Catabolic and genetic diversity of microbial communities in Australian soils are influenced by soil type and stubble management

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Abstract
Crop residues (stubble) are one of the major sources of C in low fertility agricultural soils of Southern Australia. We measured the effect of stubble addition on genetic and catabolic diversity of microbial communities in two soil types, i.e. Alfisol and Vertosol. Catabolic diversity of microbial communities was significantly affected by stubble addition in both soil types. Actinobacteria, Proteobacteria and Acidobacteria accounted for 76% of bacterial populations and bacterial community structure differed between soil types. The effect of stubble addition on the microbial metabolic potential and bacterial community structure was greatest when stubble was incorporated compared to standing stubble. Although stubble management altered microbial diversity, soil type appears to have a dominant influence in determining bacterial community structure in these soils.

Key Words
Bacterial diversity, 16S rRNA, catabolic diversity, *nif*H, soil type, stubble

Introduction

Soil biological function is an outcome of population diversity and activity of microbial communities as limited by edaphic and environmental constraints. In many Australian agricultural soils, carbon availability is the most limiting constraint of microbial function hence management of biologically available carbon is the key to changes in microbial diversity and improvement of function (Gupta *et al.* 2009). Crop residues (stubble) are one of the major C sources in low fertility agricultural soils of Southern Australia. It is generally believed that stubble retention can provide benefits from improvements to different biological properties (Roper and Gupta 1995). However, in different cropping regions of Australia, the nature and extent of these benefits and changes on microbial diversity are unknown. Soil organic C levels in these soils are generally low and soil biota generally experience boom-bust cycles of C availability. The depletion of carbon-rich microsites can affect the distribution, diversity and metabolic status of microbial communities and can impact on the overall biological resilience.

Soil type, residue quality and environment can significantly impact microbial populations and their metabolic activities, potentially influencing the timing and extent of the biological benefits derived from stubble retention. The rate of stubble decomposition during the initial six month period is known to vary with soil type, litter chemistry and climate (temperature and moisture). In a three year study, we monitored changes in soil biological and chemical properties as influenced by stubble management treatments on two soil types. Field based experiments were used to determine stubble effects on soil biological function and to identify linkages with crop yield. Glasshouse experiments were conducted to examine the short-term effects on microbial structure and activity in response to different stubble management strategies (slashed, slashed & incorporated, no stubble) with or without added chemical fertiliser.

Methods

Experimental setup: Soil profiles (0-10 cm) were recreated in tubs (41 x 64 x 28 cm) in a glasshouse assuming bulk densities of 1.2 and 1.1 g/cm^3 for Waikerie (Alfisol; organic C 0.6% and total N 0.055%, clay 3%) and Tarlee (Vertosol; organic C 1.5%, total N 0.16%, clay 43%) soils from the South Australian agricultural region, respectively. Wheat stubble, cut into segments ~ 5 cm long, was applied at rates equivalent to 3.5 and 4.8 t/ha for Waikerie and Tarlee soils, respectively; these rates represent dry matter yields seen in the field for each region. In the stubble incorporated treatment, stubble was incorporated to a depth of ~ 5 cm. After eleven months (5 months with weekly irrigation and 6 months undisturbed condition), stubble treatments were reintroduced. An additional treatment with fertiliser was added (@ 15 kg P and 40kg N and 20 kg P and 70kg N/ha, for Waikerie and Tarlee soils, respectively) and mixed to a depth of approximately 2 cm to ensure uniform distribution. All treatments were replicated four times. Tubs were maintained at constant temperatures of 20 °C and 12 °C (12h each) and water was applied weekly at rates

representative of rainfall in the respective region. Four months after the addition of fertilizer, surface 0-5cm soil was collected from a 2 x 5cm area in each tub and used to measure catabolic diversity and other biological properties. For molecular analysis, two 3cm dia. cores were collected from each tub, mixed and the entire soil sample (~50g) was used for DNA extraction (Ophel-Keller *et al.* 2008).

Catabolic diversity profiling: Measurement of the ability of soil microorganisms to utilize a diverse array of added C substrates provides a profile of microbial catabolic potential. Carbon substrate utilization profiles of soil microbial communities were determined using the Microresp® method (Campbell *et al.* 2003) modified with specific carbon substrates selected for Australian soils (Gupta VVSR, data unpublished). Catabolic potential for bacteria only was estimated by measuring C substrate use in soils amended with Captan, a broad spectrum fungicide.

Genetic diversity analysis (SSU Amplicon Pyrosequencing) of bacterial communities: Universal primers (~94.6% coverage) targeting the 16S rRNA gene was used for the initial amplification of soil genomic DNA. This allowed for multiple sample pyrosequencing on a single plate using the 454 Life Sciences FLX sequencing platform. Three independent replicate genomic DNA extractions and amplifications were performed per sample. Raw pyrosequencing reads were first filtered then aligned using INFERNAL 8.1 (Nawrocki and Eddy 2007) and a SSU rRNA secondary-structure model (Cannone *et al.* 2002). Sequences were then clustered by the complete-linkage method at the desired distance and assigned to specific taxa using the RDP Classifier (http://pyro.cme.msu.edu; Wang *et al.* 2007).

Data analysis: Multivariate statistical comparison of C-substrate utilization data was done using Genstat 12.1 (VSN International Ltd). Community level physiological profile (CLPP) analysis was used to differentiate microbial communities under different stubble and fertilizer treatments. For the DNA sequence data, the relationship among sites based solely on bacterial community structure was calculated using abundance-based adjusted Jaccard and Sorensen indices with EstimateS followed by hierarchical clustering (e.g. UPGMA tree construction). Indicator species analysis was used to identify specific clusters that significantly varied among treatments and between soil types.

Functional gene analysis: Nitrogenase reductase (*nif*H) gene fragments were amplified using primers described by Rösch *et al*. (2002) and quantified using the Stratagene Mx3000P qPCR system.

Results

Microbial biomass (MB) carbon levels ranged between 200 to 400µg C/g soil and accounted for 2-5% of soil organic C levels. Stubble retention increased MB C and N and microbial activity in both soils (data not presented) and the effect of fertilizer application was highest with stubble incorporation.

Table 1. Effect of fertilizer addition and stubble management on the catabolic potential of microbial communities (average CO_2 response for 22 substrates) measured using modified Microresp® methods.

Treatments		Tarlee (Vertosol)			Waikerie (Alfisol)		
		Total	Bacteria[1]	Fungi[1]	Total	Bacteria	Fungi
		µg CO2/g soil (5h assay)					
Without fertilizer	No Stubble	0.464	0.269	0.196	0.104	0.058	0.046
	Slashed	0.671			0.117		
	Incorp	0.729	0.352	0.377	0.158	0.056	0.103
	Standing	0.616			0.121		
With fertilizer	No Stubble	0.496	0.271	0.225	0.095	0.060	0.036
	Slashed	0.764			0.138		
	Incorp	0.807	0.386	0.421	0.211	0.058	0.153
	Standing	0.658			0.127		
Treat	**LSD (0.05)**	0.124	0.029	0.098	0.021	ns	0.033
Fert	**LSD (0.05)**	0.062			0.010		
Fert x Treat	**LSD (0.05)**	ns			0.021		

[1] CO_2 evolved from soils receiving the fungicide Captan prior to the C substrate utilization assay was considered as bacterial activity and the difference between total and Captan treated was attributed to soil fungi.

Catabolic diversity is a measure of the ability of microorganisms to utilize different types of C and N

compounds and it reflects the functional capability of soil microbial communities as influenced by management and environmental factors. CLPP analysis showed significant differences between soil types and different stubble management treatments (data not shown). The effect of fertilizer addition on total catabolic response was only observed in the presence of stubble (Table 1). Catabolic response was highest in the incorporated treatments followed by slashed and standing stubble treatments. Fertilizer application increased the contribution from fungi in the sandy soil but not in the Vertosol.

The number of *nif*H gene copies was higher in the Tarlee soil compared to that in Waikerie soil (1400 and 150 copies / nq DNA in Tarlee and Waikerie, respectively) and the trend was also reflected in N fixing potential (based on acetylene reduction (AR) bioassay). Stubble addition generally increased the *nif*H copy number and AR activity in both soils. Unlike the Vertosols, the sandy soil provides little opportunity for the development of stable microaggregates that can support free-living N_2-fixing bacterial communities (Hattori 1988).

Targeted 16S rRNA gene pyrosequencing resulted in a total of 113,895 16S rRNA gene sequences that were subjected to classification and clustering using the Ribosomal Database Project Pyrosequencing Pipeline (http://rdp.cme.msu.edu/). Unclassified bacteria accounted for 12,672 of the total at a 50% confidence threshold. Members of the phylum Actinobacteria were the most abundant (42%), followed by the Proteobacteria (21%), and the Acidobacteria (13%) (Figure 1). Complete linkage clustering at 5% operational taxonomic units (OTUs) resulted in 7467 clusters of which 2923 were classified as singletons that occurred in only one sample. Shannon and Chao indices showed that diversity increased in the Waikerie soil with stubble addition. Specifically, the number of unique clusters increased 60% with stubble incorporation at a 3% OTU level (Figure 2). Conversely, Tarlee soil diversity differed only marginally among treatments at all OTU levels.

Despite no clear influence of management regime, cluster-based UPGMA trees generated at both 3 and 5% OTU cutoff values with and without the inclusion of singletons showed that management effects influenced microbial community structure to some degree in the sandy Waikerie soil with no discernible effect in the Tarlee soil. Indicator species analysis was then undertaken in order to identify bacterial populations most responsive to treatment. Cluster dendogram branch lengths were notably smaller in the Tarlee soil as compared to the Waikerie soil. No significant indicator species were identified according to treatment type. However, using a false discovery rate, 48 highly significant ($q<0.003$) clusters were found that exhibited large differences between the two soil types. The phylum Actinobacteria accounted for 64% of the indicator species with a large proportion (>50%) belonging to the subclass Rubrobacteridae. Therefore, multiple independent modes of analysis showed that despite the application of identical treatments, soil type appears to have a dominate influence in determining bacterial community structure at these sites.

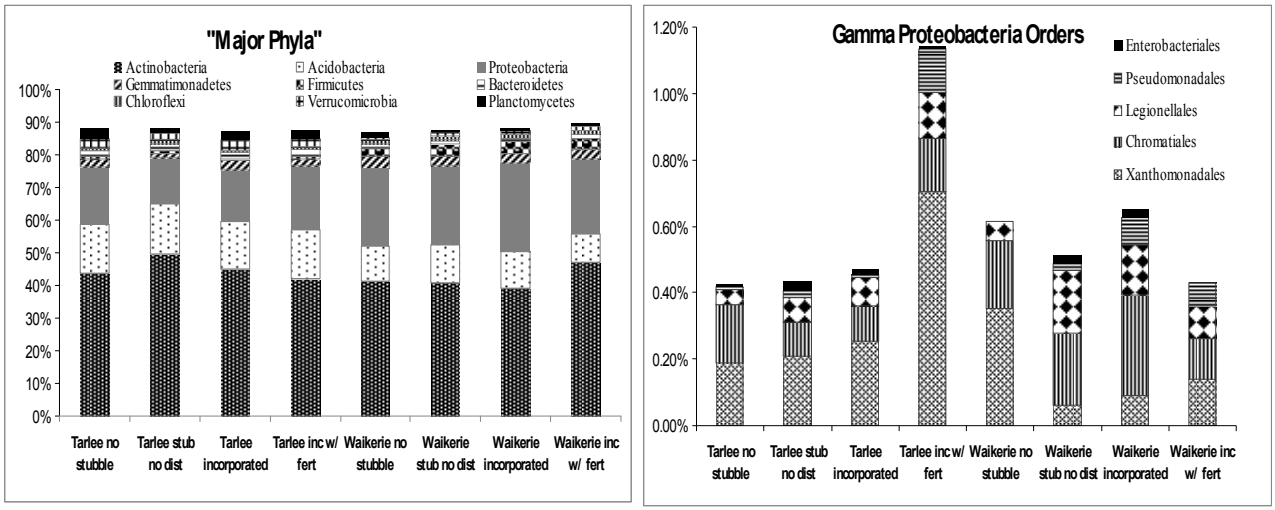

Figure 1. Effect of soil type and stubble management on the proportional distribution of bacterial taxa.

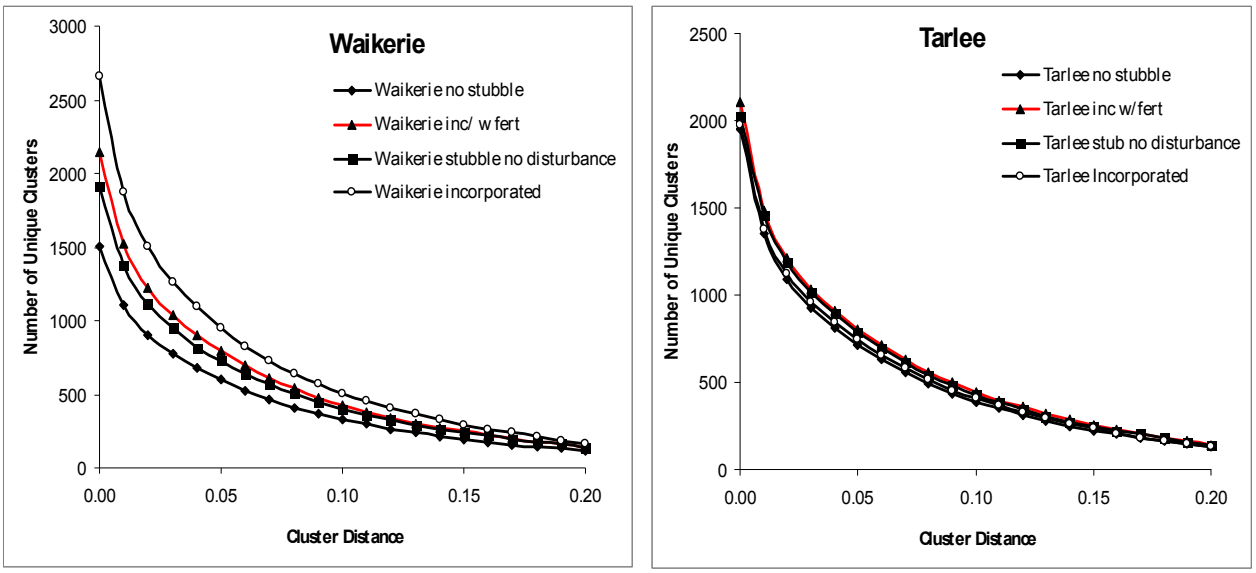

Figure 2. Changes in bacterial diversity as indicated by the number of unique clusters in the 16S sequences.

Conclusion

Stubble addition caused significant changes in the catabolic diversity of microbial communities in both soil types. Actinobacteria, Proteobacteria and Acidobacteria accounted for 76% of bacterial populations and bacterial community structure was different between soil types. The effect of stubble addition on the microbial diversity and activity, e.g. catabolic diversity of total microbial communities and genetic diversity of soil bacteria, was greatest when it was incorporated compared to standing stubble. Overall, differences in catabolic and genetic diversity of microbial communities can be attributed to the differences in the texture and chemical properties between soil types.

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