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Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities

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ABSTRACT

Understanding the influence of long-term crop management practices on the soil microbial community is critical for linking soil microbial flora with ecosystem processes such as those involved in soil carbon cycling. In this study, pyrosequencing and a functional gene array (GeoChip 4.0) were used to investigate the shifts in microbial composition and functional gene structure in a medium clay soil subjected to various cropping regimes. Pyrosequencing analysis showed that the community structure (β-diversity) for bacteria and fungi was significantly impacted among different cropping treatments. Functional gene array-based analysis revealed that crop rotation practices changed the structure and abundance of genes involved in C degradation. Significant correlations were observed between the activities of four enzymes involved in soil C degradation and the abundance of genes responsible for the production of respective enzymes, suggesting that a shift in the microbial community may influence soil C dynamics. We further integrated physical, chemical, and molecular techniques (qPCR) to assess relationships between soil C, microbial derived enzymes and soil bacterial community structure at the soil micro-environmental scale (e.g. within different aggregate-size fractions). We observed a dominance of different bacterial phyla within soil microenvironments which was correlated with the amount of C in the soil aggregates suggesting that each aggregate represents a different ecological niche for microbial colonization. Significant effects of aggregate size were found for the activity of enzymes involved in C degradation suggesting that aggregate size distribution influenced C availability. The influence of cropping regimes on microbial and soil C responses declined with decreasing size of soil aggregates and especially with silt and clay microaggregates. Our results suggest that long term crop management practices influence the structural and functional potential of soil microbial communities and the impact of crop rotations on soil C turnover varies between different sized soil aggregates. These findings provide a strong framework to determine the impact of management practices on soil C and soil health.

1. Introduction

Soil carbon (C) is a key component of terrestrial ecosystems that affects the physical, chemical and biological properties of soil and contributes greatly to its functioning (Schmidt et al., 2011; Ontl and Schulte, 2012). Maintaining the balance between soil C turnover and retention of soil C is crucial because it improves soil structure, soil fertility, crop production, and ensures long-term sustainability of agricultural systems (Six et al., 2004; Reichstein et al., 2013). Furthermore, soil can play a key role in the global C cycle by acting as a sink for atmosphere CO_2 when appropriate management practices are used (Singh et al., 2010; King, 2011; Trivedi et al., 2013a,b).

In recent decades agricultural productivity has been raised by increased fertilization and pesticide application, improved irrigation, soil management regimes and crops as well as massive land use change (Tilman et al., 2002; Pittelkow et al., 2015). However, intensive agriculture has caused 30–50% losses in the amount of

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soil C in the last century leading to the degradation of various ecosystem functions (maintenance of soil structure and fertility, soil C sequestration, nutrient cycling, and hydrological services) that impact upon plant productivity and ecosystem sustainability (Thiele-Bruhn et al., 2012). Management practices such as no or minimal till, reduction or elimination of fallow periods, intensifying cropping with the use of crop rotations and cover crops, and judicious use of inputs (e.g., pesticides, irrigation, fertilizers and manures) aim at mitigating these negative impacts in order to improve sustainable production (Gomiero et al., 2011; Thiele-Bruhn et al., 2012; Pittelkow et al., 2015). Changes in land-use or management practices are known to impact soil C turnover but the underlying mechanisms are largely unknown. The lack of a mechanistic understanding constrains their broad adoption in large-scale farm management (Tscharntke et al., 2012).

In terrestrial ecosystems, the uptake of CO_2 from the atmosphere by net primary production is dominated by higher plants, but soil microorganisms contribute greatly to the ecosystem C budgets through their multiple roles in soil C dynamics thereby modifying nutrient availability and influencing the longevity and stability of C pools (Bardgett et al., 2008; Van der Heijden et al., 2008; Singh et al., 2010; Trivedi et al., 2013a,b). Management decisions in agricultural systems can be important drivers of community change for soil microbes performing important ecosystem processess including C cycling (Six et al., 2004; Postma-Blaauw et al., 2010; Pittelkow et al., 2015). Evidence suggests that terrestrial agroecosystems can be managed and manipulated to increase soil C, however how much control the soil microbial community has on C dynamics remains a debatable topic (Hartmann et al., 2015; Singh et al., 2010). Understanding the mechanisms of microbial regulation of soil C turnover is a key challenge for predicting the loss or gain of soil C under various management practices.

The functioning of soil is, to a large degree, defined by its structure which is believed to be an important regulator of microbially mediated C storage/decomposition (Mummey et al., 2006). It is believed that soil C can be physically protected either by adsorption onto organic/inorganic clay surfaces or by the entrapment in soil aggregates and is inaccessible to degrading microbes and extracellular enzymes (Six et al., 2006; King, 2011; Vos et al., 2013). Changes in agricultural management practices influence soil structural properties including soil aggregation (Six et al., 2006; Tiemann et al., 2015). This regulates soil physical and chemical heterogeneity and consequently the distribution of microbial communities and their activities among aggregates of different sizes (Vos et al., 2013). Aggregates of different sizes and stability in soil create a composite of ecological niches differing in terms of physico-chemical and structural characteristics which promotes the colonisation and maintenance of distinct microbial assemblages within each aggregate (Davinic et al., 2012; Vos et al., 2013; Tiemann et al., 2015). Knowledge of microbial communities and their activities within different microenvironments (i.e. aggregate size) is currently poor but essential for understanding the regulation of soil C cycling which has important implications for increasing crop production and maintaining agricultural sustainability (Grundmann, 2004).

Due to high microbial diversity and complexity, it remains a daunting task to link the structure and composition of soil microbial communities to the functional activities related to ecosystem functioning (Torsvik and Øvreås, 2002; Nannipieri et al., 2003; Zhou et al., 2010). In recent years various studies have provided detailed information on microbial community structure in terrestrial ecosystems (Acosta-Martinez et al., 2008; Jangid et al., 2008, 2011; Yin et al., 2010; Ramirez et al., 2012; Singh et al., 2014). However, due to the potential (or perceived) high functional redundancy, our ability to make valid linkages between the taxonomic makeup and functional potential of microbial communities such as those related to C turnover is limited (Reeve et al., 2010; Singh et al., 2010; Thiele-Bruhn et al., 2012; Nie et al., 2014). We also have a limited understanding about the potential role of soil aggregates in structuring microbial communities, and within these microhabitats, little is known about the localization of microbial communities and their functions. In the present study our aim was to identify the response of different crop types on the structure and function of soil microbial communities and the consequences for soil processes directly linked to soil C cycling. We hypothesised that: (i) the management practices would have significant impact on the structure and function of the soil microbial community linked to C turnover and these effects would be more pronounced in larger soil structures (whole soil and macroaggregates); (ii) each aggregate-size fraction would be dominated by distinct bacterial assemblages and the abundance of bacterial groups in the aggregates would depend on C availability. To test these, hypotheses we used soil samples collected from a long term "cropping regime trial" conducted on mild-clay soil in a major Cotton/Wheat producing agro-ecosystem of Australia. We first employed advanced metagenomics/molecular approaches [pyrosequencing (Margulies et al., 2005; Hamady et al., 2008); GeoChip 4.0 (He et al., 2007); qPCR (Trivedi et al., 2013b] in concert with soil biochemical [soil enzyme (Bell et al., 2013)] approaches to determine the effect of crop management on the structural diversity and functional potential of soil microbial communities in relation to indicators of soil C turnover in whole soil samples. In the second part of the study we separated the soil into three aggregate size fractions and used chemical and molecular techniques (qPCR) to access relationships between soil C, microbial derived enzymes and soil bacterial community at the soil microenvironment scale (e.g. within different aggregate-size fractions).

2. Material and methods

2.1. Field site description

The long term "Cropping System Experiment" was located in Field 6 at the Australian Cotton Research Institute, near Narrabri (149°47′ E, 30°13′ S) in New South Wales (NSW), Australia. Narrabri has a subtropical, semi-arid climate (Kottek et al., 2006) with a mild winter and a hot summer. The hottest month is January (mean daily maximum 35 °C and minimum 19 °C) and the coldest is July (mean daily maximum 18 °C and minimum 3 °C). Mean annual rainfall is 593 mm. The soil at the experimental site is an alkaline, self-mulching, gray Vertosol, classified as a fine, thermic, smectitic, Typic Haplustert (Soil Survey Staff, 2010). Mean particle size distribution in the 0–1 m depth (per 100 g) was: 64 g clay, 11 g silt, and 25 g sand.

2.2. Experimental layout and sample collection

The experiment commenced in 1998 and included four cropping treatments replicated three times. Each plot was 16 m long and 8 m wide. These treatments included: continuous cotton where cotton was grown every two years with winter fallow (C ~ C ~ C); cotton–vetch, where cotton was grown every two years in summers and vetch (*Vicia villosa* Roth) was grown each winter (CVCV); cotton–wheat where cotton was grown every two years with wheat then fallow (CW ~ CW); and cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch (CWV). The trial followed typical management practices for crops in this area i.e. cotton crop was fully irrigated while other crops received natural rainfall. Briefly cotton crops were furrow-irrigated regularly to avoid drought stress when the soil water deficit approached

50 mm. In 2012/13, there were 6 irrigations. Wheat stubble was incorporated into the topsoil after harvest in the CW ~ CW and CWV treatments. Vetch was killed by mowing and then incorporated into the topsoil in CVCV and CWV. Importantly, the experiment used a minimum tillage system where the 1 m spaced ridges (hills) were maintained throughout the experiment with shallow (10 cm depth) tillage to maintain the furrows between each crop, to control overwintering pupae of *Helicoverpa* spp, and to incorporate herbicides and stubble. More details for the site are provided elsewhere (Rochester, 2011a,b, 2012).

Four soil cores from each treatment plot were randomly collected from 0 to 15 cm depth using a 3-cm diameter auger in September 2013 prior to fertilizer application, irrigation and Cotton sowing. These soils from each plot were then pooled in a Ziploc bag, and placed in a cooler on ice. Last standing crops before the sample collection were cotton and wheat C ~ C ~ C and CW ~ CW treatments and vetch for CVCV and CWV treatments (last standing crop is represented as the last letter for the treatments). At the time of sample collections last standing crop has been harvested. Upon return to the laboratory, 20 g of soil from each sample was collected in 50 mL centrifuge tubes for molecular analysis and stored at −80 °C, until required for DNA extraction. Approximately, 100 and 250 g of soil from each sample was collected in plastic bags for soil chemical analysis and aggregate fractionation, respectively. These samples were stored at 4 °C and the analyses were performed within a week of sample collection.

2.3. Soil chemical and biological analyses

Soil moisture content was determined by oven-drying the samples at 105 °C overnight. Soil pH was assessed using a fresh soil to water ratio of 2.5 using a Delta pH-meter (Mettler-Toledo Instruments Co., Columbus, OH, USA). Total carbon and total nitrogen were measured on a LECO macro-CN analyzer (LECO, St. Joseph, MI, USA). Dissolved organic carbon (DOC) was determined by an extraction method described by Jones and Willett (2006) and measured on a TOC analyzer (Shimadzu TOC-500A, Japan).

2.3.1. MicroResp and enzyme assays

Microplate format of MicroResp™ (Macaulay Scientific Consulting, UK; Campbell et al., 2003) was used to determine basal respiration. Approximately 0.35 g of fresh soil was added to each well of the deep well microtitre plates to which 30 μl of water was added. To avoid the practical problems of delivering field moist high clay soils to the deep well plates, samples were weighed and added to the wells individually without the use of delivery device. Twelve individual wells were filled for each soil sample. 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. A caliberation curve was generated by measuring CO_2 release by gas chromatography and the absorbance of microstrips containing the indicator dye at 570 nm by spectrophotometer using different soil types collected all across Australia as described in detail by Campbell et al. (2003). The conversion of absorbance to $\%CO_2$ was a non-linear relationship and the best fitted curve was used to obtain the formula and parameters. The following formula converts the normalized t0/t6 h data (Ai) to $\%CO_2$: $\%CO_2 = A + B/(1 + D \times Ai)$. Where $A = -0.4002$, $B = -1.298$, $D = -5.8181$. β-D-celluliosidase (CB), β-Xylosidase (XYL), α-Glucosidase (AG), and N-acetyl-β-Glucosaminidase (NAG) activities were measured using 4-methyl umbelliferyl (MUB) substrate yielding the highly fluorescent cleavage products MUB upon hydrolysis (Wallenstein and Weintraub, 2008). All the enzyme assays were set up in 96-well microplates as described by Bell et al. (2013). 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 and Weintraub, 2008). Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 460 nm emission filters. The activities were expressed as nmol h^{-1} g^{-1}dry soil.

2.4. Molecular analysis

2.4.1. Soil DNA extraction

The frozen soil (0.3 g) was used for DNA extraction with the FastDNA SPIN Kit for soil (MP Biomedicals, Heidelberg, Germany) according to the manufacturer's protocol. Extracted DNA was then purified using a 15 min incubation at 65 °C in a solution of 10% CTAB (cetyl-trimethyl ammonium bromide) and 0.7 M NaCl, followed by 24:1 chloroform:isoamyl alcohol extraction, precipitation with ethanol, and resuspension in TE buffer. DNA quality was checked on a 0.7% (w/v) agarose gel. DNA concentrations were determined using the Qubit quantification platform with Quant-iT™ dsDNA BR assay kit (Invitrogen, Carlsbad, USA). DNA was diluted to 10 ng μl^{-1} and stored in a −80 °C freezer for the following molecular analysis.

2.4.2. Quantitative PCR (qPCR)

qPCR quantifications of taxon specific 16S rDNA were performed using primers and cycling conditions described in Supplementary Table 1. qPCR reactions were carried out on extracted soil DNA from different samples using Absolute qPCR SYBR green mixes (Qiagen Inc., Valencia, CA, USA) on an ABI Prism 7500 Sequence detection system. Standard curves for real-time PCR assays were developed by PCR amplifying the respective taxa by their specific primers following methods described in detail by Trivedi et al. (2013b). Target copy numbers for each reaction were calculated from the standard curve and were used to ascertain the number of copies per g of soil. The relative fractional abundance for each of the groups was calculated by determining the copy numbers measured with each taxon-specific qPCR assay and with the "total-bacteria" assay (Fierer et al., 2005).

2.5. Barcoded pyrosequencing

Fusion primers 341F-806R and LR3-LR0R were used to amplify multiplexed bar-coded 16S rRNA and large subunit rRNA gene sequences, to profile bacterial and fungal communities, respectively. PCR products were purified, pooled and sequenced on a 454 GS FLX Titanium sequences (Roche 454 Life Sciences, Branford, CT, USA). Downstream processing and bioinformatics analysis was performed as described by Singh et al. (2014) and Barnard et al. (2013) for bacteria and fungus, respectively. Each operational taxonomic unit (OTU) was classified to the genus level and the relative abundance of each OTU in a sample was calculated across phyla, class, and genus. OTU tables used for analysis were rarefied to 1210 and 1194 sequences for bacteria and fungi, respectively, to ensure even sampling depth.

2.6. GeoChip 4.0 analysis

Geochip 4.0 analysis was performed as described by He et al. (2010). Briefly, DNA samples were labeled with fluorescent dye Cy-5 by a random priming method (Zhao et al., 2014), followed by purification with a QIA quick purification kit (Qiagen, Valencia, USA). Dye incorporation was measured by a Nano-Drop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington,

USA), and DNA was dried by a SpeedVac (ThrmoSavant, Milford, MA, USA) at 45 °C for 45 min. Thereafter DNA was hybridised with GeoChip 4.0 at 42 °C for 16 h in MAUI hybridization station (BioMicro, Salt-Lake City, UT, USA) and scanned by a NimbleGen MS200 scanned (Roche, Madison, WI, USA) at 633 nm laser, using 100% and 75% laser power and photomultiplier tube (PMT) gain, respectively. Data processing was performed as previously described (He et al., 2007, 2010) using Microarray Data Manager (http://ieg.ou.edu/microarray/).

2.7. Soil aggregate fractionation

Soil was fractionated using a wet-sieving technique as described in detail by Davinic et al. (2012) and three fractions were obtained for each sample: macroaggregates (>250 μm); microaggregates (50–250 μm); and silt-sized microaggregates and silt + clay particles (<50 μm). DNA extraction, qPCR analysis, soil properties, and soil enzyme analyses were performed as described in the previous sections.

2.8. Statistical analysis

This study was analysed as a Completely Randomized Design with each treatment as the replicate experimental unit, and each plot as a repeatedly measured unit within each treatment. Mantel test and analysis of variance (ANOVA) were performed using the VEGAN package in R version 3.0.0 (The R Foundation for Statistical Computing). Differences between treatments were compared by post hoc Fisher's least significant difference (LSD) test with Holm–Bonferroni adjustment. The significant differences were defined as $P < 0.05$, or with listed P-values. To illustrate the effect of soil properties with the cropping treatments PCO analysis was carried out using PRIMER 6.0 statistical package (Clarke and Gorley, 2006). A bootstrapped canonical analysis of principal coordinates (CAP) was performed using PRIMER 6.0 statistical package (Clarke and Gorley, 2006) to assess how structural and functional diversity determined by pyrosequencing and GeoChip, respectively could be partitioned into variations attributable to management practices.

3. Results

3.1. Effect of cropping regimes on soil processess and microbial communities in whole soil samples

The chemical and biological properties of soil are listed in Table 1. Total soil C content was significantly higher in the CVCV treatment (14.43 g kg^{-1}) while the lowest C content was observed in the CW ~ CW treatment (13.49 g kg^{-1}; Table 1). Our results from different years of sampling on total soil C showed similar trends and suggested that the differences are biologically significant and correctly represent treatment effect (Table 1). CVCV recorded the highest amount of total N (1.39 g kg^{-1}; significant at $P < 0.05$) followed by CWV (1.23 g kg^{-1}), C ~ C ~ C (1.13 g kg^{-1}), and CW ~ CW treatments (1.07 g kg^{-1}), respectively (Table 1). Soil moisture content in the C ~ C ~ C treatment was more than 2-fold higher as compared to other treatments. Basal respiration (measured as μg CO_2–C g^{-1} h^{-1}) was highest in CVCV treatments (0.31; $P < 0.05$) followed by CWV (0.28), C ~ C ~ C (0.26) and CW ~ CW (0.23) treatments (Table 1). Dissolved organic carbon (DOC) content was 68.83 and 61.23 mg 100 g^{-1} of soil for CVCV and CWV treatments, respectively which was significantly greater (at $P < 0.05$) than recorded for C ~ C ~ C (56.23 mg 100 g^{-1} of soil) and CW ~ CW (51.28 mg 100 g^{-1} of soil) treatments. The PCoA visualization revealed clear differences between the cropping treatments (Supplementary Fig. 1). About 91.0% of the total variance was explained by the first two axes, with PCoA 1 and PCoA 2 explaining 71.3% and 20.1% of variation, respectively. The PCoA biplot showed that total C, N and enzymatic activities correlated with the first ordination axis that discriminates between different treatments.

We also observed differences in the activity of extracellular enzymes between soil samples from different treatments (Table 1). The activity of all the extracellular enzymes was highest in CVCV treatment. The activity of CB and XYL in the CVCV treatment was 2 times higher as compared to the activity in C ~ C ~ C and CW ~ CW treatments. AG activity was lowest in the CWV treatment (21.3 nmol h^{-1} g^{-1}dry soil) which was approximately 3 times lower than recorded in the CVCV treatment (57.3 nmol h^{-1} g^{-1}dry soil). There was no significant differences between the activity of NAG in C ~ C ~ C and CW ~ CW treatments. NAG activity in CVCV was highest (19.3 nmol h^{-1} g^{-1}dry soil) and was 2.5 times higher than that recorded for CW ~ CW treatment (8.1 nmol h^{-1} g^{-1}dry soil).

3.2. Pyrosequencing and taxon specific qPCR analysis

Approximately 20,400 and 18,764 high quality sequence reads for bacteria and fungi, respectively were generated for the 12 replicated samples across the 4 cropping treatments. There was no significant difference in the number of reads from different treatments which were on average 1699 ± 98 and 1453 ± 63 per sample for bacteria and fungi, respectively. Community analysis using 454 pyrosequencing revealed that the α-diversity for both bacterial and fungal communities remained unchanged in the different treatments within this long term crop management experiment. We observed no significant differences in the overall microbial diversity, measured by the number of OTUs (data not shown) and Shannon, Chao1 and Simpson diversity indices (Supplementary Table 2). However, the structure of the bacterial community was markedly different among treatments as indicated by CAP analysis where a clear separation was observed between treatments (Fig. 1A). Similarly, we observed a clear separation of the fungal community between different treatments (Fig. 1B). In accordance with our hypothesis, community structure (β-diversity) for bacteria and fungi was significantly impacted among the four treatments (Fig. 2A and B). The relative abundance of Acidobacteria, Actinobacteria, Bacteroidetes and δ-proteobacteria was higher in C ~ C ~ C and CW ~ CW treatments, compared with CVCV or CWV treatments. On the other hand, the relative abundance of α, β, and γ Proteobacteria was higher in the CVCV and CWV treatments as compared with the other two treatments (Fig. 2A). Narrabri samples were dominated by fungal species belonging to phylum Ascomycota and Basidiomycota which together contributed about 80% of the total sequences among different samples (Fig. 2B). The relative abundance of fungal groups belonging to phylum Ascomycota were higher in C ~ C ~ C treatment (Fig. 2B). The relative abundance of OTUs belonging to Basidiomycota were highest in CVCV treatment. Overall the relative abundance of Basidiomycota was lower in C ~ C ~ C treatment in comparison to the other three treatments (Fig. 2B).

qPCR analysis showed that there were no difference between the total number of bacteria in C ~ C ~ C, CVCV or CWV treatments however the total bacterial numbers in the CW ~ CW treatment were significantly lower (at $P < 0.05$) in comparison to the other treatments (Supplementary Fig. 2). Taxon specific qPCR analysis showed similar trends as the pyrosequencing data with the relative abundance of α, β, and γ Proteobacteria being higher in CVCV and CWV treatments whereas the relative abundance of Actinobacteria, Acidobacteria, Fimiricutes and Bacteroidetes was higher in the C ~ C ~ C and CW ~ CW treatments (Supplementary Fig. 3). The abundance of most bacterial groups (except for Bacteroidetes) was

Table 1
Soil chemical and biological characteristics under each treatment in a long term crop rotation trial.

Soil property	Year	Treatment[a]			
		C ~ C ~ C	CW ~ CW	CVCV	CWV
Total carbon (g kg^{-1})	2013	13.73a	13.47b	14.38c	13.80a
	2012	13.78a	13.42b	14.41c	13.76a
	2014	13.80a	13.49b	14.43c	13.83a
Dissolved Organic Carbon (DOC, mg 100 g^{-1})		56.23ad	51.29 ab	68.83c	61.23d
Total nitrogen (g kg^{-1})		1.13a	1.07a	1.39b	1.23c
pH		8.22a	8.16 ab	8.05c	8.13bc
Soil moisture		6.48a	2.31b	3.16c	2.50b
Basal respiration (μg CO_2–C g^{-1} h^{-1})		0.26a	0.23b	0.31c	0.28bc
Soil enzymatic activity (nmol h^{-1} g^{-1}dry soil)					
β-D-celluliosidase (CB)		6.5a	7.6a	16.6b	10.7c
β-Xylosidase (XYL)		6.8a	6.3a	14.5b	10.2c
α-Glucosidase (AG)		44.23a	30.23b	57.3c	21.3b
N-acetyl-β-Glucosaminidase (NAG)		16.4ac	8.1b	19.3c	15.3a

[a] Values followed by same letter within a parameter are not significantly different at $P < 0.05$. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

correlated with total C content in the four treatments (Supplementary Table 3).

3.3. GeoChip analysis

It is important to link microbial community structure and their potential ecological functions with the microbial functional genes involved in major biogeochemical processes (Torsvik and Øvreås, 2002; Trivedi et al., 2013a,b). We used GeoChip because it contains functional genes involved in various ecosystem processes originating from both bacterial and fungal groups. This method has been successfully applied previously to explore the functional potential of microbial communities and has also been used to relate gene abundances with ecosystem functions (Trivedi et al., 2013b; Chu et al., 2014; Macdonald et al., 2015; Su et al., 2015). Geochip analysis detected 1897, 1799, 2120, and 2311 functional genes in C ~ C ~ C, CW ~ CW, CWV and CVCV treatments, respectively. CAP analysis of all the functional genes detected by GeoChip 4.0 revealed distinct separation of the different treatments on the first and second axis (Fig. 1C) which was similar to the pyrosequencing data.

In this study, metabolic genes involved in the degradation of starch, cellulose, hemicellulose, chitin, lignin, and pectin were detected in all the samples and the individual gene orthologs were abundant and diverse (Fig. 3). The abundance (measured as signal intensity) of genes involved in the degradation of labile C forms including those encoding α-amylase, pullulanase, Glucoamylase for starch decomposition and Arabinofuranosidase, Cellobiase and Xylanase for hemi-cellulose decomposition were significantly higher in the CVCV and CWV treatments ($P < 0.05$). For example the abundance of α-amylase, pullulanase, Glucoamylase genes was more than 2 fold higher in CVCV treatments as compared to C ~ C ~ C and CW ~ CW treatments. Overall, the CVCV treatments had significantly higher abundance for the genes encoding enzymes for labile C (Fig. 3). The genes involved in the degradation of moderately labile C such as endo-exo-glucanase involved in cellulose degradation and vanilate demethylase involved in the degradation of aromatics were significantly lower in CW ~ CW treatments ($P < 0.05$) when compared with the other three treatments. The genes involved in the degradation of hemicellulose and aromatics were highest in the CVCV treatments and their abundance in different treatments followed the order CVCV > C ~ C ~ C > CWV > CW ~ CW. Interestingly, the abundance of genes involved in the degradation of recalcitrant C such as chitin and lignin degrading genes were lowest in the CW ~ C treatment ($P < 0.05$) as compared with the other treatments. These genes showed a higher abundance in CVCV and CWV treatments.

We also performed Mantel tests to investigate the relationship between the selected genes involved in C degradation and the activity of enzymes coded by these genes. Our analyses showed that the soil enzyme activities were correlated with the intensities of functional genes detected by GeoChip ($P < 0.05$; Supplementary Table 4). These results suggested that microbial community functional gene structure could affect the soil C turnover.

3.4. Effect of cropping regimes on the soil processess and microbial communities in different size soil aggregates

In all treatments the silt and clay fractions had significantly higher C and N, followed by microaggregates and macroaggregates, respectively (Table 2). Across all treatments, total C of silt–clay aggregates was approximately 2 and 3 fold higher as compared to microaggregate and macroaggregate, respectively. We observed a trend of increased C (CVCV < CWV < CW ~ CW < C ~ C ~ C) and N (CVCV < CWV < CW ~ CW < C ~ C ~ C) in the macroaggregates in response to the treatments (Table 2).

This responsiveness appeared to decrease with the size of aggregates and there were no significant differences in the amount of C and N among cropping treatments in silt–clay fractions. In all treatments the amount of DOC was highest in the macroaggregates which was 1.5 and 2 fold higher as compared to microaggregate and silt–clay, respectively. All enzymatic activities related to C decomposition increased as the aggregate size decreased (Table 2). In all the treatments the enzymatic activities of XYL, AG, and NAG were 3 fold higher in silt–clay as compared to macroaggregates. No treatment effect was observed for enzymatic activities in microaggregates and silt–clay fractions. In the macroaggregates, the CVCV treatments had higher enzymatic activities in comparison to the other treatments.

In all treatments, the total abundance of bacteria was higher in silt–clay fractions (Fig. 4A). In the similar size aggregates within different treatments, no significant difference in total number of bacteria was observed except for CVCV treatments where the abundances were higher in macroaggregates and microaggregates as compared to other treatments. qPCR analysis also showed differences in the relative abundance of some groups of bacteria in different sizes of aggregates (Fig. 4 B–D). The relative abundance of Acidobacteria (Fig. 4C) and Bacteriodetes (Fig. 4D) increased with

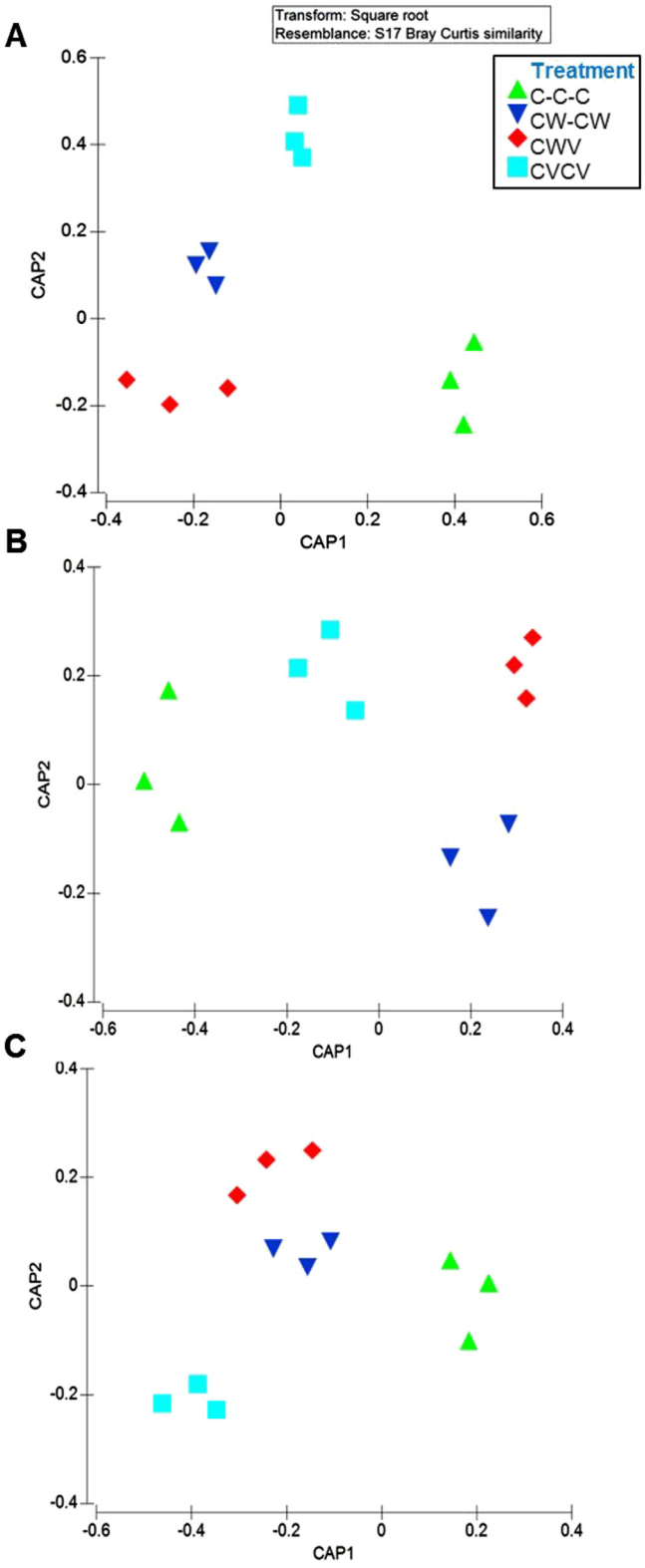

Fig. 1. Pyrosequencing based canonical analysis of principal coordinates (CAP) analysis of bacterial (A) and fungal (B) communities and GeoChip based CAP analysis of functional genes (C) under different treatments in long term crop rotation trials at Narrabri. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

the decrease in aggregate size while the opposite trend was observed for α-Proteobacteria (Fig. 4B), which was highest in macro-aggregates. Among treatments, the relative abundance of different bacterial groups varied only in the macroaggregate fractions (Fig. 4B–D). In these macroaggregates the relative abundance of α-Proteobacteria was significantly higher in the CVCV treatment while the relative abundance of Acidobacteria and Bacteroidetes were higher in C ~ C ~ C and CW ~ CW as compared to CWV and CVCV treatments.

Our analysis showed a positive correlation between the abundance of Alphaproteobacteria and the amount of DOC in different aggregates ($R^2 = 0.928$, $P < 0.01$) (Fig. 5a). On the other hand, a negative correlation was observed between the abundance of Actinobacteria ($R^2 = 0.751$, $P < 0.05$) and Bacteroidetes ($R^2 = 0.805$, $P < 0.01$) and the DOC content of different aggregates (Fig. 5 b and c).

4. Discussion

Soil is one of the most difficult environments to work with due to its complexity, therefore there are additional methodological challenges from soil sampling to sequencing analysis (Lombard et al., 2011; Lupatini et al., 2013). Our results represent a single time point and variations in phenological differences between the plants growing in different treatments according to crop management cannot be considered. Though seasonal dynamics and plant type might impact the microbial structure and abundance, previous studies have shown that long term patterns within soil microbial communities generally remain intact and reflect differences in management practices (Lupatini et al., 2013; Williams et al., 2013).

4.1. Cropping regimes affects soil properties in long term trials

We recorded the highest total soil C in CVCV and CWV treatments where crops were grown every winter and summer. The two non-legume systems had less soil C which reflects the longer fallow and fewer crops that provided stubble. From the same site, Rochester (2011a) reported that a legume crop system returned 49% more stubble-C and 133% more stubble-N than non-legume systems. Overall, the systems that returned greater quantities of stubble-C or produced stubble of higher N concentration showed greater amount of soil C. Our results are consistent with previous studies which have indicated that the use of practices such as stubble incorporation can increase the level of soil C in arable agroecosystems (Six et al., 2006; Bissett et al., 2011; Jangid et al., 2011; Bowles et al., 2014). Rochester (2011a) showed similar trends in the amount of soil C among different treatments from the same experiment site.

Similar to soil C, the amount of total N was significantly higher in the treatments that included vetch crops compared with cotton or wheat crops ($P < 0.05$). Leguminous rotation crops are constantly linked to increases in N availability, aggregate formation and stability (Hulugalle and Scott, 2006; Tiemann et al., 2015). Legume stubble has higher N content than cereal or cotton stubble. In the non-legume systems used in the experiment, the quality of the wheat and cotton stubble returned were of low N content (0.78% and 1.56% N, respectively), while legume stubble averaged 3.39% N (Rochester 2011a,b). Our results show that legume rotation may increase both the soil C and N levels which are known to improve soil quality and system productivity. Other studies have reported higher rates of C sequestration in legume cropping systems (Hulugalle, 2000; Rochester, 2012). N losses were reduced where green-manured legume crops (Vetch and clover) were incorporated compared with leaving the stubble on the surface as performed for cereal crops (Asagi and Ueno, 2009). Also, Novak et al.

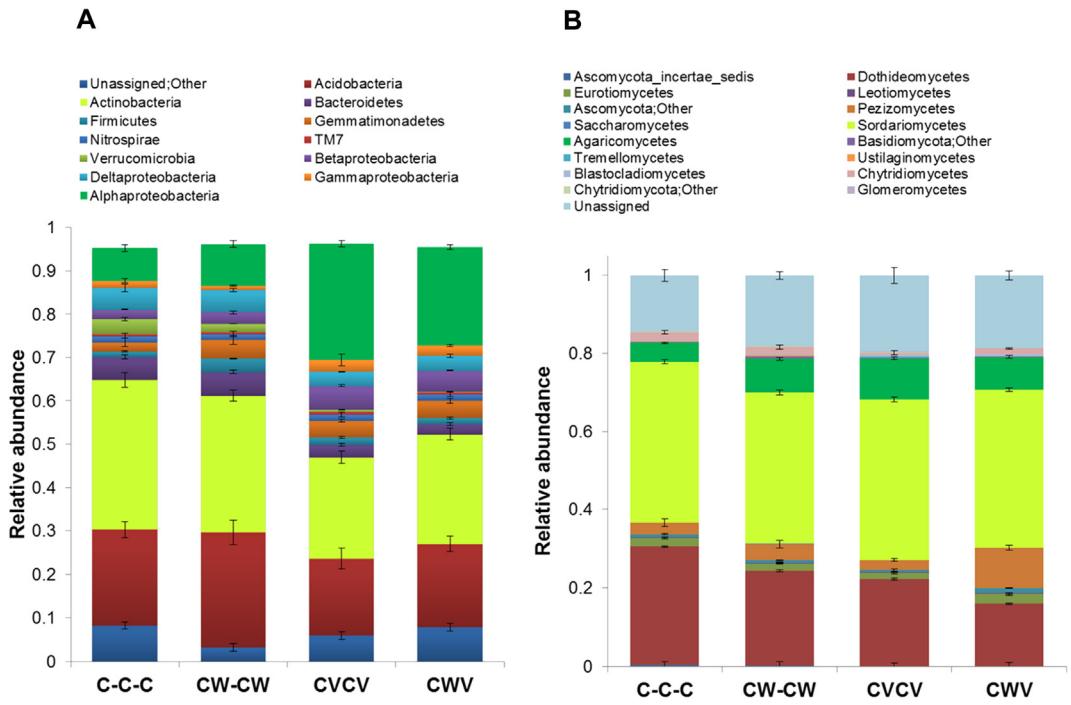

Fig. 2. Average distribution of (A) 16 S rRNA sequence classified at phylum level (class for Proteobacteria) for bacteria; and (B) large subunit rRNA sequence classified at class level for fungi as determined by Pyrosequencing analysis under different treatments in long term crop rotation trials at Narrabri. Error bars represent the standard errors between three replicates. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

(2009) showed a significant decline in topsoil soil organic C under conservation tillage where stubble was not incorporated.

The higher soil moisture content in the C ~ C ~ C treatment was related to irrigation in these plots whereas at the time of sampling the other treatments were under rain-fed conditions. An increase in the basal respiration rate could be directly related to an increase in soil C (both Total C and DOC) in CVCV and CWV plots (Supplementary Table 3). Further, the amount of soil enzymes involved in the degradation of labile forms of C was higher in CVCV and CWV treatments. This suggests that the increase in basal respiration may be the result of increased amounts of easily degradable structural plant carbohydrates (with low C:N ratio)

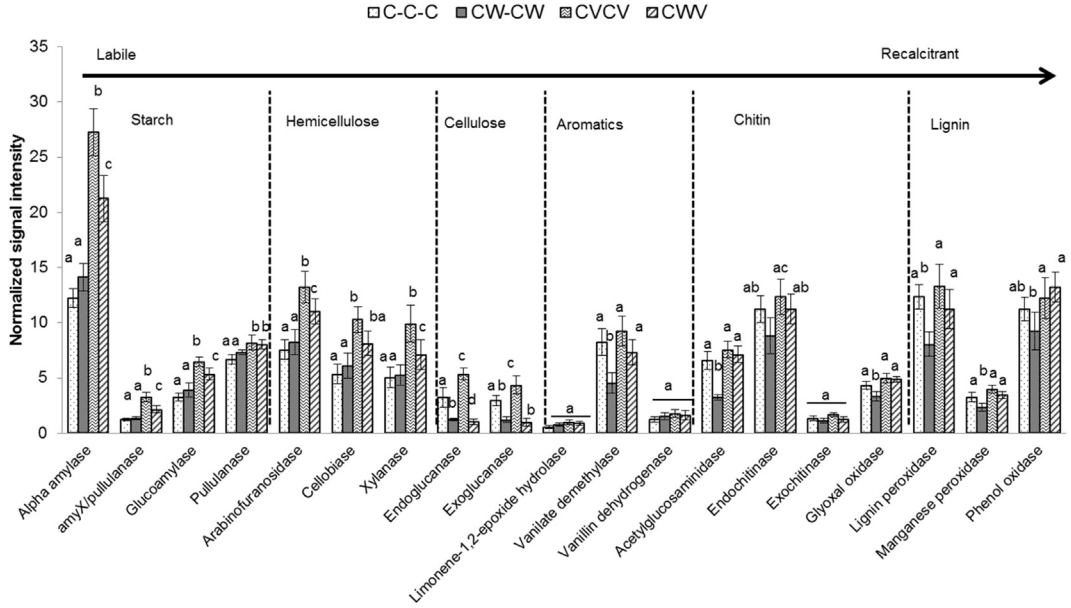

Fig. 3. The normalized signal intensity of the key gene families involved in carbon degradation. The signal intensities were the sum of individual gene sequences for each functional gene averaged over three soil samples of replicates within each treatment. The complexity of carbon is presented in the order from labile to recalcitrant. Different letters indicate statistical differences at a P value of <0.05. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

Table 2
Soil chemical characteristics of soil aggregates under each treatment in a long term crop rotation trial.

Soil properties	Treatment	Aggregates		
		Macroaggregate (>250 mm)	Microaggregate (50–250 mm)	Silt + clay (<50 mm)
Total carbon (g kg^{-1})	C ~ C ~ C	7.8Aa	11.2Bc	26.5Ca
	CW ~ CW	6.8Ab	13.23Bb	25.6Ca
	CVCV	12.1Ad	16.7Ba	26.5Ca
	CWV	9.9Ac	14.2Bb	24.2Ca
Dissolved organic carbon (DOC, mg 100 g^{-1})	C ~ C ~ C	122.49Aab	73.59Ba	59.34Ca
	CW ~ CW	109.7Ab	78.73Ba	59.96Ca
	CVCV	153.93Ac	91.81Bb	61.84Ca
	CWV	135.41Ab	86.34Bb	60.57Ca
Total nitrogen (g kg^{-1})	C ~ C ~ C	0.028Aa	0.049Ba	0.128Ca
	CW ~ CW	0.022Ab	0.056Bb	0.126Ca
	CVCV	0.038Ad	0.063Bc	0.129Ca
	CWV	0.030Ac	0.052Bb	0.119Ca
Soil enzymatic activity (nmol h^{-1} g^{-1}dry soil)				
β-D-celluliosidase (CB)	C ~ C ~ C	34.23Aa	44.23Ba	72.45Ca
	CW ~ CW	36.24Aab	45.23Ba	75.34Ca
	CVCV	44.22Ac	46.98 Ba	71.34Ca
	CWV	38.29Aab	48.23Ba	74.89Ca
β-Xylosidase (XYL)	C ~ C ~ C	9.23Aa	14.78Ba	26.65Ca
	CW ~ CW	9.97Aa	14.34Ba	26.55Ca
	CVCV	13.22Ab	15.88Ba	26.09Ca
	CWV	10.03Aa	13.90Bb	27.45Ca
α-Glucosidase (AG)	C ~ C ~ C	9.03Aa	14.78Ba	24.65Ca
	CW ~ CW	9.37Aa	13.34Bb	23.55Ca
	CVCV	10.99Ab	13.39Bb	23.09Ca
	CWV	9.23Aa	13.88Bb	23.45Ca
N-acetyl-β-Glucosaminidase (NAG)	C ~ C ~ C	7.36Aa	16.78Ba	28.65Ca
	CW ~ CW	8.22Ab	17.34Ba	27.72Ca
	CVCV	9.92Ac	16.92Ba	28.22Ca
	CWV	8.02Aab	17.88Ba	27.99Ca

Different uppercase and lowercase letters indicate statistical differences at a P value of <0.05 among similar size aggregates among treatments and different size aggregates under similar treatment. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

entering into the decomposition pathways in legume based treatments (Rochester, 2012).

4.2. Cropping regimes influenced soil microbial community structure

Both pyrosequencing and qPCR results showed an increased abundance of Acidobacteria, Actinobacteria, Bacteroidetes and δ-proteobacteria in non-legume treatments. On the other hand, the relative abundance of α, β, and γ Proteobacteria was significantly higher in legume based treatments (Fig. 2A, Supplementary Fig. 3). Based on the trophic life-histories of soil bacteria α, β, and γ–Proteobacteria have been classified as “copiotroph” (r-stategists), use labile forms of C for growth and metabolism, and grow faster in a nutrient rich environment whereas Acidobacteria, Actinobacteria, and δ-Proteobacteria are classified as “oligotrophs” (k-stategists), degrade relatively recalcitrant forms of C, grow slowly, and are dominant in nutrient poor environments (Fierer et al., 2007; Trivedi et al., 2013a). Previous reports have linked an oligotroph–copiotroph switch in ecosystems where nutrient availability has increased (Singh et al., 2010; Trivedi et al., 2013b; Macdonald et al., 2015). In our study we observed an increased availability of labile C (in form of DOC) in CVCV and CWV treatments where higher abundance of copiotrophs was also observed. Changes in the abundance of α-proteobacteria in the CVCV and CWV treatments can also be the direct result of an interaction between legumes and nutrient availability. Acosta-Martinez et al. (2010) reported higher numbers of Proteobacteria in soil where rotation crops were grown compared with continuous cotton. Proteobacteria encompass an enormous level of morphological, physiological and metabolic diversity and play significant roles in global nutrient cycling (Kersters et al., 2006). Trivedi et al. (2013a) analysed the genomic potential of different groups of bacteria to produce various C-degradation enzymes and found that as a group, Proteobacteria have a greater number of genes involved in the production of enzymes that degrade labile C. Greater abundance of Proteobacteria may therefore be associated with the higher soil enzyme activities and total C observed in CVCV and CWV treatments.

Both qPCR and Pyrosequencing analysis showed increased relative abundance of Firmicutes in the CW ~ CW as compared with other treatments (Fig. 2A and Supplementary Fig. 3). Many members of the phylum Firmicutes produce spores to overcome periods of nutrient scarcity and extreme environmental conditions (Trivedi et al., 2013a). The long fallow periods in the Wheat treatment along with the lower input of degradable C can help in explaining lower bacterial numbers and the increased abundance of Firmicutes in this cropping treatment. Another distinctive trend in this study was the significantly higher number of bacteria belonging to phylum Bacteroidetes ($P < 0.05$; Fig. 2A; Supplementary Fig. 3) in soil within the C ~ C ~ C or CW ~ CW treatments. Our results are supported by a previous study which reported higher numbers of Bacteroidetes in the cropping systems with longer fallow periods (Acosta-Martinez et al., 2010). The dominance of Bacteroidetes in soil under continuous cotton can be attributed to their ability to rapidly exploit bioavailable organic matter and colonize aggregates which may become available as crops are planted after a long winter fallow periods in cotton production systems (Abell and Bowman, 2005; Acosta-Martinez et al., 2010). Pyrosequencing results showed significantly increased abundance of phylum Verrucomicrobia in the CW ~ CW and C ~ C ~ C treatments (Fig. 2A). Verrucomicrobia are important members of the soil microbial community, and are

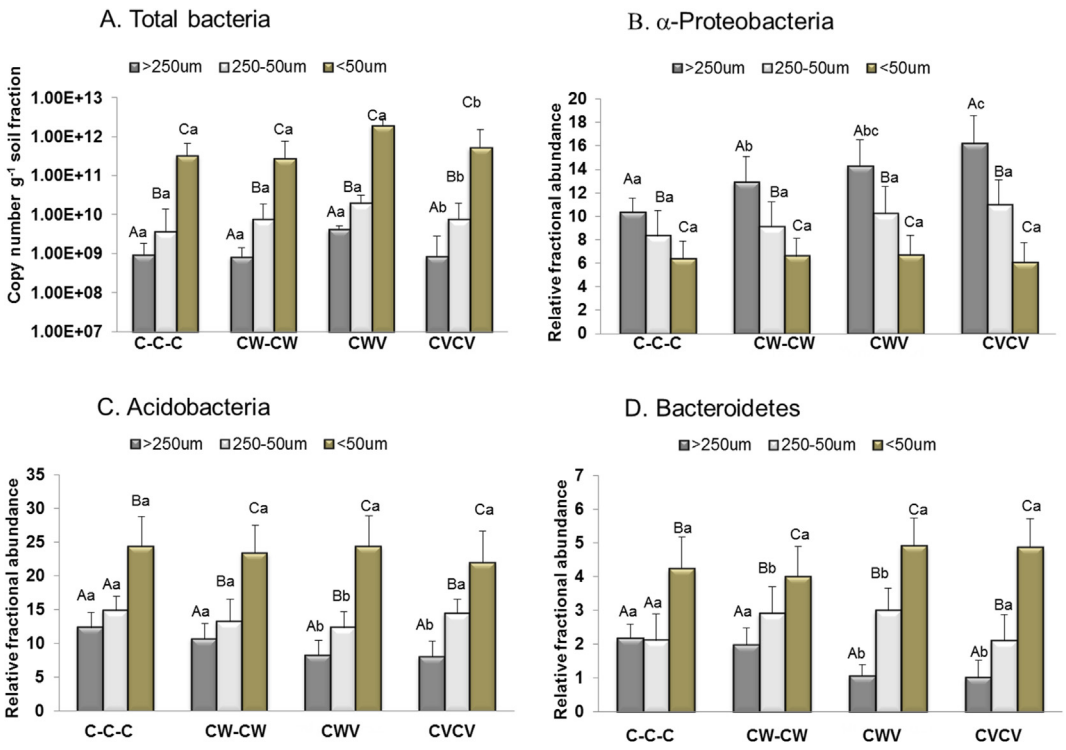

Fig. 4. Total bacterial counts (A) and relative abundance of α-Proteobacteria (B); Acidobacteria (C); and Bacteriodetes (D) in different soil aggregates across treatments in long term cropping trials determined by qPCR (n = 24). Different uppercase and lowercase letters indicate statistical differences at a P value of <0.05 among similar size aggregates among treatments and different size aggregates under similar treatment. The relative fractional abundance for each of the groups was calculated by determining the measured copy number of each group-specific qPCR assay and the 'total bacteria' assay. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

known to fluctuate with soil management practices (Buckley and Schmidt, 2001; Yin et al., 2010; Dorr de Quadros et al., 2012), however the role of these microorganisms in terrestrial ecosystems is poorly understood. The members of phylum Gemmatimonadetes are adapted to low-moisture environments (DeBruyn et al., 2011) and therefore were significantly lower in the C ~ C ~ C treatment, which is more regularly irrigated, compared with other treatments (Rochester, 2011a,b).

The low abundance of *Basidiomycetes* in C ~ C ~ C treatments can be the direct result of increased fallow periods in this treatment. *Basidiomycetes* are mainly saprophytes (Agrios 2005; Mohapatra, 2008) and their increased abundance in the treatments receiving higher plant inputs can directly be related to the increased

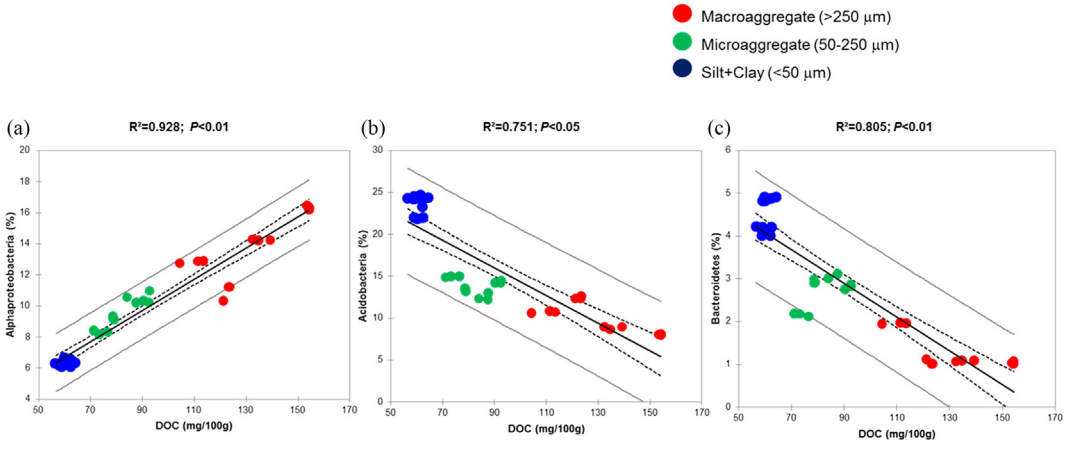

Fig. 5. Relationship (solid line) between dissolved organic carbon (mg 100 g^{-1}) and the abundance (% abundance) of Alphaproteobacteria (a), Acidobacteria (b), and Bacteroidetes (c) detected by qPCR in different aggregates from different treatments under long term crop management trials. Solid black line, black dashed lines and solid gray lines represent linear model, confidence interval (mean 95%), and confidence interval (Obs. 95%), respectively. C–C–C = continuous cotton where cotton was grown every two years with winter fallow; CVCV = cotton–vetch, where cotton was grown every two years in summers and vetch was grown each winter; CW–CW = cotton–wheat where cotton was grown every two years with wheat then fallow; and CWV = cotton–wheat–vetch where cotton was grown every two years followed by wheat and vetch. Last standing crop is represented as the last letter for the treatments.

availability of easily degradable substrates. Although both bacteria and fungi contribute significantly to ecosystem processes, Acosta-Martinez et al. (2008) have reported that in crop rotation trials which are typically tilled, bacterial populations have a much bigger role in impacting soil process as compared with non-disturbed system where shifts to higher fungal populations are found.

Our data suggest that increased availability of easily degradable C may change the soil microbial community from an oligotrophic to a copiotrophic regime. Implications of these changes can have multiple feedbacks in ecosystem processes, particularly in relation to C cycling. Including legumes in the crop rotation may lead to an increase in soil microbial respiration, as the greater labile C inputs are readily consumed by r-strategists (Adair et al., 2009; He et al., 2010) resulting in little significant long-term impact and stability on soil C stocks which are determined by more recalcitrant forms.

4.3. Long term cropping regimes have a significant effect on the functional structure of microbial communities involved in carbon cycling

Analyzing microbial functional genes encoding key enzymes involved in major biogeochemical processes is important to link microbial community structure to their potential ecological functions (Torsvik and Øvreås, 2002). Soil microbial communities have commonly been viewed as black boxes into which organic C flows and is converted into CO_2 or biomass (Allison and Martiny, 2008). This black box assumption may be valid only if microbial composition is resistant, resilient and/or functionally redundant to environmental factors (Allison and Martiny, 2008). Our study revealed major shifts in overall structure (Fig. 1A and B) and function (Fig. 1C) of the soil microbial community under different treatments in a long term crop rotation experiment. Along with previous studies, our results suggest that the composition, size, and metabolic activity of the soil microbial community may affect the degradation rates of C substrates primarily through shifts in enzymatic activities (He et al., 2010; Zhou et al., 2012; Trivedi et al., 2013b; Chu et al., 2014). Significant correlations between the functional gene data and soil enzymatic activities (Supplementary Table 4) provide novel evidence of a close linkage between indicators of soil C turnover and associated genes (Su et al., 2015) indicating that microbial communities are not resistant, resilient or functionally redundant to environmental changes.

The rate of C degradation depends on a number of factors, including availability and type of C substrates, as well as the microbial consortium present. In addition, previous reports have shown that the composition of the microbial community affects the degradation rates of soil C compounds independent of environmental variables (Yang et al., 2013; Zhang et al., 2014). Our results provide evidence that microbial community functional gene structure could significantly affect soil processes and nutrient availability. The increase in the gene intensities and corresponding enzyme activities involved in the degradation of labile forms of C in the Vetch based treatment(s) may be the consequence of stimulation of both microbial growth and activity by improved nutrient availability as well as changes in microbial community composition. Importantly, the abundance of genes involved in recalcitrant C degradation did not change significantly under various crop management practices, indicating that soil C storage may remain unaffected in the long term.

4.4. Soil aggregates determine the structure and function of the microbial community

Various techniques have been suggested to fraction soil into different aggregates each having its own inherent advantages or limitations (Schutter and Dick, 2002; Lützow et al., 2006; Dorodnikov et al., 2009). For this work, we successfully used the wet-sieving strategy described by Davinic et al. (2012). Overall the amount of C and N were significantly higher in silt–clay fractions. Other studies have also reported higher amount of nutrients in smaller aggregates regardless of the treatment type (Neumann et al., 2013; Nie et al., 2014). The response of treatments on the amount of total C and N appeared to decrease with the size of aggregates. ^{13}C NMR analyses revealed that different agricultural practices trigger compositional changes in soil organic matter, which was more pronounced in the coarse fraction than fine fractions (Kiem and Kögel-Knabner, 2003).

These results are supported by previous studies, which reported that soil aggregate size exerts strong impacts on soil C dynamics and microbial activity (Elliott, 1986; Schutter and Dick, 2002; Cheng et al., 2011; Ling et al., 2014; Nie et al., 2014). All enzymatic activities related to C decomposition increased as aggregate size decreased which is consistent with other studies (Qin et al., 2010; Lagomarsino et al., 2011; Ling et al., 2014; Nie et al., 2014). Our results further show that farm management altered enzyme activities of soil fractions only in macroaggregate fractions and cultivation practices that result in higher soil C show higher enzymatic activity (Table 2). These results are supported by previous findings that soil enzyme activities in macroaggregates are affected by soil properties (mainly organic inputs) and are significantly correlated with the organic matter content (Ling et al., 2014). Differences in enzyme activity can also depend on the type of plant inputs especially humic compounds in soil (Nannipieri et al., 2012).

Previous research has shown that soil structure can influence the distribution of bacteria in aggregates and, thereby influence microbiological processes and diversity at small spatial scales (Six et al., 2004; Neumann et al., 2013; Vos et al., 2013; Ling et al., 2014). We used taxon-specific qPCR to quantify the abundance of soil bacterial communities and differentiate their response according to their association with different aggregates from the various cropping treatments. In general, the proportion of bacteria within soil varies with aggregate size, and a greater proportion of bacteria are associated with microaggregates and a lesser proportion with macroaggregates (Monreal and Kodama, 1997; Neumann et al., 2013). The interaction between bacteria, organic matter, and clay is required for the survival of bacteria, as organic matter and clay particles offer nutrients and habitat to bacteria (Van Gestel et al., 1996; Sessitsch et al., 2001). This explains why our silt–clay fractions showed higher bacterial populations than other aggregates.

Consistent with our results based on the pyrosequencing approach Davinic et al. (2012) reported higher abundance of Acidobacteria in micro-aggregates while α-Proteobacteria had relatively high abundance in macro-aggregate fractions. We also found a significant correlation between the relative abundance of Acidobacteria, Bacteroidetes, and Alphaproteobacteria with DOC and this was related to differences in the aggregate sizes (Fig. 5a–c). The oligotrophic life strategy of Acidobacteria and Bacteroidetes might explain their dominance in micro-aggregate as these fractions are characterized by lower concentrations of new and labile C and increased amounts of physically protected and biochemically more recalcitrant C, compared with macroaggregates. On the other hand α-Proteobacteria target labile C which might be the reason of their dominance in macro-aggregates which are enriched with labile C and N originating predominantly from plant residues. A previous study has shown the higher abundance of Actinobacteria in the macroaggregates (Davinic et al., 2012). However, in our study, we observed no significant differences in the abundance of this group in different aggregate sizes (Supplementary Fig. 4). Our results provide direct

evidence on niche separation among bacterial taxa at phylum level based on habitat and nutritional quality. In accordance with another study we also observed that management practices influenced microbial abundance and C content more strongly in the larger-sized fractions than in fine clay–silt fractions (Neumann et al., 2013). While differences in the bacterial community abundance between size fractions were pronounced, these differences were only minor for the same particle size fractions and the management practices altered abundance of bacterial taxa only in coarse sand fractions (Poll et al., 2003).

Our study clearly demonstrates that increased enzyme activities related to C decomposition with decreasing aggregate size may be due to the higher C content in microaggregates compared with macroaggregates (Nie et al., 2014). One of the mechanisms through which crop management practices could influence the soil microbial community is through the inputs of labile C (Ghimire et al., 2014). Our results show that the amount of labile C decreases with aggregate size and hence the impact of treatment on soil microbial community. The copiotrophic microbes utilize the higher amounts of labile C and therefore will proliferate within the management practices that increase the availability of easily degradable C in the soil system (Carbonetto et al., 2014; Ghimire et al., 2014). However, micro-aggregates are characterized by an increase in the amount of recalcitrant C (Lal et al., 1997) and in these environments the microbial responsiveness to crop management practices will decline significantly.

5. Conclusions

Mechanistic understanding on impacts of land use changes on microbial groups with key roles in C turnover may provide valuable information on the storage and stability of C pools in agro-ecosystems. In accordance with our first hypothesis, we observed alterations in the microbial community composition in response to soil management practices. We found a shift from oligotrophic to copiotrophic microbial community in relation to management practices that increase the amount of soil C. GeoChip analysis revealed changes in the functional gene structure of soil microbial community with long term soil management practices. In particular genes related to the degradation of labile forms of soil C were affected due to changes in the availability of labile C and other soil conditions. Our results provide evidence for possible links between proxy functions (enzymes and basal respiration) and functional gene abundance.

In accordance with our second hypothesis we observed that the greater availability of relatively degradable fresh residues in macro-aggregates drives soil microbial community structure and function in coarse fractions. Our results demonstrate that microbial responsiveness to crop management practices declined in smaller aggregates. While plant material enters the bulk soil after harvest, thus explaining the differences between different rotations, the deposition and retention of C in micro-aggregates is more likely to explain stored C. Our results suggest impacts of different crop regimes on soil C and microbial communities are mediated by aggregate size distribution and these impacts are more pronounced in macro-aggregate compared to micro-aggregate sizes. These findings suggest that aggregate size should be explicitly considered to determine the impact of management practices on soil C and soil health.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.08.034.

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