.007)	4.130 (0.126)	23.22 (3.542)[b]	44.805 (1.902)[b]	29.48 (2.328)	270.94 (8.464)

Table 2b. The ratio between selected nutrients and the crude protein content in Boorowa grain samples under different field treatments. Control is untreated control. CSP is Converte seed primer. UNP in Converte universal natural plantfood. Numbers in parentheses are ± one standard error (n = 5). Numbers in the same column sharing the same letter are not statistically different ($P > 0.05$).

Treatment	C:N	N:P	N:S	N:K	Crude protein (%)
Control	15.135 (0.351)	10.185 (0.819)	16.420 (0.269)	7.854 (0527)	18.212 (0.459)
CSP	16.218 (0.713)	10.912 (0.536)	16.514 (0.344)	7.230 (0.319)	17.137 (0.860)
UNP	15.136 (0.604)	10.369 (0.538)	16.856 (0.344)	7.907 (0.289)	18.363 (0.856)
UNP+CSP	16.051 (0.727)	10.018 (0.888)	16.856 (0.432)	7.282 (0.247)	17.288 (0.816)

4.2. **LAGGAN**

4.2.1. Soil Chemical properties

Both total soil C and N was significantly greater in UNP treated soils compared to control soils in both 0-10 cm and 10-20 cm samples (Figure 11a and Figure 11b, Appendix VII.7). In the 0-10 cm layer, total soil C was 4.1% in UNP treated soils compared to 2.7% in untreated controls, while in the 10-20 cm total soil C was 3.1% in UNP treated soils compared to 2% in untreated controls. Similar trends were observed for soil N, but there was no significant effect of treatment on soil CN (Figure 11c).

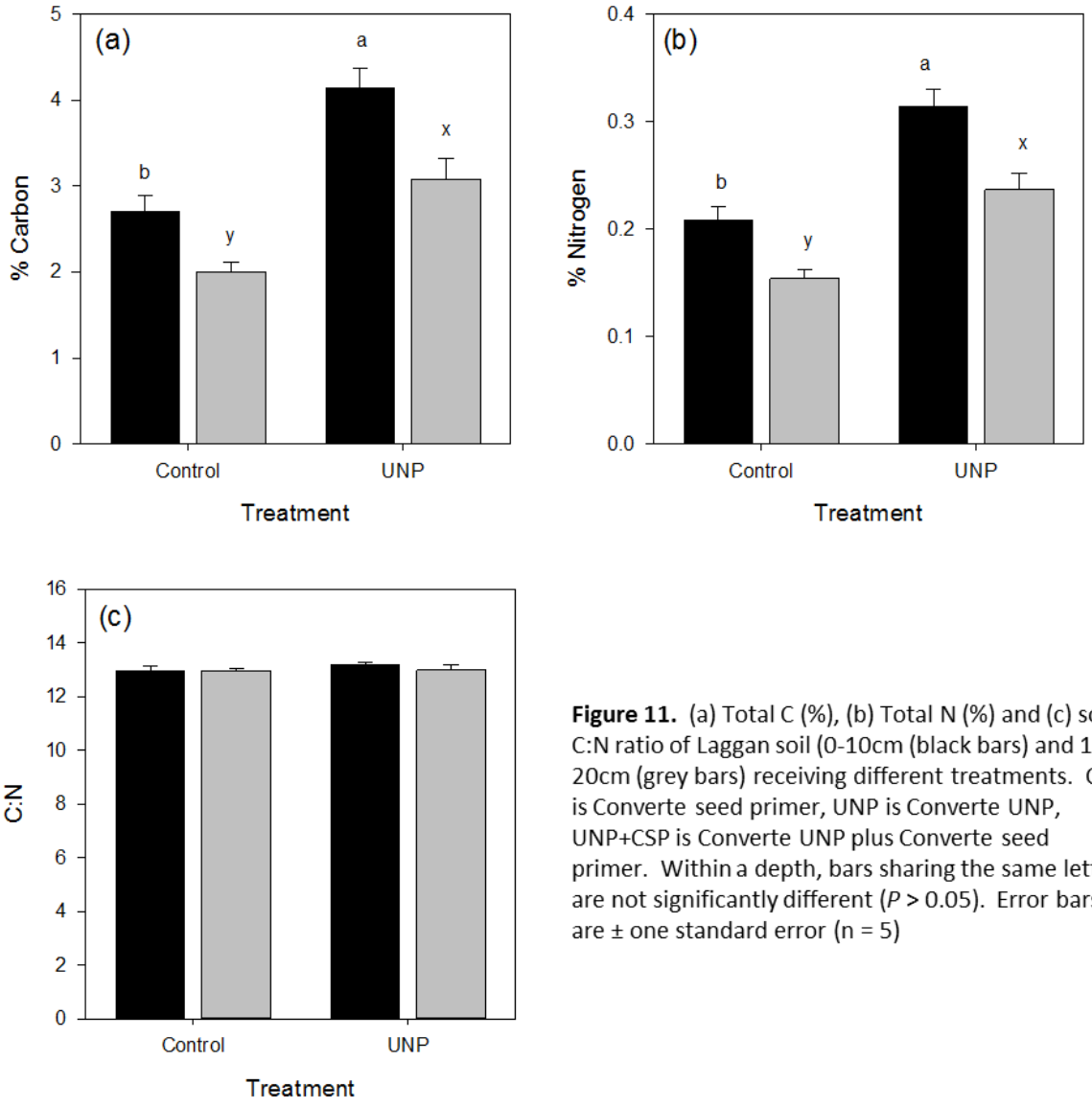

Figure 11. (a) Total C (%), (b) Total N (%) and (c) soil C:N ratio of Laggan soil (0-10cm (black bars) and 10-20cm (grey bars) receiving different treatments. CSP is Converte seed primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte seed primer. Within a depth, bars sharing the same letter are not significantly different ($P > 0.05$). Error bars are ± one standard error (n = 5)

4.2.2. Soil Biological Function

4.2.2.1. Soil Respiration and catabolic diversity as a measure of soil function

Catabolic diversity was similar across all treatments and depths and there were no significant differences (Figure 12a, Appendix VII.8).

Principal components analysis (PCA) was used to depict the level of similarity between the catabolic profiles of the samples. Samples that cluster towards each other in the PCA plot (Figure 12b) are more similar to each other than ones that do no. Analysis of similarity (ANOSIM) was used to statistically test the catabolic profile response. There was no apparent treatment effect on catabolic profiles in Laggan soils (Figure 12b, $P > 0.05$), but catabolic profiles did differ with depth in control soils (Figure 12b, $P < 0.05$).

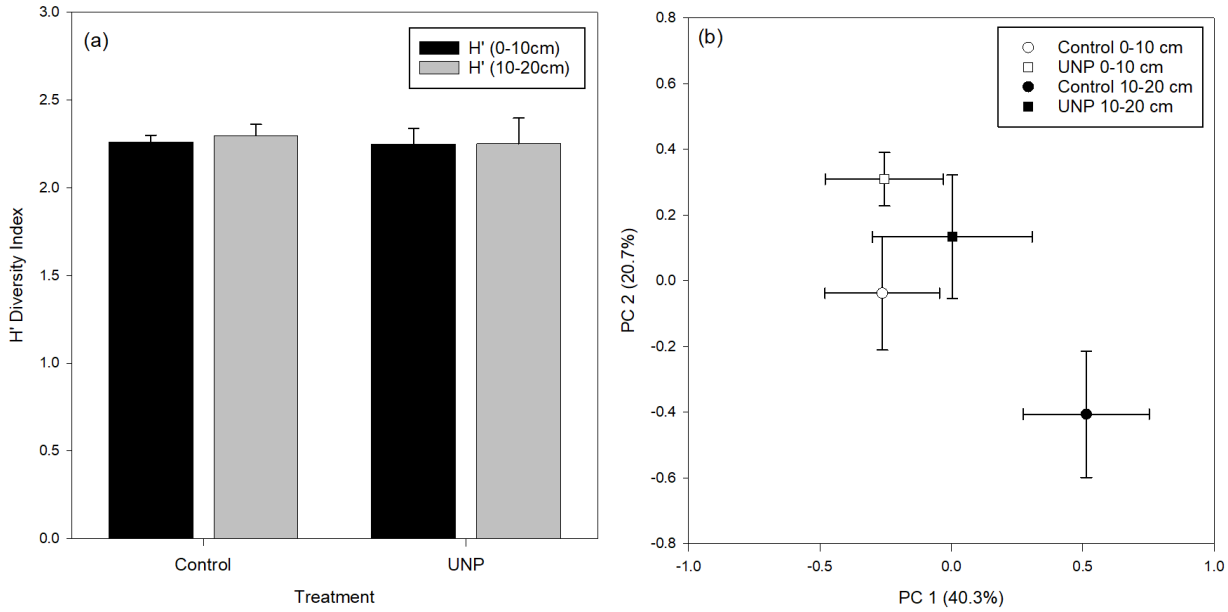

Figure 12. (a) Catabolic diversity (Shannon Diversity index (H') of microbial communities of 0-10 cm (black bars) and 10-20cm (grey bars) soils receiving different treatments at Laggan. (b) Principal components analysis (PCA) of the substrate induced respiration response to 15 different C-substrates (Appendix I) of microbial communities at Laggan. Numbers in parenthesis are percentage variation explained by the principal component. The figure can be interpreted as samples that cluster closer together are more similar in their catabolic response than those that are further apart. UNP is Converte Universal natural plantfood. Error bars are ±one standard error (n =5).

Basal Respiration was 41% greater in UNP treated soils compared to control soils (Figure 13a, Appendix VII.8 $P < 0.05$). There was no significant difference in basal respiration rates between depths (Appendix VII.8). Similarly, glucose-induced respiration was 23% greater in UNP treated soils compared to untreated control (Figure 13b), but differences were not statistically significant (Appendix VII.8, $P = 0.089$). There was no significant effect of treatment or depth on any of the other measured catabolic categories (Figure 13c-Figure 13f, Appendix VII.8 and VII.9).

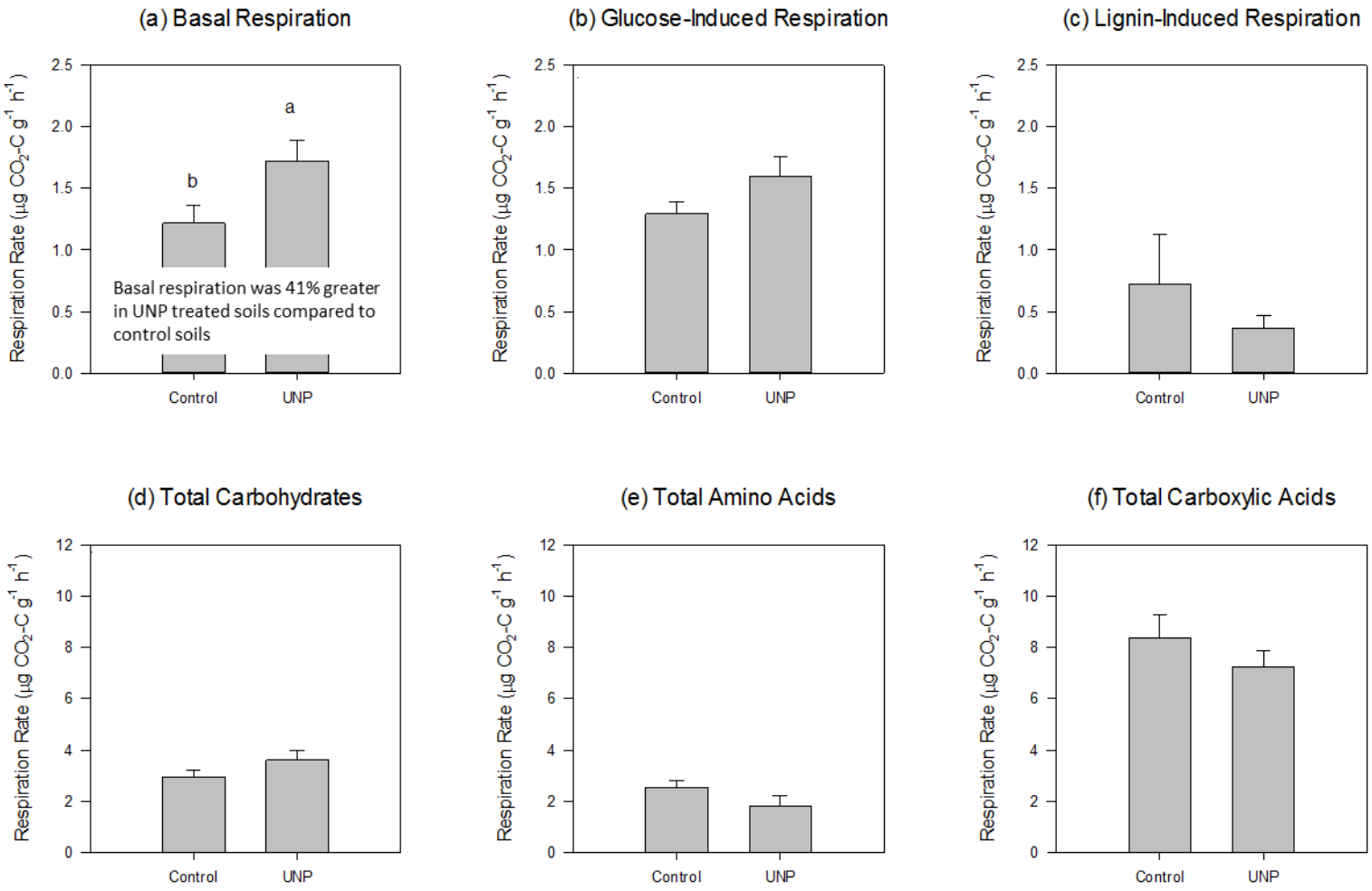

Figure 13. (a) Basal respiration, (b) glucose-induced respiration, (c) lignin-induced respiration, (d) total carbohydrate respiration, (e) total amino acid respiration and (f) total carboxylic acid respiration rates in Laggan soil (0-20 cm) receiving different treatments. Control is untreated control, UNP is Converte UNP. Error bars are ± one standard error (n=5). Bars with different letters are significantly different ($P < 0.05$).

4.2.2.2. Potential enzyme activities as a measure of soil function

Potential soil enzyme activities associated with C-cycling varied with treatment and depth (Appendix VII.10). Total activity of C-associated enzymes at 0-10cm depths, activity was 42% greater in control soils compared to UNP treated soils, while at 10-20cm depths, activity was 35% greater in UNP treated soils compared to control soils (Figure 14a).

β-Glucosidase(BG) and α-glucosidase (AG) activities were unaffected by treatment (APPENDIX XI a and b, Appendix VII.10), while cellobiosidase activity was 1.1 fold greater in control soils compared to UNP treated soil (APPENDIX XI c) at 0-10cm depths. Xylosidase activity was 61% greater in UNP treated soils at 10-20cm depths (APPENDIX XI d).

The activity of total N-associated enzymes was marginally higher in UNP than in control treated plots (Figure 14b, Appendix VII.10). This was most apparent at the 10-20cm depths where both N-acetyl-β-D-glucosaminidase and Leucine amino-peptidase (Appendix XII) showed higher activity under UNP treatment.

Similarly, phosphatase activity was significantly greater in UNP treated soils at 10-20cm depths, but not impacted by treatment at 0-10cm depths (Figure 14c).

4.2.2.3. Bacterial and Fungal abundance

Bacterial 16S rRNA and fungal gene abundance data (as assessed by qPCR) was used as a proxy for bacterial and fungal biomass within the soils. Bacterial 16S rRNA gene abundance was 138% higher in UNP-treated soil compared to control soils (Figure 15a Appendix VII.11), irrespective of depth.

Fungal gene copy numbers were highest in the 0-10 cm control treatments and 80% lower in UNP treated soils ($P < 0.05$) at this depth (Figure 15b, Appendix VII.11). This treatment effect in the 0-10 cm layer was largely driven by two very high values for field replicate 3 (1.43×10^9) and 5 (1.43×10^9) (Appendix XIII). Upon removal of these values the significant treatment effect at 0-10 cm depth disappeared, instead a significant depth effect was apparent within the control soils whereby fungal gene copies were 29% lower in 10-20 cm soils compared to 0-10 cm soils ($P < 0.05$).

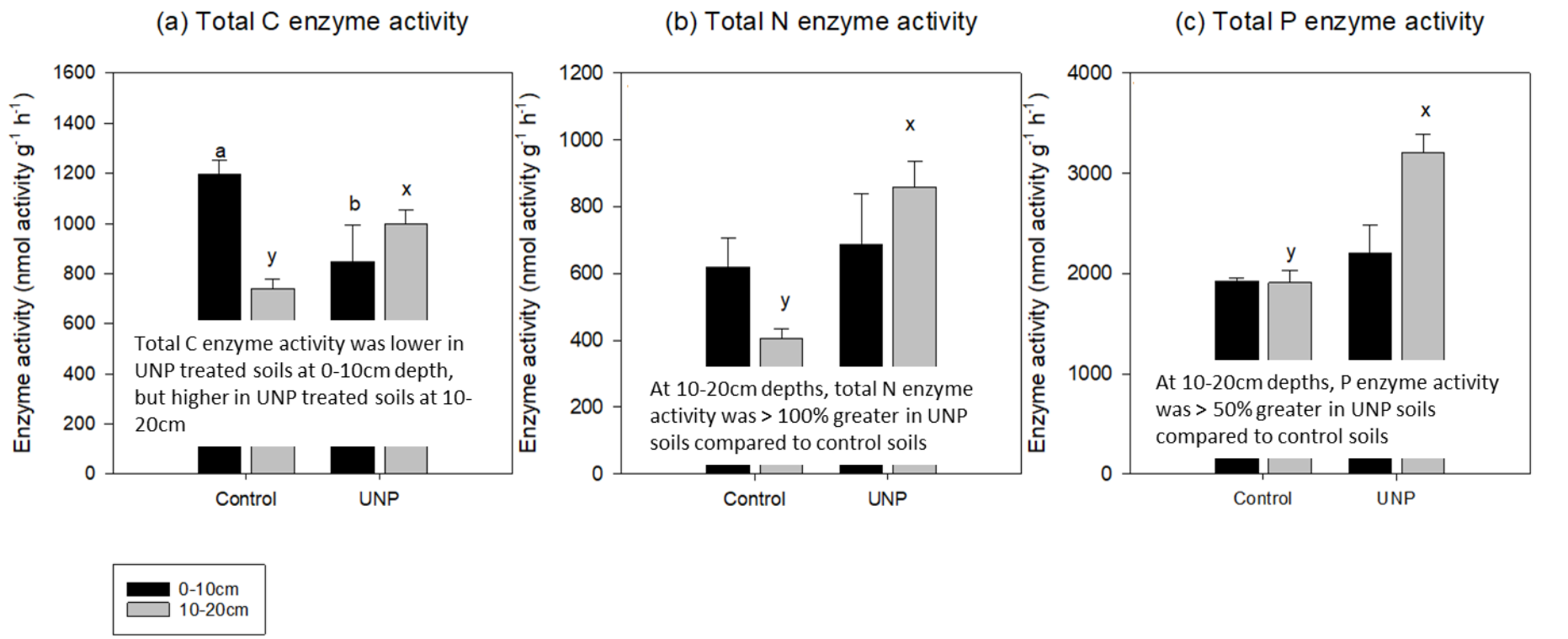

Figure 14. Total potential soil enzyme activities associated with C, N and P cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Laggan. Total C is β-Glucosidase(BG) + α-glucosidase (AG) + Cellobiohydrolase (CB) + β-Xylosidase (XYL), Total N is N-acetyl-β-D-glucosaminidase (NAG) + Leucine aminopeptidase (LAP), Total P is phosphatase. Control is untreated control, UNP is Converte UNP. Error bars are ± one standard error (n=5). Within a soil layer, bars with different letters are significantly different ($P < 0.05$).

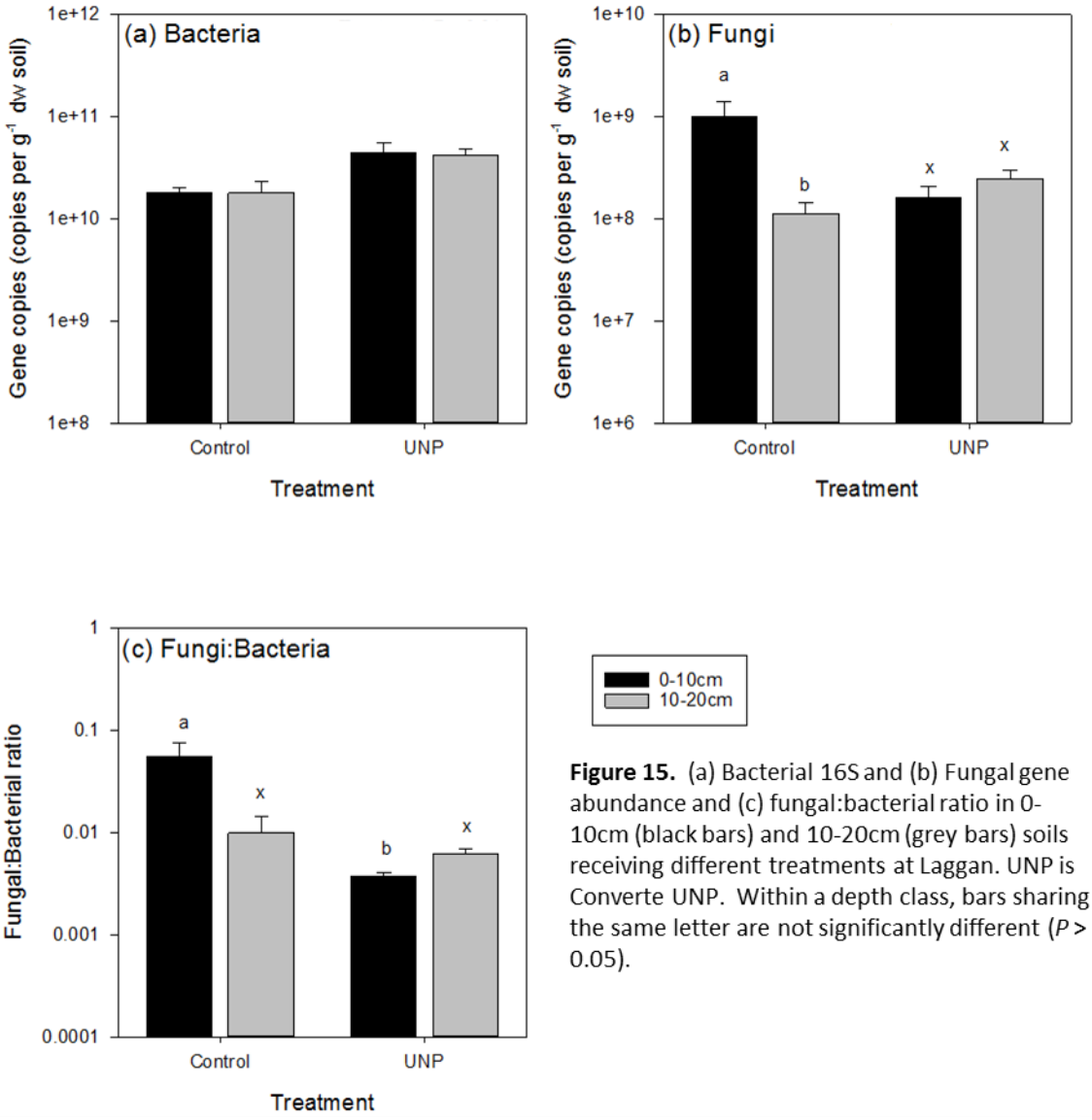

Figure 15. (a) Bacterial 16S and (b) Fungal gene abundance and (c) fungal:bacterial ratio in 0-10cm (black bars) and 10-20cm (grey bars) soils receiving different treatments at Laggan. UNP is Converte UNP. Within a depth class, bars sharing the same letter are not significantly different ($P > 0.05$).

4.2.2.4. Microbial Community Composition

Bacterial diversity was unaffected by treatment in Laggan soils (Table 3) but diversity was higher in UNP treated soils at 10-20cm compared to UNP treated soils at 0-10cm ($P < 0.01$). Although there was no statistically significant effect of treatment or depth on the fungal diversity in Laggan soils (Table 3) a similar trend to that of the bacterial diversity was observed whereby deeper soil (10-20cm) in UNP treatments have a higher level of diversity. One possible mechanism for this observation would be stimulated root growth at depth in UNP treated soils facilitating more root-derived C-inputs in to deeper soils in treated soils, stimulating microbial diversity. Further work is needed to explore this as a possible explanation for this observations.

Table 3. Shannon diversity (H') of bacterial and fungal communities in control and UNP treated soils at two different depths at Laggan as determined by amplicon sequence analysis. Numbers in parenthesis are ± one standard error.

Treatment	Depth	Bacterial H'	Fungal H'
Control	0-10 cm	9.13 (0.13)	6.46 (0.27)
	10-20 cm	9.21 (0.06)	6.41 (0.22)
UNP	0-10cm	8.86 (0.29)	6.40 (0.44)
	10-20cm	9.45 (0.09)	6.95 (0.16)

There were significant treatment ($P < 0.001$) and depth effects ($P < 0.05$) on the composition of soil bacterial communities (Figure 16a) at Laggan.

The fungal community composition was significantly influenced by UNP treatment at shallow (0-10 cm, P=0.027, PERMANOVA) and deep (10-20 cm, P=0.008, PERMANOVA) soil depths (Figure 16b). However, depth effects were not observed for this site (P=0.75). Further, no significant interaction effect between depth and treatment (UNP) was observed.

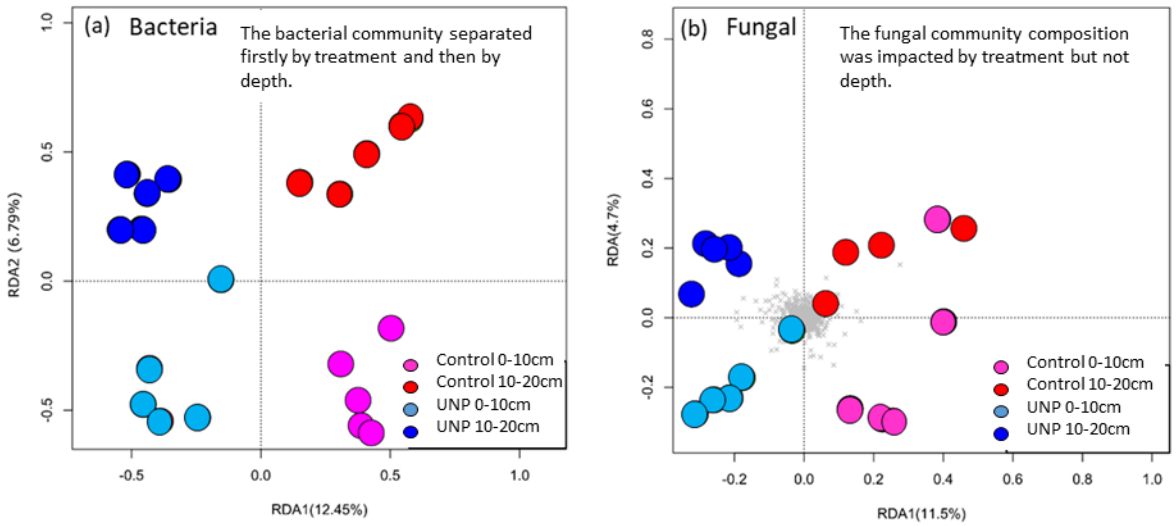

Figure 16. Redundancy analysis (RDA) biplot of (a) bacterial and (b) fungal community composition as assessed by amplicon sequence analysis of DNA extracted from soils under different treatments at Laggan at two different depths in control and Convert UNP treated soils. Numbers in parentheses are percentage variation explained by the axes. UNP is Converte universal natural plantfood UNP. PERMANOVA demonstrated a significant effect of treatment ($P < 0.001$) and depth ($P < 0.05$) for the bacterial community and significant effect of treatment on the fungal community composition at 0-10cm ($P < 0.05$) and 10-20cm ($P < 0.01$), but no effect of depth. The data can be interpreted as samples that cluster closer together on the RDA plot are more similar to each other than ones that do not

5. SUMMARY OF FINDINGS

- UNP additions stimulated basal respiration (considered a good predictor of overall biological activity in the soil) by 38% and 41% in Boorowa (wheat) and Laggan (pasture) respectively.
- There was some evidence that UNP and CSP altered the catabolic abilities of microbial communities in deeper soils (10-20cm) at Boorowa (wheat), but treatment had limited impact on the catabolic profiles in the upper (0-10cm) soil layer.
- The response of potential enzymes activities were variable, but typically stimulated under UNP+CSP additions in Boorowa (wheat) and under UNP additions at Laggan (pasture). This was particularly notable for enzymes associated with N and P cycling, suggesting increased N and P mineralisation under these treatments at the respective sites.
- UNP additions increased bacterial gene abundance (as a proxy for bacterial biomass) by 36% and 138% in Boorowa (wheat) and Laggan (pasture) respectively. At Boorowa, UNP+CSP increased bacterial gene copies by 55% providing strong evidence that UNP treatment stimulated bacterial growth in these soils.
- Fungal gene abundance (as a proxy for fungal biomass) was particularly responsive to the bio-fertiliser treatment at Boorowa (wheat) and observed in both the 0-10 cm and 10-20cm soils. There was no evidence that UNP affected fungal abundance at Laggan (pasture).
- There was no effect of bio-fertiliser treatment on the diversity of bacterial or fungal communities but each treatment did significantly alter the composition of bacterial and fungal communities at both Boorowa (wheat) and Laggan (pasture) and treatment effects were evidence at both 0-10 cm and 10-20 cm. On-going analysis will provide taxonomic resolution to these treatment effects.
- UNP significantly increased soil C and N by about 50% at Laggan (pasture). Visual observations of the field showed a greener, denser sward in UNP treated soil (Figure 2) and this likely drove higher plant-derived C-inputs into the soil and stimulated microbial (particularly fungal) growth as evidenced by higher gene copy numbers in these soils.
- Bio-fertiliser treatment increased total grain weight by 20-58% on collected samples at Boorowa. This was statistically significant in CSP treatment only.

6. REFERENCES

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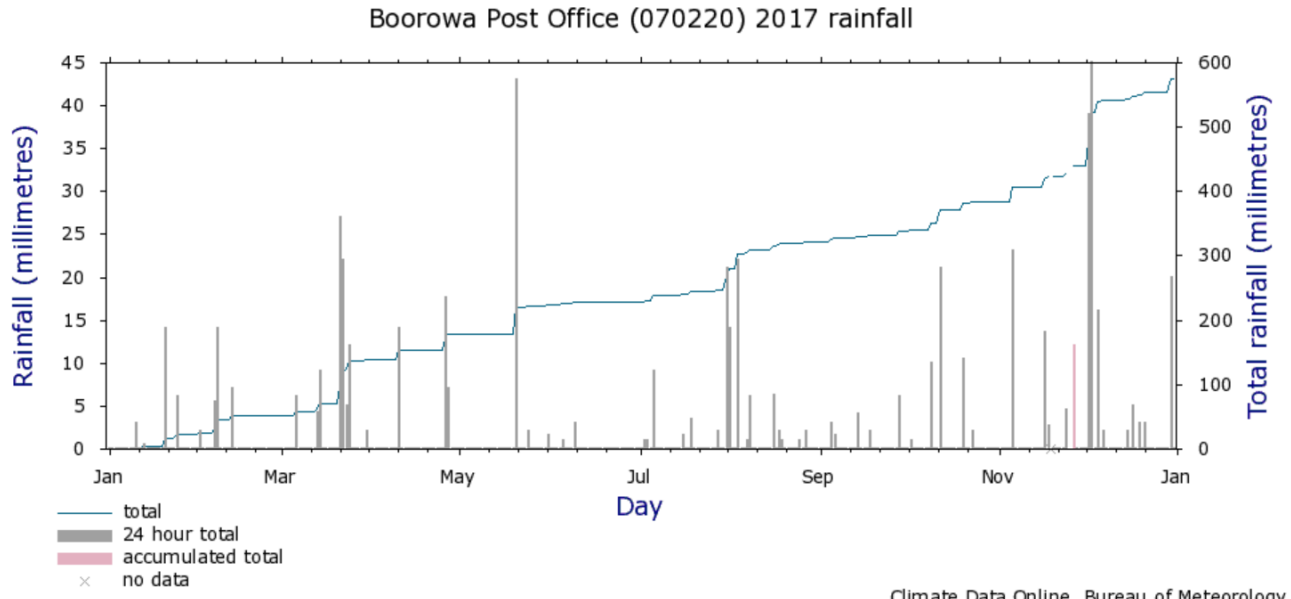
APPENDIX

APPENDIX I – Rainfall Boorowa

Daily rainfall at Boorowa Post Office (Station number 070220) in 2017. Data from the Australian Government Bureau of Meteorology accessed on 29/1/2019 (http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_display_type=dataDGraph&p_stn_num=070220&p_nccObsCode=136&p_month=13&p_startYear=2017).



Note: Data may not have completed quality control.

Product Code: IDCJAC0009

Climate Data Online, Bureau of Meteorology
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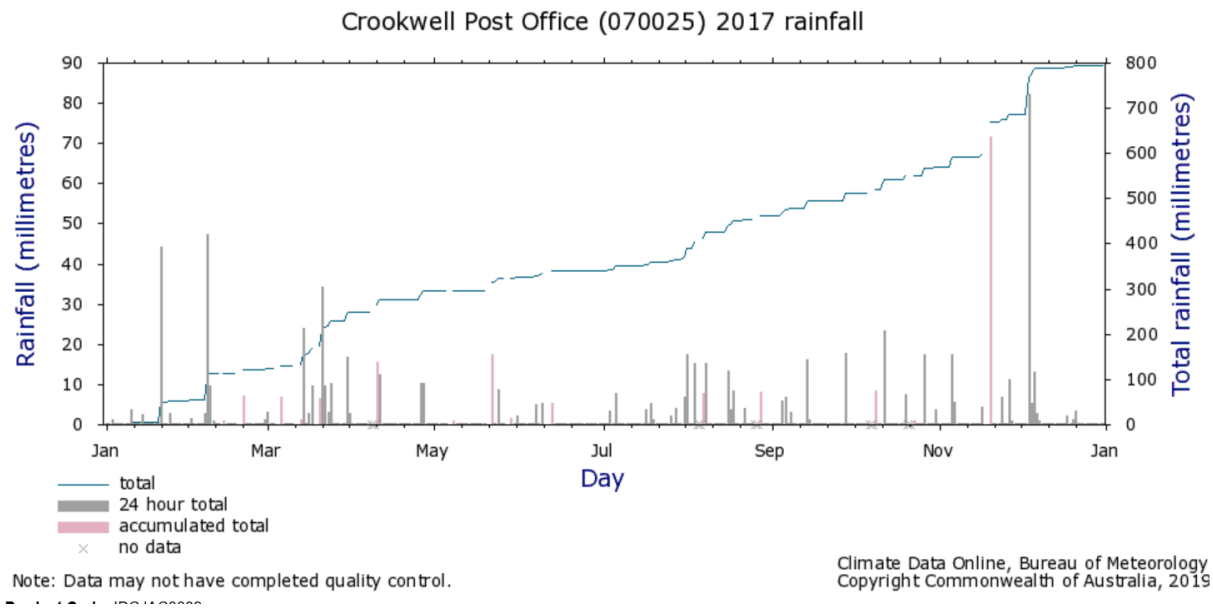
APPENDIX II - Rainfall Laggan

Daily rainfall at Crookwell Post Office (Station number 070025) in 2017. Data from the Australian Government Bureau of Meteorology accessed on 29/1/2019 (http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_display_type=dataDGraph&p_stn_num=070025&p_nccObsCode=136&p_month=13&p_startYear=2017).



APPENDIX III - Carbon substrates used in MicroRespTM

Compound type	Carbon Source
Carbohydrate	Arabinose Galactose Glucose Mannitol Sucrose
Amino Acid	Alanine Arginine Cysteine Glutamine Gamma-amino butyric acid Lysine
Carboxylic Acid	Malic Acid Oxalic Acid Alpha-keto butyric acid
Other	Lignin

APPENDIX IV - Enzymes and substrates used to assess potential soil enzyme activities

Enzymes and substrates used to assess potential soil enzyme activities associated with carbon, nitrogen and phosphorus cycling.

Enzyme	Substrate	General function
Cellobiohydrolase (CB)	4-MUB-β-D-cellobioside	Cellulose degradation
β-Glucosidase (BG)	4-MUB-β-D-glucopyranoside	Sugar degradation
α-glucosidase (AG)	4-MUB-α-D-glucopyranoside	Sugar degradation
Leucine aminopeptidase (LAP)	L-leucine-7-amido-4methylcoumarin hydrochloride	Protein degradation
N-acetyl-β-D-glucosaminidase (NAG)	4-MUB-N-acetyl-β-D-glucosaminide	Chitin degradation
Phosphatase (PHOS)	4-MUB phosphate	Phosphorus mineralisation
β-Xylosidase (XYL)	4-MUB-β-D-xylopyranoside	Hemicellulose degradation

APPENDIX V – qPCR details

Total bacterial 16S rRNA and total fungal gene copy numbers was determined using LightCycler® 480 SYBR Green I Master mix (Roche Life Science). Reactions were performed in triplicate with 7.5 µL master mix, 0.2 µL of each primer (0.4 mM), 0.2 µL of BSA (0.4 mg/ml) and 2 ng of template DNA. Details of primers and cycling conditions are listed below. All qPCR reactions were carried out in a BioRad C1000 Touch thermal cycler CFX96 Real-Time system (Bio-Rad Laboratories, USA). Calibration curves for each gene target were constructed from ten-fold serial dilutions from cloned PCR products generated using TOPO-TA cloning kit (Invitrogen) following the manufacturer's instructions. Amplification of target genes was verified by gel electrophoresis. Data is expressed as gene copies per gram dry weight soil.

Gene name	Primer name	Primer sequence (5'–3')	Product size (bp)	Source	mplification onditions
16S	Eub338-F	ACTCCTACGGGAGGCAGCAG	200	Fierer et al., 2005	95 °C – 5 min; 40 cycles of: 95 °C 15s, 53 °C 15s, 72 °C 20s
	Eub518-R	ATTACCGCGGCTGCTGG			
ITS	5.8s	CGCTGCGTTCTTCATCG		Fierer et al., 2005	95 °C – 3 min; 40 cycles of: 95 °C 15s, 53 °C 15s, 72 °C 20s
	ITS1f	TCCGTAGGTGGACCTGCGG			

Fierer, N, Jackson, J, Vilgalys, R, Jackson RB. 2005. Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. Appl. Environ. Microbiol. 71 4117-4120; DOI: 10.1128/AEM.71.7.4117-4120.2005

APPENDIX VI – Microbial Community Analysis

The diversity and composition of bacteria (16s rRNA) and fungal community were determined using Miseq Illumina profiling of ribosomal genes (Illumina Inc.) using the primer pairs 341F/805R[10,11] and ITS4[12] for bacteria and fungi respectively.

A manifest file that contains the absolute filepath of each fastq file was created using a python code. All raw Illumina sequencing data of144 fastq files were imported into QIIME2 using this manifest file[13]. QIIME plugin of dada2 was used for sequence quality control and feature table construction[14]. In this step, the forward and reverse primers were trimmed off and the forward and reverse sequences were truncated at 260 bp and 240 bp respectively to maintain those sequences with quality score >20 and were then merged. All 72 samples were normalized to 7,000 sequences, and Alpha diversity including Shannon index, observed species, Simpson index and Predicated (chao1) were then calculated respectively. Feature classifiers were trained on QIIME released 13_8 99% UNITE fungal OTUs, which was applied to our sequences for taxonomic analysis.

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APPENDIX VII - Statistical summary tables

Appendix VII.1 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on soil properties at Boorowa. Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	Total Soil C (%)	Total Soil N (%)	Soil C:N
Treatment	***P* = 0.031** F = 3.368, df = 3,31	*P* = 0.078 F = 2.501, df = 3,31	***P* < 0.001** F = 9.544, df = 3,31
Depth	***P* < 0.001** F = 182.091, df = 3,31	***P* < 0.001** F = 126.293, df = 3,31	***P* < 0.001** F = 132.602, df = 3,31
Treatment x Depth	0.471 F = 0.861, df = 3,31	*P* = 0.076 F = 2.526, df = 3,31	***P* < 0.001** F = 12.279, df = 3,31

[$] for Untreated control, 0-10cm n = 4 as T4.S1 (0-10cm) was removed from the analysis as an outlier (%C = 3.95, %N = 0.26. CN = 15.31).

Appendix VII.2 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on catabolic diversity index (H') and substrate induced respiration in Boorowa soils. Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	H'	Basal Respiration	Glucose-Induced Respiration	Lignin Induced Respiration
Treatment	*P* = 0.063 F = 2.686, df = 3,32	***P* = 0.004** F = 5.330, df = 3,32	***P* = 0.007** F = 4.786, df = 3,32	***P* = 0.036** F = 3.222, df = 3,32
Depth	*P* = 0.185 F = 1.837, df = 3,32	***P* = 0.008** F = 7.883, df = 3,32	***P* < 0.001** F = 29.532, df = 3,32	*P* = 0.185 F = 4.913, df = 3,32
Treatment x Depth	0.564 F = 0.692, df = 3,32	*P* = 0.882 F = 0.220, df = 3,32	***P* = 0.031** F = 3.365, df = 3,32	*P* = 0.291 F = 1.302, df = 3,32

Appendix VII.3 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on substrate induced respiration rates of groups of c-compounds in Boorowa soils. Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	Total Carbohydrates	Total Amino Acids	Total Carboxylic Acids
Treatment	***P* = 0.010** F = 4.430, df = 3,32	*P* = 0.345 F = 1.147, df = 3,32	*P* = 0.653 F = 0.549, df = 3,32
Depth	***P* < 0.001** F = 20.893, df = 3,32	***P* < 0.001** F = 20.748, df = 3,32	*P* = 0.099 F = 2.891, df = 3,32
Treatment x Depth	*P* = 0.063 F = 2.693, df = 3,32	*P* = 0.140 F = 1.957, df = 3,32	*P* = 0.432 F = 0.941, df = 3,32

Appendix VII.4 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on potential soil enzyme activities in Boorowa soils. Significant differences are highlighted in bold. *df* is degrees of freedom. BG is β-Glucosidase, AG is α-glucosidase, CB is Cellobiohydrolase, XY is β-Xylosidase, NAG is N-acetyl-β-D-glucosaminidase, LAP is Leucine aminopeptidase, PHOS is phosphatase. Total C is BG+AG+CB+XY. Total N is NAG+LAP.

Factor	BG	AG	CB	XYL	NAG	LAP	PHOS	Total C	Total N
Treatment	***P* < 0.001** F = 21.341, df = 3,32	***P* < 0.001** F = 20.601, df = 3,32	***P* < 0.001** F = 15.527, df = 3,32	***P* < 0.001** F = 20.873, df = 3,32	*P* = 0.063 F = 2.687, df = 3,32	***P* = 0.043** F = 3.050, df = 3,32	***P* < 0.001** F = 24.560, df = 3,32	***P* < 0.001** F = 16.652, df = 3,32	***P* = 0.021** F = 3.706, df = 3,32
Depth	***P* < 0.001** F = 57.279, df = 3,32	***P* = 0.004** F = 9.846 df = 3,32	***P* < 0.001** F = 31.091, df = 3,32	***P* < 0.001** F = 19.798, df = 1,16	*P* = 0.630 F = 0.236, df = 3,32	***P* < 0.001** F = 32.426 df = 3,32	***P* = 0.007** F = 8.154, df = 3,32	***P* < 0.001** F = 43.281, df = 3,32	***P* = 0.018** F = 6.178, df = 3,32
Treatment x Depth	***P* = 0.032** F = 3.323, df = 3,32	***P* < 0.001** F = 30.514, df = 3,32	***P* < 0.001** F = 10.768, df = 3,32	***P* = 0.001** F = 6.827, df = 3,32	*P* = 0.338 F = 1.166, df = 3,32	*P* = 0.412 F = 0.986, df = 3,32	***P* < 0.001** F = 8.680, df = 3,32	***P* < 0.001** F = 12.429, df = 3,32	*P* = 0.171 F = 1.178 df = 3,32

Appendix VII.5 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on bacterial and fungal gene copies and fungal:bacterial ratios in Boorowa soils.

Factor	Bacterial 16S rRNA gene copies	Fungal gene copies[$]	Fungal:Bacterial Ratios[#]
Treatment	***P* = 0.005** F = 5.273, df = 3,32	***P* < 0.001** F = 27.195, df = 3,30	***P* < 0.001** F = 8.148, df = 3,30
Depth	***P* < 0.001** F = 43.299, df = 3,32	***P* < 0.001** F = 27.845 df = 3,30	***P* < 0.001** F = 3.082 df = 3,30
Treatment x Depth	*P* = 0.232 F = 1.503, df = 3,32	***P* = 0.584** F = 0.659, df = 3,30	***P* = 0.426** F = 0.956, df = 3,30

[$] Data was log-transformed to fit the assumptions of the test. [#] Data was arcsin(sqrt) transformed to fit the assumptions of the test.

Appendix VII.6 *P* and F values calculated by one-way ANOVA showing the effects of treatment on wheat grain properties and nutrient content at Boorowa. Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	***P* value**	**F value**	**df**
Grain number per head	*P* = 0.126	F = 2.211	df = 3,16
Grain mass per head	*P* = 0.028	F = 3.926	df = 3,16
Mass per grain	*P* = 0.886	F = 0.213	df = 3,16
% C	*P* = 0.711	F = 0.465	df = 3,16
% N	*P* = 0.583	F = 0.669	df = 3,16
% P	*P* = 0.961	F = 0.096	df = 3,16
% K	*P* = 0.990	F = 0.037	df = 3,16
% S	*P* = 0.392	F = 1.063	df = 3,16
% Mg	*P* = 0.874	F = 0.230	df = 3,16
% Ca	*P* = 0.999	F = 0.008	df = 3,16
Cu (mg Kg^{-1})	*P* = 0.194	F = 1.767	df = 3,16
Zn (mg Kg^{-1})	***P* = 0.019**	**F = 4.445**	**df = 3,16**
Mn (mg Kg^{-1})	***P* = 0.039**	**F = 3.536**	**df = 3,16**
Fe (mg Kg^{-1})	*P* = 0.141	F = 2.096	df = 3,16
Si (mg Kg^{-1})	*P* = 0.586	F = 0.665	df = 3,16

Appendix VII.7 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on soil properties at Laggan. Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	Total Soil C (%)	Total Soil N (%)	Soil C:N
Treatment	***P* < 0.001** F = 40.21, df = 1,16	***P* < 0.001** F = 48.530, df = 1,16	***P* > 0.05** F = 0.711, df = 1,16
Depth	***P* < 0.001** F = 91.649, df = 1,16	***P* < 0.001** F = 23.679, df = 1,16	***P* > 0.05** F = 0.418, df = 1,16
Treatment x Depth	***P* > 0.05** F = 0.803, df = 1,4	***P* > 0.05** F = 0.711, df = 1,16	***P* > 0.05** F = 0.485, df = 1,16

Appendix VII.8 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on catabolic properties in Laggan soils. Within a depth class, values sharing the same letter are not significantly different (P > 0.05). Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	H'	Basal Respiration	Glucose-Induced Respiration	Lignin Induced Respiration
Treatment	*P* = 0.755 F = 0.101, df = 1,16	*P* = 0.033 F = 5.442, df = 1,16	*P* = 0.089 F = 3.272, df = 1,16	*P* = 0.398 F = 0.756, df = 1,16
Depth	*P* = 0.847 F = 0.038, df = 1,16	*P* = 0.102 F = 3.016, df = 1,16	*P* = 0.081 F = 3.460, df = 1,16	*P* = 0.192 F = 1.860, df = 1,16
Treatment x Depth	*P* = 0.866 F = 0.030, df = 1,16	*P* = 0.932 F = 0.001, df = 1,16	*P* = 0.069 F = 3.801, df = 1,16	*P* = 0.337 F = 0.980, df = 1,16

Appendix VII.9 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on catabolic properties in Laggan soils. Within a depth class, values sharing the same letter are not significantly different (P > 0.05). Significant differences are highlighted in bold. *df* is degrees of freedom.

Factor	Total Carbohydrates	Total Amino Acids	Total Carboxylic Acids
Treatment	*P* = 0.172 F = 2.049, df = 1,16	*P* = 0.121 F = 2.678, df = 1,16	*P* = 0.275 F = 1.276, df = 1,16
Depth	*P* = 0.986 F = 0.00, df = 1,16	*P* = 0.179 F = 1.977, df = 1,16	*P* = 0.079 F = 3.511, df = 1,16
Treatment x Depth	*P* = 0.209 F = 1.714, df = 1,16	*P* = 0.333 F = 0.997, df = 1,16	*P* = 0.438 F = 0.633, df = 1,16

Appendix VII.10 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on potential soil enzyme activities Laggan soils. Significant differences are highlighted in bold. *df* is degrees of freedom. BG is β-Glucosidase, AG is α-glucosidase, CB is Cellobiohydrolase, XY is β-Xylosidase, NAG is N-acetyl-β-D-glucosaminidase, LAP is Leucine aminopeptidase, PHOS is phosphatase. Total C is BG+AG+CB+XY. Total N is NAG+LAP.

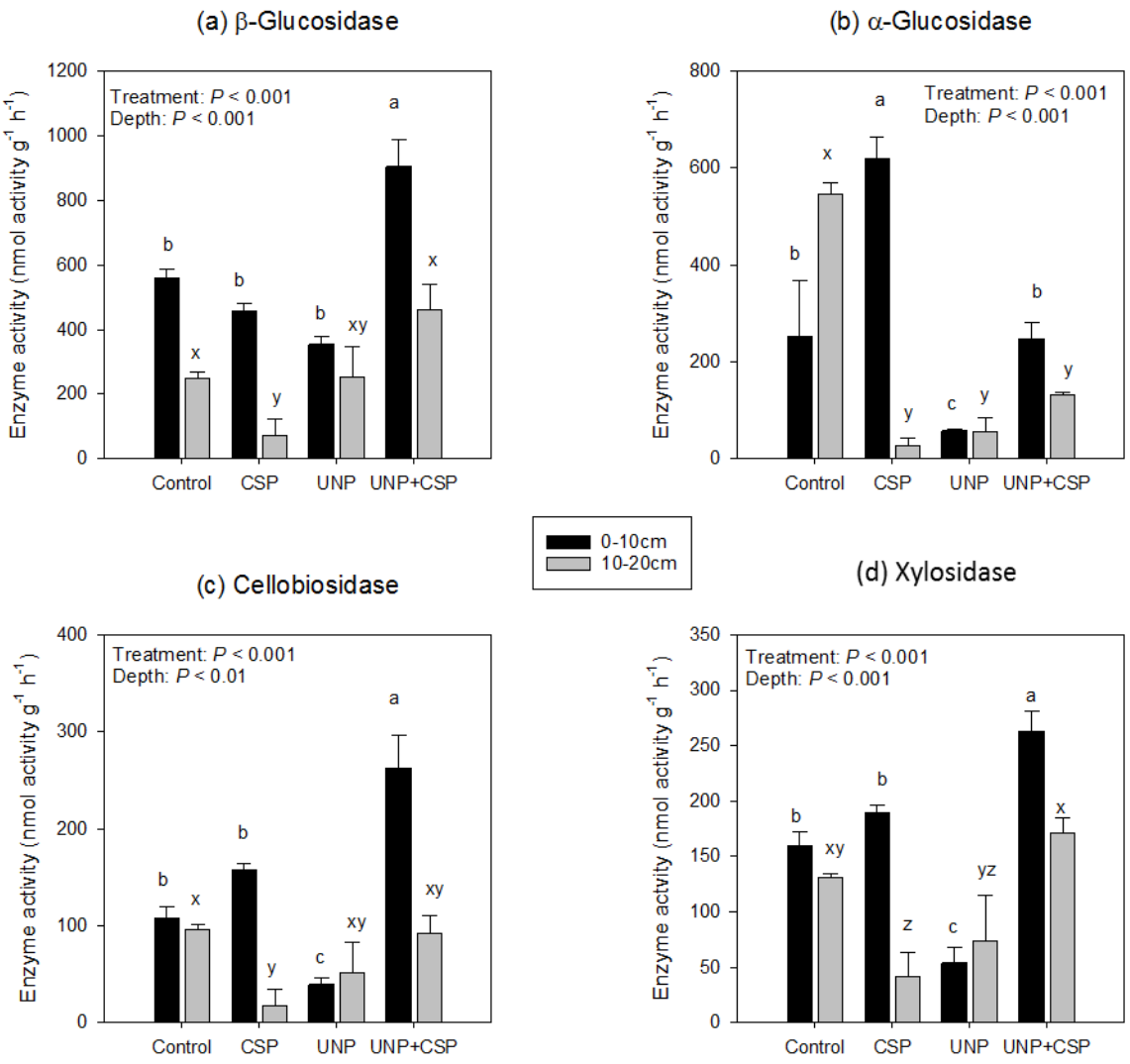
Factor	BG	AG	CB	XYL	NAG	LAP	PHOS	Total C	Total N
Treatment	*P* = 0.973 F = 0.001, df = 1,16	*P* = 0.289 F = 1.204, df = 1,16	***P* = 0.010** F = 8.666, df = 1,16	*P* = 0.055 F = 4.281, df = 1,16	***P* < 0.001** F = 24.430, df = 1,16	*P* = 0.567 F = 0.341, df = 1,16	***P* < 0.001** F = 20.707, df = 1,16	*P* < 0.602 F = 0.284, df = 1,16	***P* < 0.015** F = 7.349, df = 1,16
Depth	*P* = 0.091 F = 3.235, df = 1,16	*P* = 0.113 F = 2.815, df = 1,16	*P* = 0.283 F = 1.236, df = 1,16	*P* = 0.167 F = 2.092, df = 1,16	*P* = 0.215 F = 1.666, df = 1,16	*P* = 0.241 F = 1.481, df = 1,16	***P* = 0.012** F = 8.055, df = 1,16	*P* = 0.090 F = 3.249, df = 1,16	*P* = 0.825 F = 0.051, df = 1,16
Treatment x Depth	***P* = 0.012** F = 8.114, df = 1,16	*P* = 0.154 F = 2.241, df = 1,16	***P* = 0.033** F = 5.413, df = 1,16	***P* = 0.001** F = 14.922, df = 1,16	***P* < 0.001** F = 17.065, df = 1,16	*P* = 0.926 F = 0.009, df = 1,16	*P* = 0.10 F = 8.530, df = 1,16	***P* = 0.002** F = 12.897, df = 1,16	*P* = 0.063 F = 3.989, df = 1,16

Appendix VII.11 *P* and F values calculated by two-way ANOVA showing the effects of treatment and depth and their interaction on bacterial and fungal gene copies and fungal:bacterial ratios in Laggan soils.

Factor	Bacterial 16S rRNA gene copies	Fungal gene copies[$]	Fungal:Bacterial Ratios[#]
Treatment	***P* = 0.003** **F = 12.476,** **df = 1,16**	*P* = 0.114 F = 2.803, df = 1,16	***P* = 0.003** F = 12.476, df = 1,16
Depth	*P* = 0.837 F = 0.0439, df = 1,16	*P* = 0.074 F = 3.662, df = 1,16	*P* > 0.05 F = 0.0439, df = 1,16
Treatment x Depth	*P* = 0.844 F = 0.0399, df = 1,16	***P* = 0.034** **F = 5.343,** **df = 1,16**	*P* > 0.05 F = 0.0399, df = 1,16

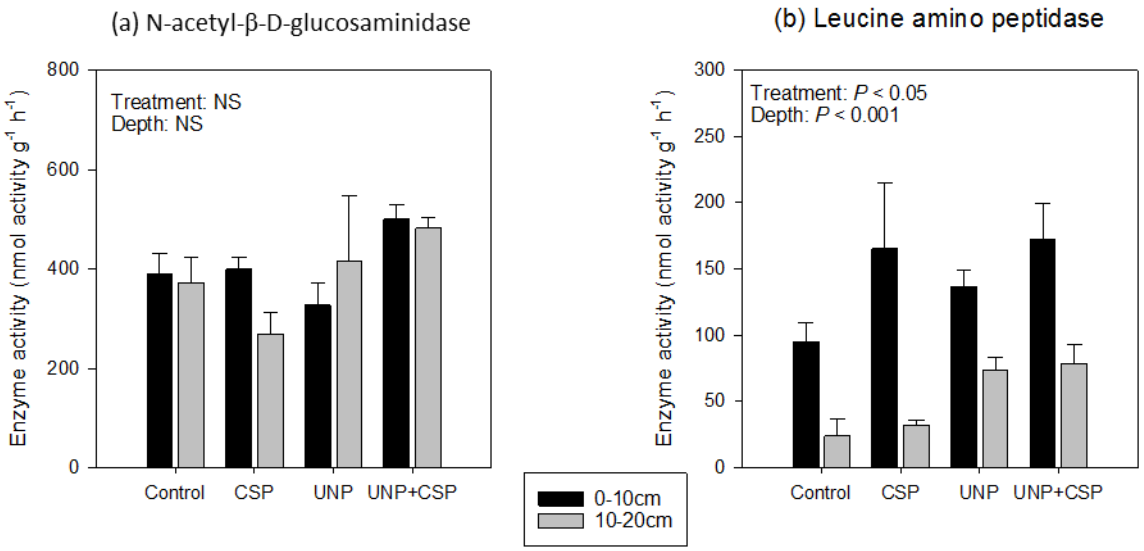
[$] Data was log-transformed to fit the assumptions of the test. [#] Data was arcsin(sqrt) transformed to fit the assumptions of the test. Field replicate 3 ($1.43x10^9$) and 5 ($1.43x10^9$)

APPENDIX VIII - Potential soil enzyme activities associated with C-cycling - Boorowa



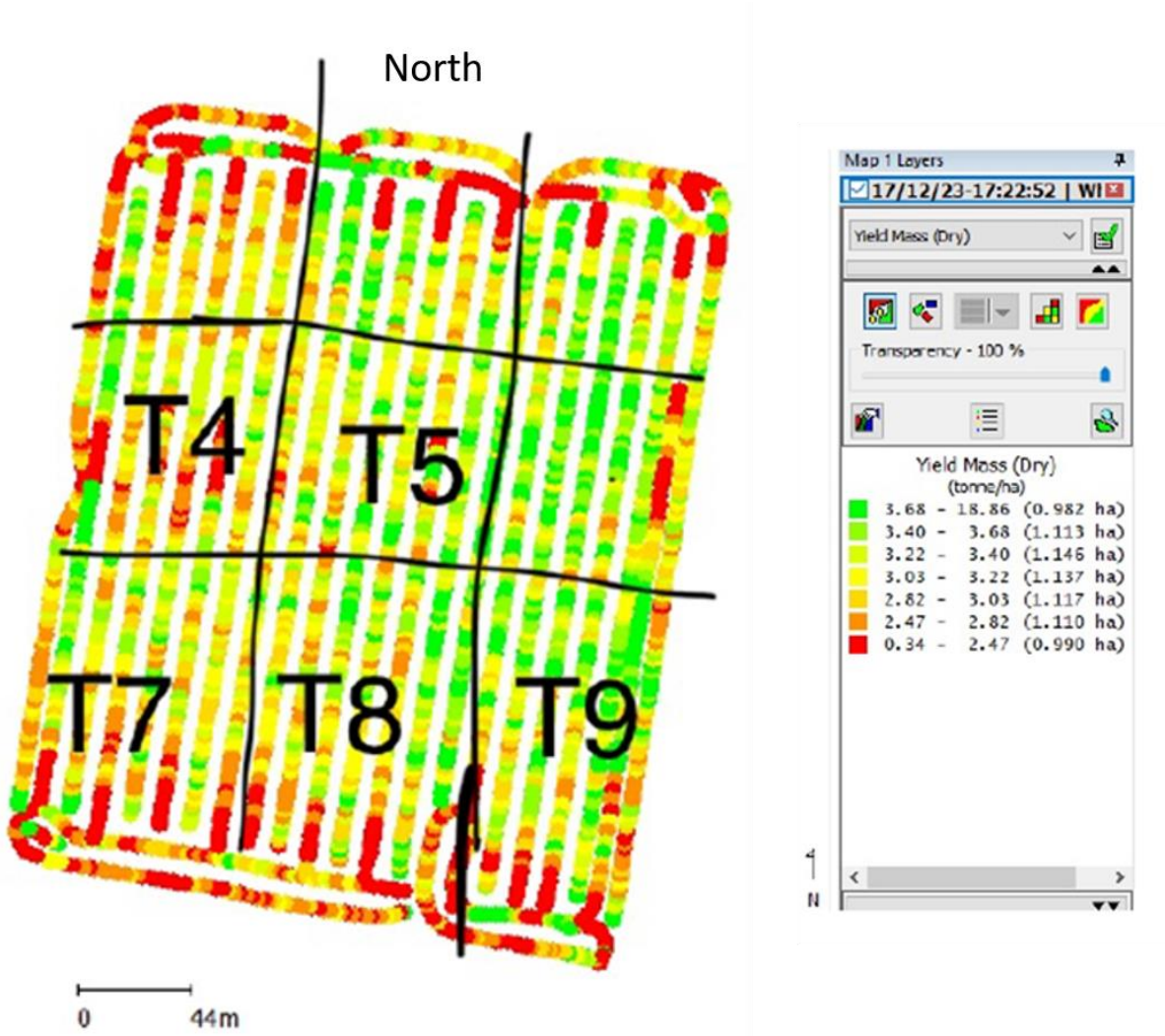
Potential soil enzyme activities associated with C-cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Boorowa. Control is untreated control, CSP is Converte Seed Primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte Seed Primer. Error bars are ± one standard error (n=5). Within a soil layer, bars sharing the same letter are not significantly different ($P > 0.05$).

APPENDIX IX - Potential soil enzyme activities associated with N-cycling - Boorowa



Potential soil enzyme activities associated with N cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Boorowa. Control is untreated control, CSP is Converte Seed Primer, UNP is Converte UNP, UNP+CSP is Converte UNP plus Converte Seed Primer. Error bars are ± one standard error (n=5). Within a soil layer, bars sharing the same letter are not significantly different ($P > 0.05$).

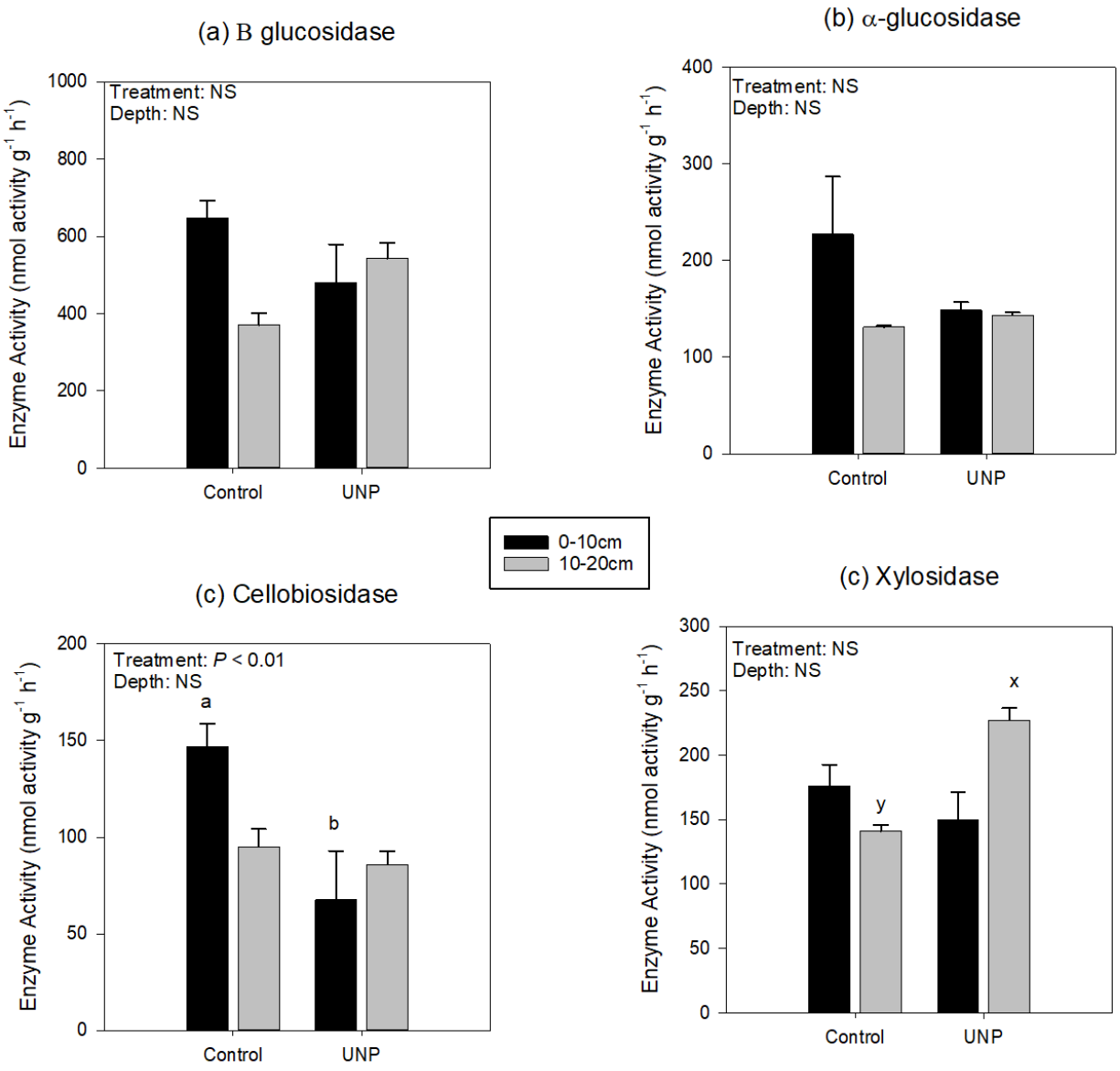
APPENDIX X – Yield map for Boorowa Trial Plot 2017



Yield map for Boorowa wheat trial 2017 (image provided by John Ridley, Converte Pty. Ltd.) Data is derived directly from the grain yield monitor at time of harvest with approximate location of treatment plots superimposed. T4 is untreated control, T5 is Converte Seed primer, T7 is Converte UNP, T8 is Converte seed primer plus UNP. T9 is not applicable to this report.

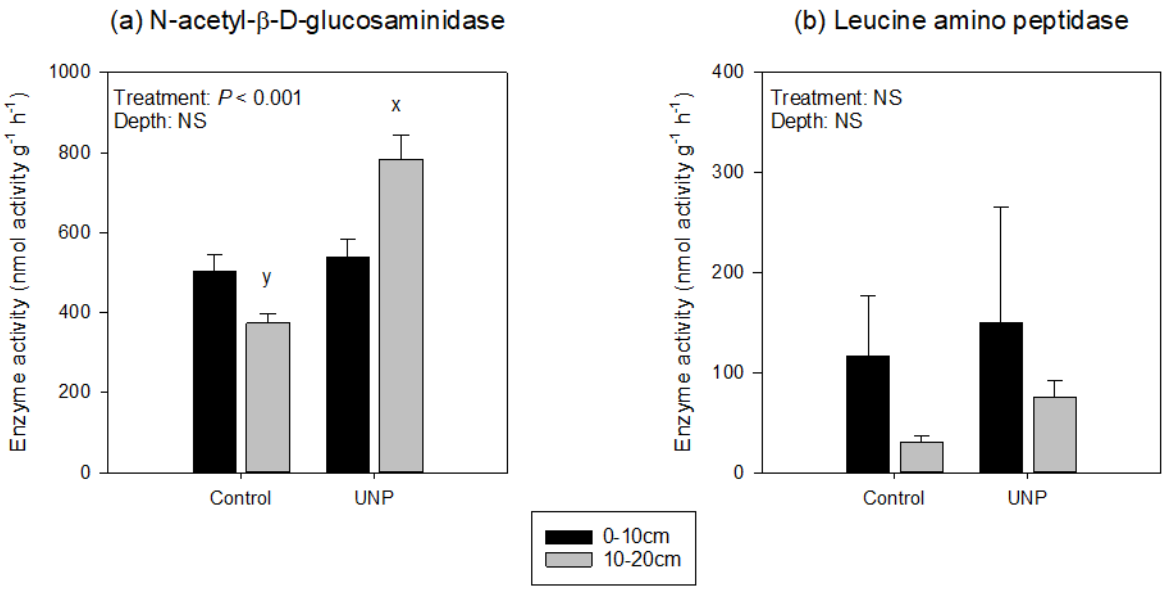
It is noted that areas flooded in recent years along the northern edge of the paddock have generally underperformed the other areas of the paddock, and there is a noticeable edge effect due to increased applications as spray equipment turns. Spatial variation in other soil parameters across the field, such as high zinc along the western edge must be taken into consideration when interpreting these results (John Ridley, pers. com.).

APPENDIX XI - Potential soil enzyme activities associated with C-cycling – Laggan



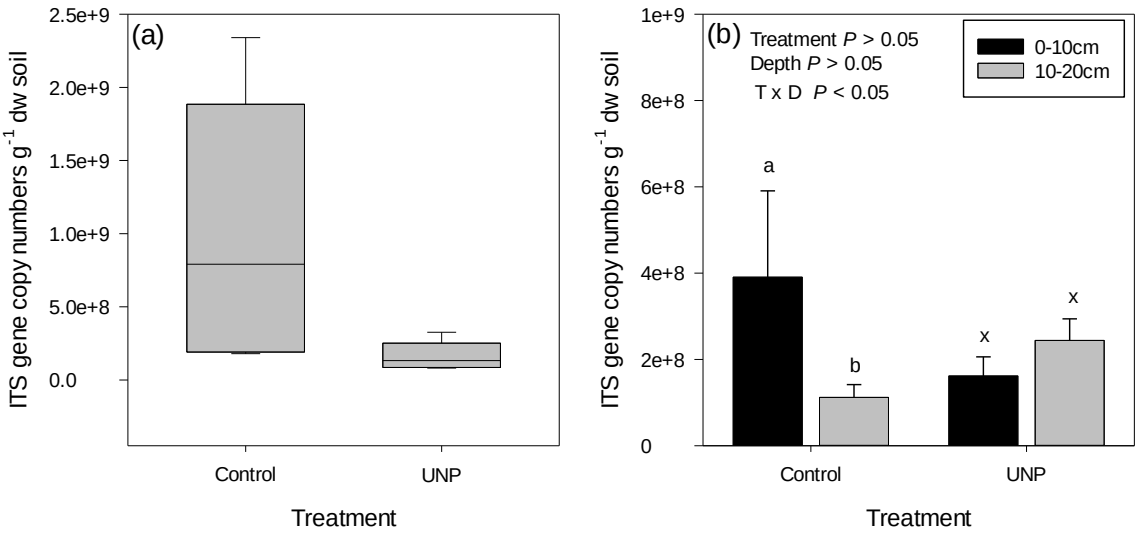
Potential soil enzyme activities associated with C-cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Laggan. Control is untreated control, UNP is Converte UNP. Error bars are ± one standard error (n=5). Within a soil layer, bars with different letters are significantly different ($P < 0.05$).

APPENDIX XII - Potential soil enzyme activities associated with N-cycling – Laggan



Potential soil enzyme activities associated with N cycling of 0-10 cm (black bars) and 10-20 cm (grey bars) soil receiving different treatments at Laggan. Control is untreated control, UNP is Converte UNP. Error bars are ± one standard error (n=5). Within a soil layer, bars with different letters are significantly different ($P < 0.05$).

APPENDIX XIII – Fungal Gene copy numbers - Laggan



(a) Box plot showing the variation in fungal gene copy number in control and UNP-treated soil (0-10cm) at Laggan. (b) Fungal ITS gene abundance in 0-10cm (black bars) and 10-20 cm (grey bars) soils under different treatments. UNP is Converte Universal nutrient plantfood. Error bars are ± one standard error (n = 5). Within a treatment, bars with different letters are significantly different ($P < 0.05$).