Title page

Title:

Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content.

Authors:

Pankaj Trivedi[1,2*], Manuel Delgado-Baquerizo[1,3], Thomas C Jeffries[1], Chanda Trivedi[1], Ian C Anderson[1], Matthew McNee[4], Kenneth Flower[5], Bhupinder Pal Singh[6], David Minkey[4], Brajesh K. Singh[1,7*]

[1] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith South, NSW 2751, Australia

[2] Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523, USA

[3] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA

[4] Western Australian No-Tillage Farmers Association, Leeuwin Centre, CSIRO, 65, Brockway Road, Floreat, WA 6014, Australia.

[5] School of Plant Biology and Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[7] NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, NSW 2568, Australia

[7] Global Centre for Land Based Innovation, Western Sydney University, Building L9, Locked Bag 1797, Penrith South, NSW 2751, Australia

***Authors for correspondence:** Pankaj Trivedi, Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523, USA

Phone: (+1)9704914019

Email: Pankaj.trivedi@colostate.edu; p.trivedi@westernsydney.edu.au

Brajesh K Singh, Global Centre for Land Based Innovation, Western Sydney University, Building L9, Locked Bag 1797, Penrith South, NSW 2751, Australia

Phone: (+61) 245701329

Email: b.singh@westernsydney.edu.au

Running title: Microbes in soil aggregates impact carbon content

Originality-Significance Statement

This work explores the relative contribution of microbial groups to soil organic carbon (SOC) turnover in different aggregate-size fractions under contrasting management practices. This work is significant, as it demonstrates that different aggregate sizes and their associated microbial communities modify the effects of management practices on soil C turnover and these are more pronounced in macro- compared to micro-aggregates. Specialised soil microbes present in the micro-aggregates modulate the retention of recalcitrant forms of C in the micro-aggregates thus contributing towards soil C storage.

Summary

Soil carbon (C) stabilization is known to depend in part on its distribution in structural aggregates, and upon soil microbial activity within the aggregates. However, the mechanisms and relative contributions of different microbial groups to C turnover in different aggregates under various management practices remain unclear. The aim of this study was to determine the role of soil aggregation and their associated microbial communities in driving the responses of soil organic matter (SOM) to multiple management practices. Our results demonstrate that higher amounts of C inputs coupled with greater soil aggregation in residue retention management practices has positive effects on soil C content. Our results provide evidence that different aggregate size classes support distinct microbial habitats which supports the colonization of different microbial communities. Most importantly our results indicate that the effects of management practices on soil C is modulated by soil aggregate sizes and their associated microbial community and are more pronounced in macro-aggregate compared to micro-aggregate sizes. Based on our findings we recommend that differential response of management practices and microbial control on the C turnover in macro-aggregates and micro-aggregate should be explicitly considered when accounting for management impacts on soil C turnover.

Key words:

Soil aggregates; Residue management; Soil carbon; Extracellular enzymatic activities; Microbial community

Introduction

Soil organic matter (SOM) is an essential component of soil fertility as it comprises almost 50% of carbon (C) in terrestrial biosphere; holds nutrients for plant uptake; promotes the structure, biological and physical health of soil; provides a buffer against harmful substances; and partially mitigates anthropogenic gas emissions (Victoria *et al*., 2012). Soil C levels have dropped by up to half of pre-agricultural levels in many areas because of agricultural activities such as fallowing, cultivation, stubble burning or removal and overgrazing (Chan 2008; Smith *et al*., 2016). With 40% of the Earth's surface currently occupied by agricultural activities (Hooke *et al*., 2012), there is an urgent need to develop innovative management practices not only to increase productivity to feed an ever increasing human population but also to support soil C storage in agro-ecosystems (Lal, 2004; Foley *et al*., 2005).

Conservation agriculture is one such agricultural practice that represents a set of three crop management principles: (1) no/minimal till; (2) residue retention; and (3) crop rotation (Coughenor and Chamala, 2000; Hobbs *et al*., 2008; Pittelkow *et al*., 2015). There are examples where conservation agriculture practices have been widely adopted (e.g. in Western Australia), however there is still an incomplete understanding of the challenges, limitations, and potential of conservation agriculture in impacting yields, C turnover, and sequestration (Tscharntke *et al.*, 2012; Pittelkow *et al*., 2015) which constrains its broad adoption in large-scale farm management (Tscharntke *et al*., 2012).

Changes in land-use or management practices are known to impact soil C turnover but the underlying mechanisms are largely unknown. Thus, there is growing interest to elucidate the relationships between management practices and below-ground processes and to seek a mechanistic understanding of the contribution of soil biota and associated processes to ecosystem functioning (Wardle *et al*., 2004; Xiao *et al*., 2007; De Deyn *et al*., 2008; Jin *et al*., 2010). In agro-ecosystems, soil microorganisms have an integral role in virtually all

ecosystem processes including nutrient cycling and decomposition of organic matter (Singh *et al*., 2010; Trivedi *et al*., 2013). The rates to which these processes occur are largely dictated by the functional diversity of microbial taxa (Heemsbergen *et al*., 2004). An improved knowledge of the interactions between management practices and microbial communities in facilitating SOM decomposition and soil C sequestration is increasingly recognized as key to improve farm productivity and sustainability (Singh *et al*., 2010; MacDonald and Singh, 2013). However, owing to the complexity of below-ground processes as well as technical difficulties to experimentally manipulate soil microbial structures and activities, significant gaps remain in our current understanding on how soil microbial communities are controlled by complex interactions of biotic and abiotic site factors and how the structural shifts in soil microbial communities are linked to alterations of their functioning, such as in mediating SOM dynamics (Hackl *et al*., 2005; Brockett *et al*., 2012).

A particularly important variable controlling SOM dynamics and soil fertility is the process of soil aggregation (Gupta and Germida, 1998; Six *et al*., 2004; 2006). SOC turnover in different sized soil aggregates is influenced by a number of variables including management practices, organic matter distribution and protection, mechanical disturbance, temperature and moisture, along with functional groups of soil microbes (Six *et al*., 2002; 2006; Tiemann *et al*., 2015; Trivedi *et al*., 2015). Moreover, current data suggests that arable systems with less mechanical disturbance store more soil C which is correlated with an increase in micro-aggregate formation (Six *et al*., 2006; Trivedi *et al*., 2015). Microbial community functions such as the production of extracellular enzymes and aggregate binding agents, and decomposition of aggregate-associated SOM, have previously been linked to aggregate stabilization and SOM persistence/accrual (Six *et al*., 2006; Wilson *et al*., 2009; Tiemann and Grandy, 2015). According to "resource economic theory" (Litchman and Klausmeier, 2008) trade-offs in life-history strategies of soil microbes result in growth trait

variation and impact soil C turnover. It has been postulated that spatial distribution of resources within different sized soil aggregates provides niches for the heterogeneous distribution of contrasting functional groups of microorganisms that regulates ecosystem-level decomposition and nutrient mineralization (Davinic *et al*.; 2012; Tiemann and Grandy, 2015; Trivedi *et al*., 2015). Despite this knowledge, the mechanisms and relative contributions of different microbial groups to SOC turnover in different aggregates remains a topic of debate.

In recent years, many studies have addressed the role of soil microbes across different soil types and management regimes to explain soil C dynamics in agricultural systems (Trivedi et al., 2016 and references within). However, we have a very limited understanding on the interactions between different micro-environments (i.e. aggregate-size fractions) and their resident microbial communities and how their interactions regulate C process rates at cropping system scale. The aim of this study was to determine linkages between different aggregate sizes and their bacterial communities, which control SOM storage in response to different management practices, including rotations and residue management practices in a no-till system. Furthermore, we evaluate the relative importance of agricultural management, microbial communities, and soil C on the activities of enzymes involved in C degradation in aggregates of different sizes. We hypothesized that management practices will have a strong influence on the structure of microbial communities and their associated functions in larger sized aggregates where greater availability of labile forms of C will be linked to nutrient cycling and productivity; while these controls will decrease in lower sized aggregates where the deposition and retention of recalcitrant C in micro-aggregates that is protected from microbial attack will be more likely to explain stored C.

To test these hypotheses, we used a unique, established, experiment to determine the long-term benefits of the key components of conservation agriculture (crop residue retention,

diverse rotations, minimal soil disturbance and controlled traffic) in Western Australian cropping systems (Table 1). We separated the soils into three aggregate size fractions that captures both the earliest stages of SOM formation and stabilization [mega-aggregate (>2 mm) and macro-aggregate (0.25-2mm] and longer term, more stable SOM that is protected from microbial attack [micro-aggregate (0.053-0.25 mm)] (Tiemann and Grandy, 2015). Moreover, here for the first time we used MiSeq technology to determine the bacterial community composition in different aggregate fractions. Finally, we assessed linkages between soil aggregates, management practices, soil bacterial community, and their associated functions involved in soil C degradation.

Results

Aggregate size distribution and stability

Aggregate size distributions varied with management practices, with more water stable mega-aggregates in residue retention plots as compared to residue removed ($P < 0.001$) (Figure 1). Within residue retention treatments BOW(P1)-FR and LCW(P2)-FR have higher water stable mega-aggregates as compared to WWW(P3)-FR suggesting that crop rotation effects the amount of water stable mega-aggregates. We found the greatest mega-aggregate stability in WWW(P3)-FR treatment. Overall the mega-aggregate stability of residue retentention treatments were significantly greater than residue removed ($P < 0.001$) treatments. Total C ($P < 0.01$) and nitrogen ($P < 0.01$) in bulk soil was greater in residue retained as compared to residue removed treatments (Supplementary Fig. 1).

Bacterial community structure in different aggregate fractions

CAP analysis plot of taxa (Bray-Curtis) revealed that the bacterial community was markedly different between aggregates wherein samples from different sized aggregates

formed distinct clusters in the ordination plot (Permanova, $P = 0.001$; Fig. 2). We observed differences between treatments only in mega-aggregates (Permanova, $P = 0.01$) where full residue retention treatments formed seperate cluster from low residue treatments (Fig. 2).

Our analysis further described the microbial groups that drive the differences between mega-, macro-, and micro-aggregates (Fig. 2; Supplementary Fig. 2). We verified trends from MiSeq analysis by performing taxon specific qPCR analysis of selected bacterial groups. Our results showed that both the analysis of relative abundances of various bacterial groups as determined by qPCR and MiSeq analysis are highly correlated ($P< 0.0001$; Supplementary Table 1). This improved our confidence about the relative abundances of various bacterial groups in different aggregates from the dataset obtained by MiSeq analysis. The qPCR analysis also revealed that the total number of bacteria were significantly different ($P <0.001$) between aggregates and followed the order mega-<macro-<micro-aggregates. We observed significantly higher relative abundance of *Acidobacteria* ($P = 0.014$); *Armatimonadetes* (formerly known as OP10; $P< 0.0001$); *Chloroflexi* ($P = 0.017$); *Gemmatimonadetes* ($P< 0.0001$); *Plactomycetes* ($P< 0.0001$); δ-Proteobacteria ($P< 0.001$) and *Verrucomicrobia* ($P< 0.001$) in micro- as compared to mega-aggregates (Supplementary Fig. 2). Similar trends were observed while comparing the relative abundances of these groups between micro-aggregates (all higher) and macro-aggregates. The relative abundance of *Actinobacteria* ($P< 0.0001$), *Alphaproteobacteria* ($P < 0.001$; Bacteroidetes ($P < 0.001$); *Betaproteobacteria* ($P< 0.0001$); *Gammaproteobacteria* ($P < 0.001$) was higher in mega-aggregates as compered to microaggregates. As can be expected the relative abundance of different groups in macro-aggregates was intermediate between mega- and micro-aggregate.

In mega- and macro-aggregate we observed significant differences in the abundance of various bacterial groups according to treatments (Supplementary Fig. 3a and 3b) while we didn't observe treatment differences in the micro-aggregates (Supplementary Fig. 3c). In

mega-aggregates the relative abundances of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Planctomycetes*, *Betaproteobacteria*, and *Gammaproteobacteria* were significantly higher ($P< 0.001$) with full residue retention [BOW(P1)-FR, LCW(P2)-FR, and WWW(P3)-FR] as compared to low residue treatments [BOW(P1)-LR, LCW(P2)-LR, and BLW(P4)-LR]. In macro-aggregates the relative abundaces of *Bacteroidetes*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Verrucomicrobia* were significantly higher ($P< 0.001$) with residue retention [BOW(P1)-FR, LCW(P2)-FR, and WWW(P3)-FR] as compared to low residue treatments [BOW(P1)-LR, LCW(P2)-LR, and BLW(P4)-LR]. Conversely, in both mega- and macro-aggregates, the relative abundance of *Firmicutes* was about 4 fold higher in low residue as compared to residue retention treatments.

Soil chemical and biological properties

There were significant differences in soil chemical and biological properties ($P < 0.001$) between different sized aggregates (Table 2). Total carbon and nitrogen increased significantly ($P< 0.001$) with decreasing aggregate size. Total C content in different sized aggregates followed the order mega-aggregates (7.9±1.5 mg g^{-1}) < macro-aggregates (13.9±2.2 mg g^{-1}) < micro-aggregates (25.0±1.3 mg g^{-1}). Similarly total N content in micro-aggregates (2.1±0.10 mg g^{-1}) was two and five times higher than in the macro-aggregates (1.1±0.10 mg g^{-1}) and mega-aggregates (0.4±0.1 mg g^{-1}), respectively. In contrast, the dissolved organic carbon (DOC) content was significatly higher ($P< 0.01$) in mega-aggregates (85.38±8.62 mg $100g^{-1}$) as compared to macro-aggregate (67.90 ± 6.46 mg $100g^{-1}$) and micro-aggregates (56.77±3.49 mg $100g^{-1}$). On the other hand content of recalcitrant C was highest in micro-aggregate (116.88±7.50 mg $100g^{-1}$) as compared to macro-aggregate (85.83 ± 8.42 mg $100g^{-1}$) and mega-aggregate (71.66±6.90 mg $100g^{-1}$). Basal respiration

differed significantly ($P < 0.001$) between aggregates and was 0.98 ±0.15; 1.41±0.13; and 2.55±0.18 μg CO_2–C g^{-1} $h^{-1}$for mega-, macro-, and micro-aggregates, respectively.

We observed significant effects of treatments on various soil properties in different size aggregates (Table 2). In mega- and macro-aggregates total C, total N, DOC, recalcitrant C and basal repiration of residue retained treatments [BOW(P1)-FR, LCW(P2)-FR, and WWW(P3)-FR] was significatly higher ($P< 0.01$) than the equivalent low reidue [BOW(P1)-LR, LCW(P2)-LR] and BLW(P4)-LR treatments. There were no significant treatment effects in the contents of total C, total N, DOC, and recalcitrant C in micro-aggregates. In micro-aggregates the basal respiration of BLW(P4)-LR treatment was significantly greater ($P< 0.001$) than other treatments [BOW(P1)-FR, LCW(P2)-FR, P3-FRWWW(P3)-FR, BOW(P1)-LR, and LCW(P2)-LR].

Extracellular enzymatic activity

We observed a significant increase ($P< 0.0001$) in the activities of enzymes involved in the degradation of labile C (CB, AG, BG) with the decrease in the aggregate size (Fig. 3a-c). For example, the AG, BG, CB activities were 327.7±75.8; 1489.7±104.6; 326.1±32.4 and 502.7±12.3; 2038.6±111.8; 458.17±26.1 nmol h^{-1} g^{-1}dry soil for megaaggregates and microaggregates, respectively. On the other hand the activity of NAG which is involved in the degradation of a recalcitrant form of C (chitin) significantly increased ($P< 0.001$) with an increase in the aggregate size and was 277.8±27.5; 246.8±21.26 and 146.8±11.26 nmol h^{-1} g^{-1}dry soil for mega-aggregate, macro-aggregate, and micro-aggregates, respectively (Fig. 1d). The activity of all the extracellular enzymes was negatively correlated with total C ($P< 0.0001$) in the macro- and mega-aggregates. However, in micro-aggregates we didn't observe significant correlations between enzymatic activities and total C (Supplementary Table 2).

The activity of enzymes involved in breaking down C was higher with low residue retention than full residue retention, specifically in the macro and mega aggregates (Fig. 3a-d). In micro-aggregates, in majority of treatments there were no significant differences in the activity for all the four enzymes. In mega- and macro-aggregates the activity of CB, AG and BG was higher in BOW(P1)-LR, LCW(P2)-LR and BLW(P4)-LR treatments as compared to BOW(P1)-FR, LCW(P2)-FR and WWW(P3)-FR treatments. Similarly the activity of NAG involved in the degradation on relativeley recalcitrant form of C was lower in the FR than LR treatments the larger aggregates (Fig. 3d). We did not observe any significant trends between different philosophies within the same residue management treatment (fully retained or low residue), suggesting that residue management has higher effect on the production of extracellular enzymes in comparision to crop rotations.

To determine the C-degrading enzymatic activity per unit of C present in the samples we divided enzymatic activities by the amount of total C present in the respective samples. The enzymatic activities per unit C in the micro-aggregates was significantly ($P<0.001$) less for all the studied enzymes as compared to mega-aggregates and macro-aggregates for most of the treatments (Supplementary Fig. 4a-d). The trends between treatments were similar to those observed when presenting the absolute enzymatic activity within different aggregates. For example,in all the studied enzymes the activity per unit C of low residue [BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR] was significantly higher ($P< 0.001$) than residue retained [BOW(P1)-FR; LCW(P2)-FR] treatments in mega- and macro-aggregates. In micro-aggregates, there was no significant differences in the activity per unit C among treatments for all the four enzymes (Fig 3a-d).

Correlations between relative abundances of bacterial groups and soil biochemical properties

Relative abundance of various bacterial groups were significantly correlated with different biochemical properties across all the aggregate-size fractions in the soil (Table 3). The relative abundance of *Acidobacteria*, *Armatinomonadetes*, *Gemmatinonadetes*, *Planctomycetes*, and *Verrucomicrobia* was positively correlated with the concentration of total and recalcitrant C. In contrast the relative abundance of *Actinobacteria*, *Firmicutes*, and *Betaproteobacteria* was negatively corrrelated with the content of total C and recalcitrant C while being positively correlated with labile DOC. In most cases the bacterial groups that were negatively correlated with the concentration of total C were positively correlated with the extracellular enzymatic activities in the soil samples while an opposite trend was observed for bacterial groups that were positively correlated with the concentration of total C.

Direct and indirect effects of management practices, bacterial community composition, total C on the activity of C degrading enzymes

We used SEM approach to determine the direct and indirect effects of management practices, bacterial community composition, total C on the activities of different enzymes involved in C degradation (AG, BG, NAG, CB shown as functions in the SEM) in different sized aggregates (Fig. 4). SEM explained higher percent of variations in both the amount of C and enzymatic activities of mega-aggregates and macro-aggregates (all greater than 92.0%). However, in micro-aggregates SEM explained significantly lower variations in the amount of soil C (24%) and enzymatic activities (59%) as compared to both large sized aggregates. In mega-aggregates, management practices had a direct and significant effect on the structure of microbial communities; total C; and functions (Fig. 4a). The control of management practices on these same variables was maintained in macro-aggregates however, the effect was not as strong as observed for mega-aggregates. Effect of microbial community on functions was not apprant in mega- or macro-aggregates (Fig. 4b). In micro-aggregates the combined effect of

management practices on microbial community composition; total C and functions decreased significantly as compared to macro- and mega-aggregates (Fig. 4c). However, we observed a significant and direct effect of microbial community composition on the enzymatic activities that was not present in mega- or macro-aggregates. Interestingly, we observed a significant interaction between total C and microbial community structure only in micro-aggregates.

Discussion

To date, there are only a few studies describing the interactions between soil aggregate structure, crop management, microbes, and nutrient cycling (Davinic *et al*., 2012; Constancias *et al*., 2014; Trivedi et al., 2015; Tiemann *et al*., 2015) and to best of our knowledge limited studies have used advanced sequencing techniques (454 pyrosequencing; Davinic *et al*., 2012; Baily et al., 2013a,b; Constancias *et al*., 2014) to look at bacterial community across individual aggregates and none has employed the enhanced resolution of MiSeq technology to profile the bacterial diversity associated with different sized aggregates. The aim of the research undertaken here was to develop a framework of how soil structure interacts with microbial composition and activity, and management practices, and in particular how these interactions affect soil processes. The widely-held view that conservation principles like residue retention will increase C turnover is too general. Our results suggest that site/soil specific factors, in this case soil aggregate class structure, mediate the efficiency of C turnover due to residue retention. Our study provides new evidence that soil aggregate size and their associated microbial communities modify the effects of agricultural management, in this case residue management and crop rotation. Thus, this aspect should be considered when assessing the impact of management practices on soil health.

Impact of management practices on aggregate size distribution and stability

There is a broad body of literature demonstrating that management practices that implement residue retention possess more soil C as compared to conventional systems (Six *et al*., 2000; 2004; Roger-Estrade *et al*., 2010; Kong *et al*., 2011). This trend has been linked with an increased stability of soil aggregates, which in turn fosters a protective environment for soil C retention and reduced decomposition rate (Bronick and Lal, 2005; Ashagrie *et al*., 2007). Our results demonstrate that management practices impact soil aggregation and chemical/physical heterogeneity of soils resulting in variable effects on soil C content. It has been reported that management practices that increase soil biological activity and have positive effects on the stability of mega-aggregates, enhance C and N concentrations (Grandy and Robertson, 2007; Tiemann and Grandy, 2015; Tiemann *et al*., 2015). Our results clearly demostrates that residue retention has positive effects on aggregates formation; their stability; and soil C turnover (both in context of soil C storage and nutrient release), and supports the use of residue retentition as a viable management practice for soil sustainability and productivity.

Impact of soil aggregate size on soil carbon and enzymatic activities in different management practices

Soil structure is believed to be an important regulator of microbially mediated C storage/decomposition in terrestrial ecosystems (Six *et al*., 2002; 2006). In our study, irrespective of the treatment effects, we observed higher content of total C and recalcitrant C in micro-aggregates, however, DOC (proxy of labile C) was higher in mega- and macro-aggregates (Nei *et al*., 2012; Trivedi *et al*., 2015). Similar to our results, previous studies have reported differences in the content of organic C and its chemistry in different sized aggregates (Qin *et al*., 2010; Nie *et al*., 2014; Trivedi *et al*., 2015; Tiemann *et al*., 2015). The relatively labile nature of C in macro- and mega-aggregates may, at least in part, explain the

high influence of agricultural management on the total soil C concentration in these aggregates. Contrary to this, the greater concentration of recalcitrant C in micro-aggregates compared to macro- and mega-aggregates may provide a better control on C functions, turnover and storage in response to management practices in this aggregate-size fraction.

Factors such as microbial composition and enzymatic activities have been reported to strongly regulate the tunrover of SOM in different aggregate sizes (Trivedi *et al*., 2015; Schnecker *et al*., 2015). Our results showed that the effect of management practices on the activity of microbial enzymes decreased with aggregate size. Soil enzyme activities in mega and macro-aggregates are affected by management practices and are significantly correlated with the quantity of residues entering the soil (Ling *et al*., 2014; Trivedi *et al*., 2015; Tiemann *et al*., 2015; Tiemann and Grandy 2015). The production of enzymes involved in degrading different forms of C substrate is directly correlated with the demand for the available resources (Tiemann *et al*., 2015; Tiemann and Grandy 2015). We observed an increase in the activities of enzymes involved in the degradation of labile C (CB, AG, BG) with the decrease in the aggregate size. This result is consistant with other studies that reported increased activity of enzymes involved in degradation of labile C with a decrease in aggregate size (Qin *et al*., 2010; Lagomarsino *et al*., 2012; Ling *et al*., 2014; Nei *et al*., 2014). On the other hand the activity of NAG (i.e. chitin degradation) increased with the increase in the aggregate size. However, it can be argued that the higher activities of enzymes involved in degradation of labile C will result in higher SOC turnover thus leading to lower C levels in micro-aggregates. Similar to our study, Tiemann *et al*., (2015) and Trivedi *et al*., (2015) reported relative high rates microbial activity in terms of production of extracellular enzymes in micro-aggregates where the SOC content was higher as compared to mega- or macroaggregates. Tiemann *et al*., (2015) explained this inconsistancy based on the potential limitation of the enzymatic assays that activate residual microbial bound enzymes

(Wallenstein and Weintraub 2008) and increased microbial investment in enzymes in response to more severe resource limitation (Tiemann *et al*. 2015). We believe that the inconsistency in the enzymatic activity reported by Tiemann *et al*. (2015) and also in our study, results from the significant differences in the amount of C and its stability and forms among the aggregate fractions. When we normalized the enzyme activity to per unit of C among different aggregates, significantly less enzymatic activities per unit C in the micro-aggregates further supports our arguments (Supplementary Fig. 4a-d).

Dynamics of microbial communities in different size aggregates from different management practices

In general, the proportion of bacteria within soil varies with aggregate size, and a greater proportion of bacteria are associated with micro-aggregates and less with macro-aggregates (Monreal and Kodama, 1997; Neumann *et al*., 2013). Our results suggest that different aggregate size classes provide distinct microbial habitates which supports the colonization of distinct microbial communities (Davinic *et al*., 2012; Trivedi *et al*., 2015). Our results support copiotroph/oligotroph hypothesis that suggests that C type and availability provides strong selective pressures for defining lifestyle statergies among soil microbes (Fierer *et al*. 2007, Trivedi *et al*. 2013; Leff *et al*. 2015). According to this hypothesis "oligotrophs" (equivalent to slower-growing *K*-selected plant species e.g. Acidobacteria) are slow growing bacteria; have high C use efficiency; and are abundant in niches with high amounts of recalcitrant organic matter (Fierer *et al*. 2007; Trivedi *et al*. 2013). On the other hand "copiotrophs" (equivalent to fast-growing *r*-selected plant species e.g. *Alphaproteobacteria*, *Betaproteobacteria*) prefer using labile C fractions and grow at higher rates as a consequence. In our study, micro-aggregates are considered to possess oligotrophic rich communities since they contain higher concentration of recalcitrant C

(Table 2), while mega-aggregates favour copiotrophic communities due to higher concentration of DOC (Table 2) and relatively labile coarser particulate organic matter (Six *et al*., 2002; Kong *et al*., 2011). Under this assumption, bacteria in micro-aggregates are expected to be *K*-selected and to present low growth rates and very efficient nutrient uptake systems with higher substrate affinities (Trivedi *et al*. 2013; 2015). In contrast, bacteria in mega- and macro-aggregates soils are expected to be *r*-selected and to have higher rates of activity per biomass unit, higher turnover rates and faster growth rates. Our findings are generally consistent with our hypothesized shifts in general life history strategies with bacterial taxa that are faster growing and more copiotrophic (Fierer *et al*., 2007; Trivedi *et al*., 2013; Leff *et al*., 2015) being favoured by large aggregates, while slow growing and more oligotrophic dominate micro-aggregates. We further found stong positive correlations between oligotrophs and the concentration of recalcitrant C present in the aggregates (Table 2). On the other hand, copiotrophic organisms such as *Actinobacteria* and *Betaproteobacteria* showed significant correlations with the concentration of DOC present within the aggregates, which was higher in the mega-aggregates.

In accordance with our results, previous studies that reported differences in the relative abundances of groups of bacteria with contrasting trophic lifestyles in different sized aggregates suggesting that these groups occupy different ecological niches (Davinic *et al*., 2012; Constancias *et al*., 2014; Trivedi *et al*., 2015). Community analysis using MiSeq reveled that samples from different aggregate sized grouped seperately; however clear groups among samples based on the management practices was only observed in mega- and macro-aggregates. This observation is supported by earlier studies that that reported similar results for different soil types and land management practices (Mummey *et al*., 2006; Davinic *et al*. 2012; Constancias *et al*., 2014; Trivedi *et al*. 2015). These observations might be related to the processes of community assemblages resulting from the balance between the effects of

management practices versus soil aggregate structure (Martiny *et al*., 2006). By providing the greatest and most permanent level of protection for bacterial community, micro-aggregates host a specific community structure, which may result mainly from historical contingencies. In contrast, in less stable and less protected macro-aggregates, the community structure may result mainly from the contemporary perturbations and, thus would also reflect the effect of current land management practices.

In mega- and macro-aggregates we observed higher fractional abundances of both copiotrophs and oligotrophs in the full residue retention as compared to the low residue retention treatments. This is not surprising as high quantity of labile POM and DOC, but also recalcitrant C protected in micro-aggregates and silt+clay within macro- and mega-agggregates might promote the growth of both bacterial groups in the high residue retention treatment. Overall we observed that residue management has a more pronounced effect on the microbial community and their associated functions as compared to other managment practices such as crop rotation. Govaerts *et al*. (2007; 2009) have reported that residue retention has significant effect on the catabolic potential of soil microbial community as compared to other management practices such as tillage intensity and crop rotations. The relative abundance of *Firmicutes* was higher in the low residue plots which might be the result of some effect of burning of crop residue before removal in windorws. The members of phylum *Firmicutes* form spores and can tolerate heat and dessication that might be induced by residue burning. We also observed significant positive correlations between abundance of *Actinobacteria*, *Betaproteobacteria,* and *Firmicutes* with the activity of different enzymes involved in degradation of labile (CB, AG, and BG) and recalcitrant C (NAG). These group of bacteria are known to possess higher numbers of genes involved in the production of respective enzymes as compared to other groups (Trivedi *et al*., 2013). It must be noted that breakdown products of extracellular enzymes can be used as "public good" and can be taken

up by "cheaters" that don't necessarily produce the enzymes. In this case again "copiotrophs" with more transporters that can assimilate C substrate for growth have an selective advantage as compared to "oligotrophs" that contain fewer and substrate specific enzymes (Trivedi *et al*. 2013).

Controls of enzymatic activities and soil C content among different sized aggregates

SEM clearly showed the greater microbial control of the activities of extracellular enzymes involved in C degradation in micro-aggregates as compared to mega- or macro-aggregates. In the mega-and macro-aggregates, the production of extracellular enzymes was controlled primarily by the management practices. In these aggregates, management practices have a strong influence on the soil C content and also on the bacterial community composition. Our results demonstrated that microbial responsiveness to crop management practices declined in micro-aggregates. While the variation in plant materials entering the soil after harvest may explain differences in total C content and biochemical processes between the management practices, the occlusion and retention of microbial metabolites in micro-aggregates is more likely to enhance the recalcitrance of C in the soil. Interestingly, the control of soil microbial communities on the enzymatic activities increased significantly in the micro-aggregates as compared to mega- and macro-aggregates. We further observed contrasting correlations between the activity of extracellular enzymes and total C concentration among different sized aggregates. For example, the enzymatic activities were negatively correlated with the concentration of C in mega- and macro-aggregates while there were no significant correlations in the micro-aggregates (Supplementary Table 2). Our results suggest impacts of different crop management regimes on soil C concentration, microbial communities, and the production of extracellular enzymes vary in different sized aggregates with more pronounced in macro-aggregate compared to micro-aggregate sizes. In micro-aggregates significantly higher variations in the amount of soil C and enzymatic activities

remained un-explained as compared to larger sized aggregates, suggesting different control mechansisms regulating soil C turnover. It has been shown pore geometry and connectivity; oxygen diffusion rates; and bacterial community structure have more pronounced effects on the mechanics of soil C dynamics in micro-aggregates as compared to larger sized aggregates (Six *et al*., 2004; Vos *et al*., 2013; Rabbi *et al*., 2016) . Conceptually, micro-aggregate conditions are understood to be responsible for the protection of soil C, however quantitative knowledge on the specific factors driving SOC dynamics in micro-aggregates is still lacking (Kravchenko *et al*., 2016). Considering the relative importance of micro-aggregates in regulating C storage (Six *et al*., 2006; Tiemann *et al*., 2015; Trivedi *et al*., 2015), more research is required to further understand both the structure and function of soil microbial community in micro-aggregates.

Despite its clear benefits, there is an ongoing "use" vs "save" debate on how to manage soil C for sustainable agriculture production (Wood *et al*., 2016). While decomposition of organic matter to liberate nutrients that will be taken up by plants in short term will increase productivity (Janzen 2006); prioritizing the formation and long-term stabilization of soil C will enhance long-term soil quality through nutrient retention and water holding capacity (Lal 2004). Our study clearly demonstrates that complex interactions between soil microbial communities; different soil organic fractions; and management practices that occur at the scale of soil aggregates could regulate "release" or "retention" of soil C. This is a key finding in relation to the emerging paradigm in SOC dynamics that suggest that different soil C pools may have different underlying drivers and potentially leading to different relationships with management outcomes (Schmidt *et al*., 2011; Woods *et al*., 2016). Our study highlight the need of modelling the differential effects of soil aggregate level processes for representing soil C dynamics that otherwise are lost in continuum or mean field models that ignore dynamic interactions of microbial communities subjected to abiotic

constrains modulated by soil aggregate size (Manzoni and Porporato 2009; Vogel *et al*., 2015; Ebrahimi and Or 2016).

Overall our results demonstrate that management practices impact soil chemical and physical heterogeneity among aggregates of different size and consequently the distribution of microbial communities and their activities. Although our experimental set up has a combination of management practices that also included crop-diversity, we observed that residue management had the main effect in driving soil microbial community and their associated functions. Recently it has been reported that with the equal amounts of plant biomass inputs, SOC gains and microbial dynamics was impacted mainly by the diversity within crop rotations as compared to monoculture (Tiemann *et al*., 2015). This discrepancy can be due to the higher diversity (3-5 vs 3 crop species); higher C content (19.3 vs 10.12 mg g^{-1}); and longer term of trails (12 vs 6) in the site used by Tiemann *et al*. (2015), relative to our study. We expect that although crop rotations may play important role in mediating below ground processes (MaDaniel *et al*., 2014; Tiemann *et al*., 2015), our experimental site is generally poor in C, and practices that supported greater organic C input and availability may have played a major role in mediating microbial processes as compared to other crop diversity in the rotational regime of the crop management practices. Furthermore, the short-term effects of crop rotation and residue management on SOC dynamics are often complex and variable and may vary with soil types (Al-Kaisi and Yin 2005; Chivenge *et al*., 2007). Also, crop diversity would have greater effect in the growing season because of the impact of belowground root C input on soil processes, which would decrease after harvesting and during the fallow period, while the residue input effect is likely to predominate.

Conclusions:

The data we present suggest that residue retention practices change microbial community structure and activity in larger aggregates, with positive effects on aggregate

formation/stability and soil C accrual, and supports the adoption of residue retention as a viable option for promoting soil sustainability particularly in context of low-input conservation agriculture practices. Our study clearly demonstrates that soil microbes exist in spatially distinct communities reflecting the heterogeneity of soil structure. We further provide evidence that different drivers regulate complex processes involved in the soil C dynamics in macro- and micro-aggregates. Microbial regulation is strongest in the micro-aggregate where the greatest concentration of stable form of organic C resides, including the likely stabilisation of microbial metabolites (i.e. highly decomposed organic matter) via organo-mineral interactions. Based on our findings we propose that differential response of management practices and microbial control on the C turnover in macro-aggregates (wherein higher amount of labile C was present) and micro-aggregate (wherein higher amount of recalcitrant C was present) should be explicitly considered when accounting for management impacts on soil functions including C storage. Quantifying the relative importance of different microbial groups for C storage and functions in aggregate-size fractions will significantly advance this area of science by providing the empirical data required for models that predict global soil C sequestration (Weider *et al*., 2015). This information can be incorporated in decision support system tools for increased farm productivity and profitability.

Experimental Procedures

Site description

The long-term conservation agriculture cropping system experiments was started in 2007 at the Cunderdin College of Agriculture (117°14´E, 31°38´S) in Western Australia. The site has a Mediterranean-type environment with a 20 year average rainfall of 300 mm. The Cunderdin

soil was red sandy clay loam with pH of 6.6, increasing with depth to 7.9. The treatments in the trials were based on four different cropping philosophies:

- P1–maximum carbon input (continuous cereal; no-tillage disc seeder)
- P2– maximum diversity (cereal/legume/brassica; no-tillage disc seeder)
- P3–controls (continuous wheat; no-tillage disc seeder)
- and P4–maximum profit (cereal/cereal/break crop (or fallow); no-tillage knife-point seeder with higher levels of disturbance)

Additional details on crop sequences are provided in Flower *et al*. (2012). Each treatment was replicated three times in a randomised complete block design: the P1, P2 and P4 philosophies had a three-year rotation with each phase presented every year, while P3 was continuous wheat. From 2010 (start of the second three year rotation cycle) all of the "P1–maximum carbon input" and "P2– maximum diversity" sequences were split for full stubble retention (original treatment with full retention e.g. BOW(P1)-FR) and windrow burning (low residue level e.g. BOW(P1)-LR. The straw and chaff were windrowed after harvesting and then burnt prior to seeding). P3 had full residue retention (WWW(P3)-FR). P4 was windrow burnt from 2010 onwards (e.g. BLW(P4)-LR). Plots of 36 m x 80 m were sown with 4.5 m disc or knife-point no-till seeders in May each year. All crops were sown at 300 mm row spacing and normal agronomic practices were followed.

Sampling protocol

Samples were taken 7-9 March 2013 before seeding in the last year of the second 3 year phase (i.e. after 6 years of treatments). To reduce the "hidden effect" of the variation in current crop type on soil properties (Tiemann *et al*. 2015), we sampled soils following the wheat phase across all the treatments (i.e. all plots sampled had fresh wheat residue). Soils were sampled from the top 10 cm of soil using a 5.5- cm-diameter slide-hammer soil coring

device (Giddings Machine Company, Windsor, CO, USA). Five intact soil cores were collected from each plot and placed in plastic bags, stored on ice, and transported to the laboratory. Each core was gently broken up along natural points of weakness and passed through an 8-mm sieve, removing large roots and stones. Replicated cores were combined into one composite sample for each plot.

Determination of aggregate stability

Aggregate stability was determined using the same sieve and shaker, but applying a lid at the top, fitted with a nozzle and hose attached to a deionised water tap as described by Tiemann and Grandy (2015). Sand content (>0.053) was determined by dispersing aggregates in a 5% (w/v) sodium hexametaphosphate solution and sieving through a 0.053-mm mesh sieve. After correcting for sand content, the percentage of field moist mega-aggregates that remain as water stable mega-aggregate was used to represent aggregate stability.

Soil aggregate separation

Soil samples were prepared for soil aggregate isolation utilizing an optimal moisture approach to standardize soil moisture content and minimize disturbance to microbial communities (Bach and Hofmockel, 2015). Briefly, sieved soils were placed in open, sterilized plastic containers and dried to approximately 10% gravimetric water content at 4 °C and subjected to the following fractionation procedure. We employed dry sieving to minimise the effects of wet sieving procedure (e.g. Six *et al.,* 2006) on microbial communities and activities, while producing aggregates fractured along natural planes (Kristiansen *et al*., 2006). We used a rotary sieve shaker (Retsch 200; Verder Scientific Inc., Newton, PA, USA) shaken at 200-250 rpm for 3 minutes to separate soils into three aggregate size classes: >2mm mega-aggregates; 0.25-2mm macro-aggregates; and 0.053-0.25 mm micro-aggregates

(Tiemann *et al*., 2015; Bach and Hofmockel 2015). After aggregate fractionation samples were subsampled for various chemical, biological, and molecular analyses. The samples for molecular analysis and long term storage were stored at -80 °C, until required for DNA extraction. The samples for soil chemical and biological analyses were stored at 4 °C and were processed for downstream analyses within a week after performing soil aggregate separation.

Microbial Community structure

Soil DNA was extracted from 0.25 g of soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. We characterized bacterial diversity and composition in the samples from different aggregate sizes by using the IlluminaMiseq profiling of ribosomal genes Illumina Miseq plataform (Illumina Inc.) and the 341F/805R (Herlemann *et al*., 2011) primer set (see details in Appendix S1). Initial sequence processing and diversity analyses were conducted using the QIIME package (Caporaso *et al*., 2010). Initially, low quality regions (Q<20) were trimmed from the 5' end of sequences and paired ends were joined with FLASH (Magoč *et al*., 2011). Sequences were de-multiplexed and a further round of quality control was conducted to remove sequences containing ambiguous bases (N), and reads containing bases with a quality score below 25. Chimeric 16S rDNA sequences were detected using the UCHIME algorithm from the USEARCH package (Edgar *et al*., 2010; 2011) implemented within VSEARCH (https://github.com/torognes/vsearch). The RDP training dataset V950 was used as a reference for chimera detection, as recommended by the UCHIME documentation. The remaining high quality chimera free sequences were used for downstream analysis. Operational Taxonomic Units (OTUs) were defined as clusters of 97% sequence similarity using UCLUST (Edgar *et al*., 2010). Taxonomy was assigned using

UCLUST against the Greengenes database version 13_85 (DeSantis *et al*., 2006; McDonald *et al*., 2010) for 16S rDNA OTUs. The resultant OTU abundance tables were filtered to remove singletons and rarefied to an even number of sequences per samples to ensure an equal sampling depth (11925 sequences). Bacterial richness (i.e. number of OTUs) was calculated on these rarefied OTU tables using QIIME (Caporaso *et al*., 2010).

To confirm the trends obtained by MiSeq analysis we quantified the abundance of total bacteria and other groups (*Acidobacteria, Actinobacteria, α, β, γ Proteobacteria; Bacteroidetes*) using taxon specific qPCR by primers and conditions described in details in Trivedi *et al*., (2013) (Supplementary Table 3)

Soil chemical and biological properties in different sized aggregates

Soil pH was assessed using a fresh soil to water ratio of 2.5 using a Delta pH-meter (Meter-Toledo Instruments Co., Columbus, OH, USA). Total carbon and total nitrogen were measured on a LECO macro-CN analyzer (LECO, St. Joseph, MI, USA). The amount of recalcitrant C in the different sized aggregates was determined by the acid hydrolysis method as described in Leavitt *et al*., (1996). The resistance to acid hydrolysis is a common property of most recalcitrant organic polymers (lignin, chitin, suberin, resins, waxes) and the method has been used to quantify recalcitrant forms of C in various studies (for example Rovira *et al*., 2002 and references within).

Soil respiration was measured using MicroResp^{TM} (Macaulay Scientific Consulting, UK; Campbell *et al*., 2003). Approximately 350 mg of soil was added to deep well microtitre plates to which 30 µl of water was added in each well. A rubber sealing mat was used to seal the deep well plate to an indicator plate, and plates were incubated in the dark over 6 h at 25 °C as previously described in Campbell *et al*. (2003). After incubation, the CO_2 production

rate (μg CO_2-C g^{-1} h^{-1}) was calculated based on the change in absorbance (A_{570}) of the indicator plate.

β-Glucosidase (BG), β-D-celluliosidase (CB), α-Glucosidase (AG), and N-acetyl-β-Glucosaminidase (NAG) activities were measured using 4-methylumbelliferyl (MUB) substrate, yielding the highly fluorescent cleavage products MUB upon hydrolysis (Wallenstein *et al*., 2008). All the enzyme assays were set up in 96-well microplates as described by Bell *et al*. (2012). Twelve replicate wells were set up for each sample and each standard concentration. The assay plate was incubated in the dark at 25 °C for 3 h to mimic the average soil temperature. Enzyme activities were corrected using a quench control (Wallenstein *et al*., 2008). Fluorescence was measured using a microplate fluorometer (EnSpire® 2300 Multilabel Reader, Perkin Elmer, USA) with 365 nm excitation and 460 nm emission filters. The activities were expressed as nmol h^{-1} g^{-1}dry soil or nmol h^{-1} g^{-1}dry soil C^{-1} (enzyme activity per unit of total C).

Data analysis

Two-way PERMANOVAs (Anderson, 2001) were used to determine the effects of aggregate-size fractions and management practices (BOW(P1)-FR, BOW(P1)-LR, LCW(P2)-FR, LCW(P2)-LR, WWW(P3)-FR and BLW(P4)-LR; see Table 1 for details on the different practices) on soil C, and enzyme variables. Both aggregate size and management practices were included as fixed factors in these analyses. Post hoc analyses were done to explore significant differences among management practices within each aggregate-size fraction when an interaction for aggregate size x management practices was observed in the PERMANOVA analyses. To further differentiate various parameters among treatments within individual aggregate size we performed one-way ANOVA by aggregate size fraction, with treatments as a fixed effect and 'block' as a random affect using PROC MIXED (SAS

Institute, Cary, NC, USA). Waller–Duncan k-ratio post hoc tests were used to evaluate differences between cropping systems.

Spearman correlation analyses were performed to evaluate relationships between soil chemical properties and microbial activity by XLSTAT. A bootstrapped canonical analysis of principal coordinates (CAP) was performed using PRIMER 6.0 statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK, Clarke and Gorley, 2006) to assess changes in the structure of microbial community in different aggregate size fractions. We used CAP analysis because this is one of the non-parametric discriminative methods based on permutation tests that do not rely on assumptions that are commonly too stringent for most ecological data sets (Anderson 2001). CAP uses principal coordinate ordination followed by canonical discriminant analysis to provide a constrained ordination that maximizes the differences among *a priori* groups and reveals patterns that can be cryptic in unconstrained ordinations (Anderson and Willis 2003). Two way PERMANOVA analysis with aggregate size and management practices as fixed factors on the Bray–Curtis distance matrix was conducted using the PERMANOVA+ for PRIMER statistical package.

We used a structural equation modelling approach (SEM) to build a system-level understanding on the linkages among microbial communities, soil C and function in different aggregate size classes (Colman and Schimel 2013; Grace 2006). We constructed a conceptual base model (Supplementary Figure 5) using management practices (described above) microbial community (CCA axis 1 and 2), and soil total C as direct and indirect factors controlling soil functions (standardized averaged of activities related to AG, CB, NAG, BG as in Delgado-Baquerizo *et al*., 2016; Trivedi *et al*., 2016). We merged all four enzyme activities into a single variable called "function" because of two main reason: (1) simplicity and (2) it was our aim to identify the role of micro-aggregates and management in driving soil function as a whole rather than on a particular soil enzyme (e.g. NAG). In addition,

because we were interested in identifying the indirect effects of management on soil function and carbon via shifts in the entire microbial community composition rather than in particular taxa, we included in our SEM the axes from a CCA (CCA axis 1 and 2) ordination aiming to represent the composition of the entire microbial community.

Soil total carbon was log-transformed prior to modelling to improve the normality and linearity of our data. The models were run for each of the different sized aggregates (mega-, macro-, and micro-aggregates). When these data manipulations were completed, we parameterized our model using our dataset and tested its overall goodness of fit. There is no single universally accepted test of overall goodness of fit for SEM, applicable in all situations regardless of sample size or data distribution. Here, we used three commonly used indexes to evaluate the goodness of fit of our model: the Chi-square test ($\chi 2$), goodness of fit index (GFI) and root mean square error of approximation (RMSEA) (Schermelleh-Engel *et al*., 2003). Our *a priori* model attained an acceptable fit by all criteria, and thus no post hoc alterations were made. All the SEM analyses were conducted using AMOS 20.0 (AMOS IBM, USA).

Acknowledgments

We thank Dr W. Dougherty and Prof J. Six for valuable discussions on soil fractionation protocol and Dr Jasmine Grinyer for providing critical comments. The Grains Research and Development Corporation (GRDC), Australia via Grant number UWS000008 and Australian Research Council via Grant number DP13010484 funded this work. The long-term field experiment was funded by GRDC, Australia.

References:

Al-Kaisi, M.M. and Yin, X., 2005. Tillage and crop residue effects on soil carbon and carbon dioxide emission in corn–soybean rotations. J Envir Qual 34: 437-445.

Anderson, M.J. (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol **26:** 32-46.

Anderson, M.J. and Willis, T.J. (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecology* **84:** 511-525.

Ashagrie, Y., Zech, W., Guggenberger, G., and Mamo, T. (2007) Soil aggregation, and total and particulate organic matter following conversion of native forests to continuous cultivation in Ethiopia. *Soil Till Res* **94:**101-108.

Bach, E.M. and Hofmockel, K.S. (2015) A time for every season: soil aggregate turnover stimulates decomposition and reduces carbon loss in grasslands managed for bioenergy. *GCB Bioenergy* **8:** 588-599.

Bailey, V.L., Bilskis, C.L., Fansler, S.J., McCue, L.A., Smith, J.L. and Konopka, A. (2012) Measurements of microbial community activities in individual soil macroaggregates. *Soil Biol Biochem* **48:**192-195.

Bailey, V.L., Fansler, S.J., Stegen, J.C. and McCue, L.A. (2013) Linking microbial community structure to β-glucosidic function in soil aggregates. *ISME J* **7**:2044-2053.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K. and Wallenstein, M.D. (2013) High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *J Visualized Exp: JoVE*, (81).

Brockett, B.F.T., Prescott, C.E., and Grayston, S.J. (2012) Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biol Biochem* **44:** 9–20.

Bronick, C.J. and Lal, R. (2005) Soil structure and management: a review. *Geoderma*, **124:**3-22.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., and Potts, J.M. (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl Environ Microbiol* **69:** 3593-3599.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., and Knight, R. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *P Natl Acad Sci USA* **108:** 4516-4522.

Chan, Y. (2008) Increasing soil organic carbon of agricultural land. *Primefact* **735:** 1-5.

Chivenge, P.P., Murwira, H.H., Giller, K.E., Mapfumo, P., and Six, J. (2007) Long term impact of reduced tillage and residue management on soil carbon stabilization: implications for conservation agriculture on contrasting soils. *Soil Tillage Res* **94:** 328 337.

Clarke, K.R., and Gorley, R.N. (2006) PRIMER v6: User manual/tutorial, PRIMER-E, Plymouth UK, 192pp.

Colman, B.P., and Schimel, J.P. (2013) Drivers of microbial respiration and net N mineralization at the continental scale. *Soil Biol Biochem* **60:** 65–76.

Constancias, F., Prévost-Bouré, N.C., Terrat, S., Aussems, S., Nowak, V., Guillemin, J.P., Bonnotte, A., Biju-Duval, L., Navel, A., Martins, J.M., and Maron, P.A. (2014) Microscale evidence for a high decrease of soil bacterial density and diversity by cropping. *Agron Sustain Dev* **34:** 831-840.

Coughenour, C. M., and Chamala, S. (2007). *Conservation tillage and cropping innovation: constructing the new culture of agriculture*. John Wiley & Sons.

Coughenour, C.M. (2003) Innovating conservation agriculture: The case of no-till cropping. *Rural Sociology* **68:** 278-304.

Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderón, F.J., Cox, S.B., Dowd, S.E., Allen, V.G., Zak, J.C., and Moore-Kucera, J. (2012) Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. *Soil Biol Biochem* **46:** 63-72.

De Deyn, G.B., Shiel, R.S., Ostle, N.J., McNamara, N.P., Oakley, S., Young, I., Freeman, C., Fenner, N., Quirk, H., and Bardgett, R.D. (2011) Additional carbon sequestration benefits of grassland diversity restoration. *J Appl Ecol* **48:** 600-608

Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D. and Singh, B.K. (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat Commun* **7.**

Ebrahimi, A. and Or, D. (2016) Microbial community dynamics in soil aggregates shape biogeochemical gas fluxes from soil profiles–upscaling an aggregate biophysical model. *Global Change Biol* **22:**3141-3156.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27:** 2194-2200.

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007) Toward an ecological classification of soil bacteria. *Ecology* **88:** 1354-1364.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., and Helkowski, J.H. (2005). Global consequences of land use. *Science* **309:** 570-574.

Govaerts, B., Mezzalama, M., Sayre, K.D., Crossa, J., Lichter, K., Troch, V., Vanherck, K., De Corte, P., and Deckers, J. (2008) Long-term consequences of tillage, residue management, and crop rotation on selected soil micro-flora groups in the subtropical highlands. *Appl Soil Ecol* **38:** 197-210.

Govaerts, B., Mezzalama, M., Unno, Y., Sayre, K.D., Luna-Guido, M., Vanherck, K., Dendooven, L., and Deckers, J. (2007) Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. *Appl Soil Ecol* **37:** pp.18-30.

Grace, J.B. (2006) Structural Equation Modeling and Natural Systems. New York, Cambridge University Press.

Grandy, A.S. and Robertson, G.P. (2007) Land-use intensity effects on soil organic carbon accumulation rates and mechanisms. *Ecosystems* **10:**59-74.

Gupta, V.V.S.R. and Germida, J.J. (1988) Distribution of microbial biomass and its activity in different soil aggregate size classes as affected by cultivation. *Soil Biol Biochem* **20:** 777-786.

Hackl, E., Pfeffer, M., Donat, C., Bachmann, G., and Zechmeister-Boltenstern, S. (2005) Composition of the microbial communities in the mineral soil under different types of natural forest. *Soil Biol Biochem* **37:** 661-671.

Heemsbergen, D.A., Berg, M.P., Loreau, M., Van Hal, J.R., Faber, J.H., and Verhoef, H.A. (2004) Biodiversity effects on soil processes explained by interspecific functional dissimilarity. *Science* **306:** 1019-1020.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J.J., and Andersson, A.F. (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J* **5:** 1571-1579.

Hobbs, P.R., Sayre, K., and Gupta, R. (2008) The role of conservation agriculture in sustainable agriculture. *Philos T R Soc B* **363:** 543-555.

Hooke, R.L., Martín-Duque, J.F., and Pedraza, J. (2012) Land transformation by humans: a review. *GSA Today* 22: 1-4.

Janzen, H. H. (2006) The soil carbon dilemma: Shall we hoard it or use it? *Soil Biol Biochem*

38:419–424.

Jastrow, J.D., Amonette, J.E. and Bailey, V.L. (2007) Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Climatic Change* **80:** 5-23.

Jin, H., Sun, O.J. and Liu, J. (2010) Changes in soil microbial biomass and community structure with addition of contrasting types of plant litter in a semiarid grassland ecosystem. *J Plant Ecol* **3:** 209-217.

Kong, A.Y., Scow, K.M., Córdova-Kreylos, A.L., Holmes, W.E., and Six, J. (2011) Microbial community composition and carbon cycling within soil microenvironments of conventional, low-input, and organic cropping systems. *Soil Biol Biochem* **43:** 20-30.

Kravchenko, A.N., Negassa, W.C., Guber, A.K. and Rivers, M.L. (2014) Protection of soil carbon within macro-aggregates depends on intra-aggregate pore characteristics. *Sci Rep-UK* **5:**16261-16261.

Lagomarsino, A., Grego, S., and Kandeler, E. (2012) Soil organic carbon distribution drives microbial activity and functional diversity in particle and aggregate-size fractions. *Pedobiologia* **55:** 101-110.

Lal, R. (2004) Soil carbon sequestration impacts on global climate change and food security. *Science* **304:** 1623-1627.

Leavitt, S.W., Follett, R.F., Paul, E.A. (1996) Estimation of slow- and fast-cycling soil organic carbon pools from 6N HCl hydrolysis. *Radiocarbon* **38:** 231–239.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M., and McCulley, R.L. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *P Natl Acad Sci USA* **112:** 10967-10972.

Ling, N., Sun, Y., Ma, J., Guo, J., Zhu, P., Peng, C., Yu, G., Ran, W., Guo, S., and Shen, Q. (2014) Response of the bacterial diversity and soil enzyme activity in particle-size fractions of Mollisol after different fertilization in a long-term experiment. *Biol Fertil Soils* **50:** 901-911.

Litchman, E., and Klausmeier, C.A. (2008) Trait-based community ecology of phytoplankton. *Annu Rev Ecol Evol* S **39:** 615-639.

Litchman, E., Edwards, K.F. and Klausmeier, C.A. (2015) Microbial resource utilization traits and trade-offs: implications for community structure, functioning, and biogeochemical impacts at present and in the future. *Front Microbiol* 6.

Macdonald, C. and Singh, B. (2014) Harnessing plant-microbe interactions for enhancing farm productivity. *Bioengineered* **5:** 5-9.

Manzoni, S. and Porporato, A. (2009) Soil carbon and nitrogen mineralization: theory and models across scales. *Soil Biol Biochem* **41:**1355-1379.

Magoč, T. and Salzberg, S.L. (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27:** 2957-2963.

Martiny, J.B.H., Bohannan, B.J., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., and Morin, P.J. (2006) Microbial biogeography: putting microorganisms on the map. *Nat RevMicrobiol* **4:** 102-112.

McDaniel, M.D., Tiemann, L.K., and Grandy, A.S. (2014) Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecol Appl* **24:** 560–570.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., and Hugenholtz, P. (2012) An improved Greengenes

taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* **6:** 610-618.

Monreal, C.M., and Kodama, H. (1997) Influence of aggregate architecture and minerals on living habitats and soil organic matter. *Can J Soil Sci* **77:** 367-377.

Mummey, D., Holben, W., Six, J. and Stahl, P. (2006) Spatial stratification of soil bacterial populations in aggregates of diverse soils. *Microbial Ecol* **51:** 404-411.

Neumann, D., Heuer, A., Hemkemeyer, M., Martens, R., and Tebbe, C.C. (2013) Response of microbial communities to long-term fertilization depends on their microhabitat. FEMS Microbiol Ecol **86:** 71-84.

Nie, M., Pendall, E., Bell, C., and Wallenstein, M.D. (2014) Soil aggregate size distribution mediates microbial climate change feedbacks. *Soil Biol Biochem* **68:** 357-365.

Pittelkow, C.M., Liang, X., Linquist, B.A., Van Groenigen, K.J., Lee, J., Lundy, M.E., van Gestel, N., Six, J., Venterea, R.T. and van Kessel, C. (2015). Productivity limits and potentials of the principles of conservation agriculture. *Nature* **517:** 365-368

R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org.

Rabbi, S.M.F., Daniel, H., Lockwood, P.V., Macdonald, C., Pereg, L., Tighe, M., Wilson, B.R. and Young, I.M., 2016. Physical soil architectural traits are functionally linked to carbon decomposition and bacterial diversity. *Sci Rep-UK* **6**.

Rovira, P., and Vallejo, V.R. (2002) Labile and recalcitrant pools of carbon and nitrogen in organic matter decomposing at different depths in soil: an acid hydrolysis approach. *Geoderma* **107:** 109–141.

Schermelleh-Engel, K., Moosbrugger, H., and Müller, H (2003) Evaluating the fit of structural equation models: Tests of significance and descriptive goodness-of-fit measures. *Meth Psychological Res online* **8:** 23-74.

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A. and Nannipieri, P. (2011) Persistence of soil organic matter as an ecosystem property. *Nature* **478:**49-56.

Singh, B.K., Bardgett, R.D., Smith, P., and Reay, D.S. (2010) Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Rev Microbiol* **8:** 779-790.

Six, J., Bossuyt, H., Degryze, S., and Denef, K. (2004) A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Till Res* **79:** 7–31.

Six, J., Paustian, K., Elliott, E.T., and Combrink, C. (2000) Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Sci Soc Am J* **64:** 681–689.

Six, J., S.D. Frey, R.K. Thiet, and K.M. Batten (2006) Bacterial and fungal contributions to C- sequestration in agroecosystems. *Soil Sci Soc Am J* **70:**555-569.

Smith, P., House, J.I., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., Rumpel, C., and Paustian, K. (2016). Global change pressures on soils from land use and management. *Global Change Biol* **22:** 1008-1028.

Schnecker, J., Wild, B., Hofhansl, F., Alves, R. J. E., Bárta, J., Čapek, P., et al. (2014). Effects of soil organic matter properties and microbial community composition on enzyme activities in cryoturbated arctic soils. *PloS one*, **9:** e94076

Tiemann, L.K., and Grandy, A.S., 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. *GCB Bioenergy* **7:** 161-174.

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E. and McDaniel, M.D. (2015) Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecol Lett* **18:** 761-771.

Trivedi, P., Anderson, I.C., and Singh, B.K. (2013) Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends Microbiol* **21:** 641-651.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J., and Singh, B.K. (2016) Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. The ISME Journal.

Trivedi, P., Rochester, I. J., Trivedi, C., Van Nostrand, J. D., Zhou, J., Karunaratne, S., Anderson, I.C., and Singh, B. K. (2015). Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. *Soil Biol Biochem*, **91:** 169-181

Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J. and Whitbread, A. (2012) Global food security, biodiversity conservation and the future of agricultural intensification. *Biol Conserv* **151:** 53-59.

Victoria, R., Banwart, S.A., Black, H., Ingram, H., Joosten, H., Milne, E. et al. (2012). The benefits of soil carbon: managing soils for multiple economic, societal and environmental benefits. Foresight Chapter. In: UNEP Year Book 2012: Emerging Issues in Our Global Environment. UNEP: Nairobi, Kenya, pp 19–33

Vogel, L.E., Makowski, D., Garnier, P., Vieublé-Gonod, L., Coquet, Y., Raynaud, X., Nunan, N., Chenu, C., Falconer, R. and Pot, V. (2015) Modeling the effect of soil meso-and macropores topology on the biodegradation of a soluble carbon substrate. *Adv Water Resour* **86:** 123-136.

Vos, M., Wolf, A.B., Jennings, S.J. and Kowalchuk, G.A. (2013) Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol Rev* **37:**936-954.

Wallenstein, M.D., and Weintraub, M.N. (2008) Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes. *Soil Biol Biochem* **40:** 2098-2106.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., and Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science* **304:** 1629-1633.

Wilson, G.W., Rice, C.W., Rillig, M.C., Springer, A., and Hartnett, D.C. (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecol Lett* **12:** 452-461.

Wood, S.A., Sokol, N., Bell, C.W., Bradford, M.A., Naeem, S., Wallenstein, M.D., and Palm, C.A. (2016) Opposing effects of different soil organic matter fractions on crop yields. *Ecol Appl* **26:**2072-2085.

Xiao, C., Janssens, I.A., Liu, P., Zhou, Z., and Sun, O.J. (2007) Irrigation and enhanced soil carbon input effects on below-ground carbon cycling in semiarid temperate grasslands. *New Phytol* **174:** 835-846.

Figure legends:

Figure 1. Impacts of land management practices on aggregation (circles) and aggregate stability (bars, which represent percentage of > 2 mm field moist aggregates that were stable). Points and bar represent means ± SD (n = 6) and different letters indicate significant differences ($P < 0.001$) between treatments. Details of treatments including abbreviations are provided in Table 1.

Figure 2. MiSeq based canonical analysis of principal coordinates (CAP) of bacterial communities in mega-, macro-, and micro-aggregates of soil from different treatments in a long term crop management trial at Cunderdin, WA. Details of treatments including abbreviations are provided in Table 1.

Figure 3. Impacts of soil management practices on the activities of enzymes involved in C degradation among different aggregate sizes. Points and bar represent means ± SD (n = 6). Different capital and small letters indicate significant differences (P< 0.001) between treatments within the similar sized aggregates and between different aggregates from similar treatments, respectively. Details of treatments including abbreviations are provided in Table 1.

Figure 4. Structure equation models based on the effects of management practices, soil C, microbial community composition on the activities of enzymes involved in soil C turnover (functions) in mega-(a-1); macro-(b-1); and micro-aggregates (c-1). Numbers adjacent to the arrows are standardized path coefficients, analogous to partial regression weights and indicative of effect size of the relationship. Arrow width is proportional to the strength of path coefficients. R^2 indicates the proportion of variance explained. Model fitness details are provided adjacent to the path analysis. Details of treatments including abbreviations are provided in Table 1.

Table 1. Description of long-term conservation agriculture cropping system experiments at the Cunderdin College of Agriculture, Western Australia.

Philosophy	**Description**	**Residue retention (Weighted Biomass, T/ha)**	**Treatment name**
P1 Low crop diversity	Maximum carbon input (Barley-Oat-Wheat) Three year rotation with cereals only and every phase presented every year. Crops seeded with a minimum disturbance disc opener.	High (5.14±1.1) Low (1.92±0.21)	**BOW(P1)-FR**[†] **BOW(P1)-LR**[†]
P2 Maximum Diversity	High crop diversity (Lupin-Canola-Wheat) Diverse three year rotation (cereal – legume – canola) with every phase presented every year. Crops seeded with a minimum disturbance disc opener. Sequences P2/S4, P2/S5 and P2/S6.	High (5.48±0.82) Low (1.85±0.30)	**LCW(P2)-FR** **LCW(P2)-LR**
P3 Control	Continuous wheat with maximum residue retention. Continuous wheat. Crops seeded with a minimum disturbance disc opener. Sequences P3/S7 (wheat).	High (5.54±0.57)	**WWW(P3)-FR**
P4 Maximum profit	Current farmer practice with low residue retention (Barley-Lupin-Wheat) Three year rotation (cereal – cereal – legume) with every phase presented every year. Crops sown with a higher disturbance tine and knife-point no-till seeder. Sequences P4/S9, P4/S10 and P4 S11.	Low (1.47±0.63)	**BLW(P4)-LR**

[†]FR = full retention of residue, LR = low retention of residue (windrow and burn the straw and chaff after harvesting)

Table 2. One-way ANOVA analysis for the effects of management practices (philosophies and residue retention) on soil C and N pools and microbial activities by aggregate size fractions. Details of treatments including abbreviations are provided in Table 1.

	Mega-aggregate		Macro-aggregate		Micro-aggregate	
	Mean+ SD	Treatment effects	Mean+ SD	Treatment effects	Mean+ SD	Treatment effects
Total C (mg g^{-1})	7.9+ 1.5A**	P1-FRBOW(P1)-FR, LCW(P2)-FR, WWW(P3)-FR > BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR* BOW(P1)-FR>BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR**	13.9+ 2.2B**	BOW(P1)-FR > LCW(P2)-FR, WWW(P3)-FR > BOW(P1)-LR, LCW(P2)-LR > BLW(P4)-LR* BOW(P1)-FR > BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR**	25.0+ 1.3C**	
Total N (mg g^{-1})	0.4+ 0.1A**	LCW(P2)-FR > BOW(P1)-FR, P4-FR > LCW(P2)-LR > WWW(P3)-FR, BOW(P1)-LR* LCW(P2)-FR > BOW(P1)-LR, WWW(P3)-FR, LCW(P2)-LR** BOW(P1)-FR > BOW(P1)-LR** LCW(P2)-LR > LCW(P2)-LR**	1.1+ 0.1B**	LCW(P2)-FR > LCW(P2)-LR > BOW(P1)-FR > BOW(P1)-LR > BLW(P4)-LR; WWW(P3)-FR*	2.1+ 0.1C**	
Dissolved organic carbon (DOC, mg $100g^{-1}$)	85.38+ 8.62A*	LCW(P2)-FR, BOW(P1)-FR > WWW(P3)-FR > LCW(P2)-LR, BOW(P1)-LR > BLW(P4)-LR* LCW(P2)-FR, BOW(P1)-FR > BOW(P1)-LR, BLW(P4)-LR, LCW(P2)-LR**	67.9+ 6.46B*	LCW(P2)-FR > BOW(P1)-FR; WWW(P3)-FR > LCW(P2)-LR, BOW(P1)-LR, BLW(P4)-LR* LCW(P2)-FR > LCW(P2)-LR, BOW(P1)-LR, BLW(P4)-LR**	56.77+ 3.49C*	
Recalcitrant Carbon (RC, mg $100g^{-1}$)	71.66+ 6.90A**	BOW(P1)-FR > LCW(P2)-FR > WWW(P3)-FR > BOW(P1)-LR, LCW(P2)-LR > BLW(P4)-LR* BOW(P1)-FR, LCW(P2)-FR > BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR**	85.83+ 8.42B**	LCW(P2)-FR > BOW(P1)-FR > WWW(P3)-FR, BOW(P1)-LR, LCW(P2)-LR > BLW(P4)-LR* LCW(P2)-FR, BOW(P1)-FR > BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR**	116.88+ 7.50C**	
Basal respiration ($\mu g\ CO_2$-C $g^{-1}\ h^{-1}$)	0.98+ 0.15A**	LCW(P2)-FR, BOW(P1)-FR, WWW(P3)-FR, > BOW(P1)-LR, LCW(P2)-LR, BLW(P4)-LR* LCW(P2)-FR> BOW(P1)-LR, LCW(P2)-LR**	1.41+ 0.13B**	LCW(P2)-FR, WWW(P3)-FR > BOW(P1)-FR > BLW(P4)-LR, BOW(P1)-LR, LCW(P2)-LR * LCW(P2)-FR > BLW(P4)-LR, BOW(P1)-LR, LCW(P2)-LR **	2.55+ 0.18C**	BLW(P4)-LR > LCW(P2)-LR, BOW(P1)-LR, WWW(P3)-FR, LCW(P2)-FR, BOW(P1)-FR**
Basal respiration/ C	1.26+ 0.21A**	BLW(P4)-LR > LCW(P2)-LR, BOW(P1)-LR> WWW(P3)-FR, LCW(P2)-FR> BOW(P1)-FR* BLW(P4)-LR> BOW(P1)-FR**	1.09+ 0.15B**	BLW(P4)-LR > LCW(P2)-LR, BOW(P1)-LR, WWW(P3)-FR, LCW(P2)-FR> BOW(P1)-FR* BLW(P4)-LR > BOW(P1)-FR**	1.02+ 0.13B**	BLW(P4)-LR > LCW(P2)-LR, BOW(P1)-LR, WWW(P3)-FR, LCW(P2)-FR > BOW(P1)-FR*

* P < 0.01; ** * P < 0.001

Table 3. Spearman's rank correlations among the relative abundances of bacterial groups at phylum level (class for Proteobacteria), soil properties and enzymatic activities. Blank cells represent non-significant correlations at P>0.01. Green and yellow coloured bacterial groups represent oligotrophs and copiotrophs, respectively. No colour represent that the groups are not functionally categorized.

Variables	**Carbon (mg g^{-1})**		**Dissolved organic carbon (mg 100 g^{-1})**		**Recalcitrant Carbon (mg 100 g^{-1})**		**Basal respiration (µg CO_2-C g^{-1} h^{-1})**		**Enzymatic activities (n mol g^{-1} soil h^{-1})**							
									α-Glucosidase (AG)		**β-Glucosidase (BG)**		**β-D-celluliosidase (CB)**		**N-acetyl-β-Glucosaminidase (NAG)**	
	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value	ρ	*P* value
Acidobacteria	0.295	0.040	-0.402	0.001	0.339	0.010	-0.371	0.006	-0.346	0.011			-0.305	0.010		
Verrucomicrobia	0.483	0.000	-0.298	0.004	0.370	0.006			-0.273	0.010	-0.377	0.005	-0.350	0.010	-0.366	0.007
Deltaproteobacteria	0.398	0.002			0.423	0.001	-0.423	0.001	-0.323	0.001			-0.423	0.001		
Planctomycetes	0.410	0.002	-0.385	0.010	0.655	< 0.0001	-0.460	0.001	-0.679	< 0.0001	-0.724	< 0.0001	-0.690	< 0.0001	-0.700	< 0.0001
Alphaproteobacteria			0.423	0.006					-0.389	0.001						
Betaproteobacteria	-0.354	0.005	0.369	0.010	-0.373	0.001					0.386	0.01			0.369	0.006
Actinobacteria	-0.384	0.001	0.735	0.003	-0.546	< 0.0001	0.556	0.005	0.357	0.008	0.543	< 0.0001	0.450	0.001	0.586	< 0.0001
Firmicutes	-0.317	0.010	0.312	0.010	-0.378	0.005			0.578	< 0.0001	0.455	0.001	0.513	< 0.0001	0.324	0.01
Bacteroidetes									-0.389	0.004						
Gammaproteobacteria	-0.390	0.001	0.423	0.001	-0.650	<0.0001	0.6523	<0.0001	0.623	<0.0001			0.478	0.002		
Armatinonadetes	0.285	0.037			0.433	0.001			-0.443	0.001	-0.476	0.000	-0.449	0.001	-0.459	0.001
Gemmatimonadetes	0.422	0.002			0.370	0.006	-0.416	0.002	-0.349	0.010	-0.446	0.001	-0.369	0.006	-0.445	0.001

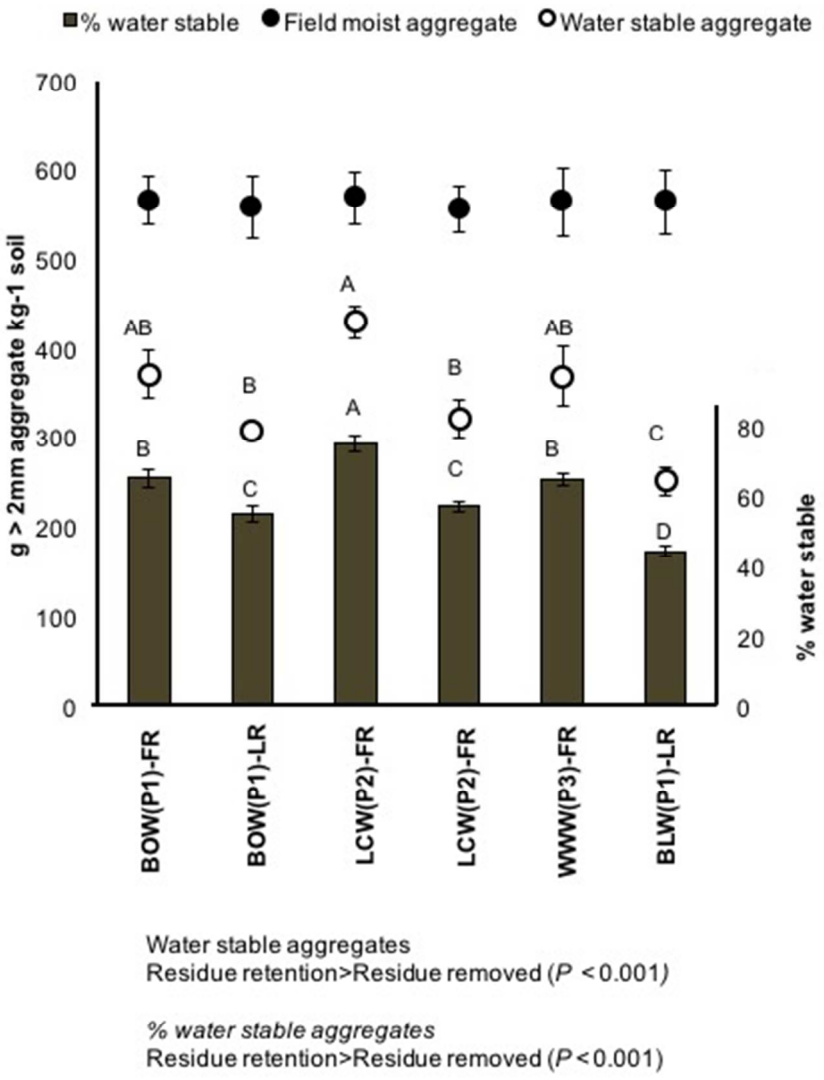

Figure 1. Impacts of land management practices on aggregation (circles) and aggregate stability (bars, which represent percentage of > 2 mm field moist aggregates that were stable). Points and bar represent means ± SD (n = 6) and different letters indicate significant differences (P < 0.001) between treatments. Details of treatments including abbreviations are provided in Table 1.

150x205mm (72 x 72 DPI)

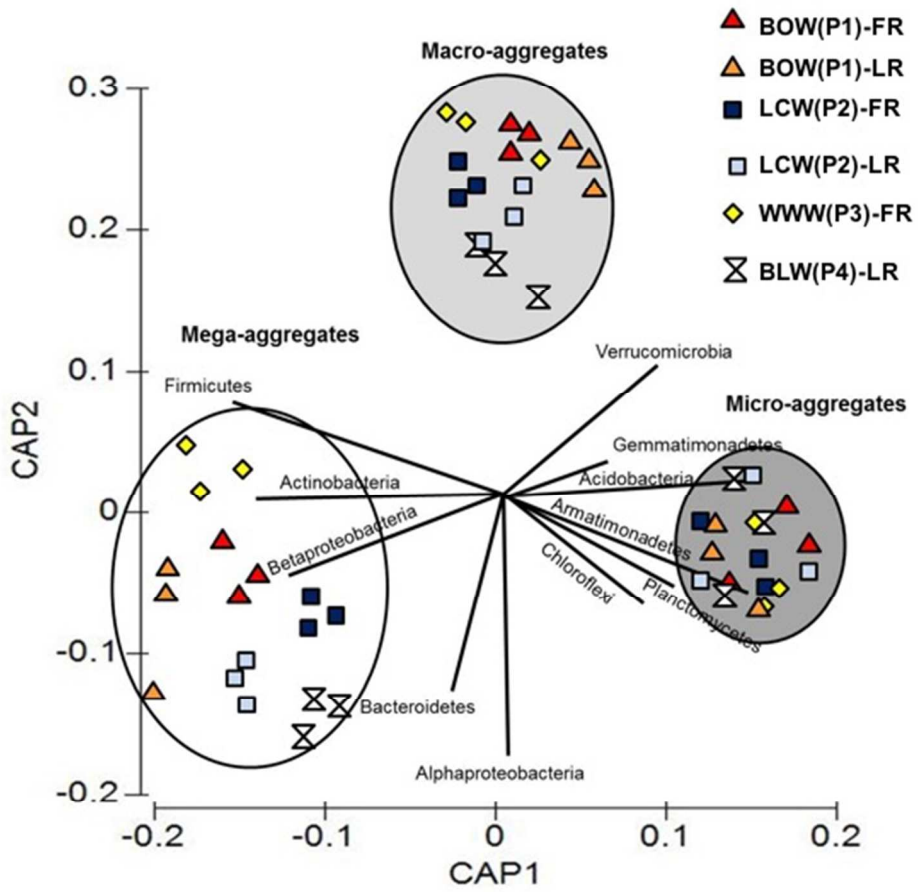

Figure 2. MiSeq based canonical analysis of principal coordinates (CAP) of bacterial communities in mega-, macro-, and micro-aggregates of soil from different treatments in a long term crop management trial at Cunderdin, WA. Details of treatments including abbreviations are provided in Table 1.

207x190mm (72 x 72 DPI)

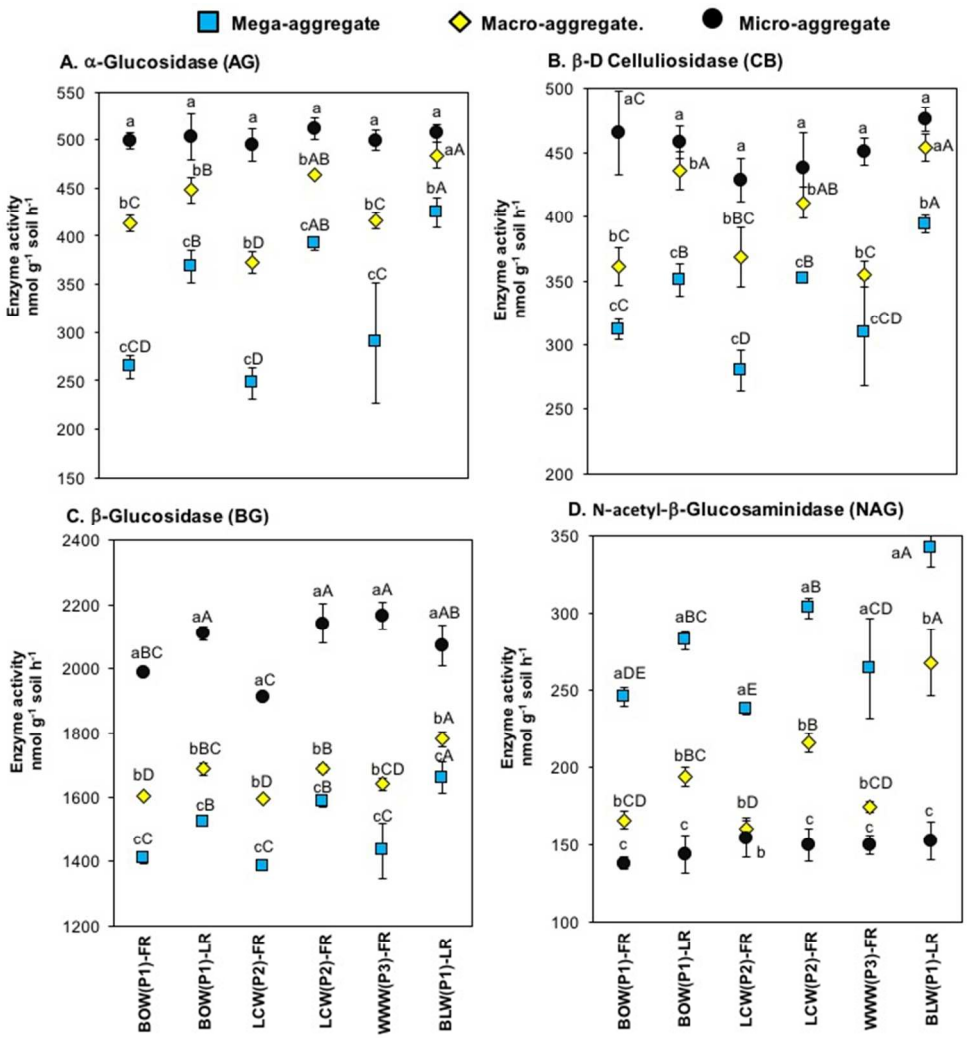

Figure 3. Impacts of soil management practices on the activities of enzymes involved in C degradation among different aggregate sizes. Points and bar represent means ± SD (n = 6). Different capital and small letters indicate significant differences (P< 0.001) between treatments within the similar sized aggregates and between different aggregates from similar treatments, respectively. Details of treatments including abbreviations are provided in Table 1.

260x270mm (72 x 72 DPI)

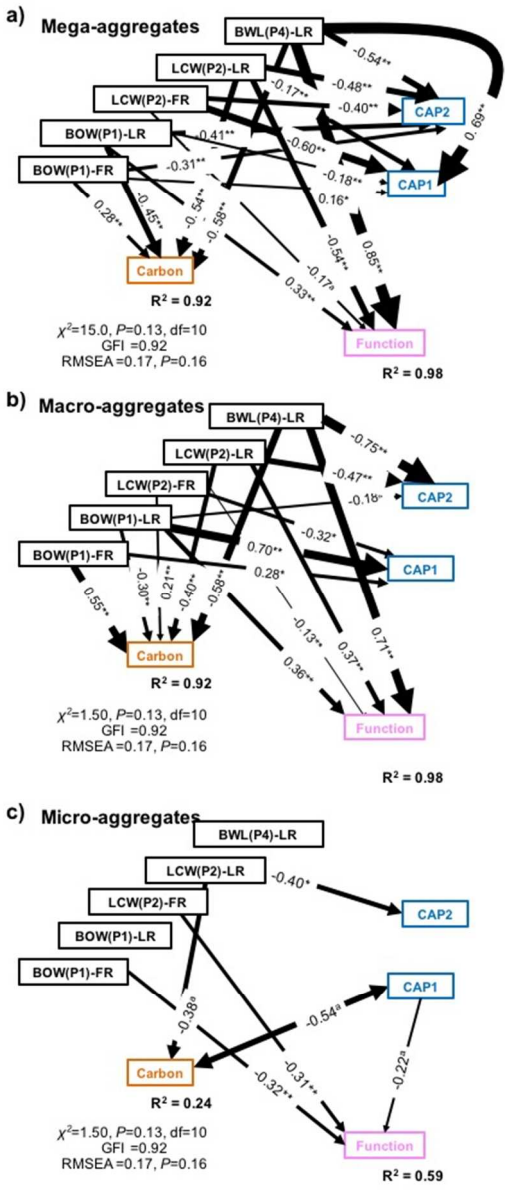

Figure 4. Structure equation models based on the effects of management practices, soil C, microbial community composition on the activities of enzymes involved in soil C turnover (functions) in mega-(a-1); macro-(b-1); and micro-aggregates (c-1). Numbers adjacent to the arrows are standardized path coefficients, analogous to partial regression weights and indicative of effect size of the relationship. Arrow width is proportional to the strength of path coefficients. R2 indicates the proportion of variance explained. Model fitness details are provided adjacent to the path analysis. Details of treatments including abbreviations are provided in Table 1.

149x351mm (72 x 72 DPI)