Crop rotation and fallowing can affect the functional resilience of microbial communities in a rainfed cropping system in southern Australia

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Abstract
The availability of biologically available carbon and soil moisture are the two key factors affecting the level of soil biological functions in southern Australian dryland cropping soils. In a long-term field experiment conducted on an alfisol, effects of crop rotation, fallowing and tillage treatments on soil microbial and chemical properties were investigated. Microbial biomass C and N and microbial activity were generally lower during the fallow phase of the rotation compared to continuous crop treatments. Crop rotation and fallow also influenced the catabolic diversity of microbial communities and the quantity of functional genes involved in N mineralization most likely due to differences in the quantity and quality of C inputs. The response of biological functions to stresses, e.g. repeated wet-dry cycles, differed between cropping systems with lower resilience in soils from a fallow-crop rotation than under continuous cropping.

Key Words
Microbial activity, resilience, catabolic diversity, microbial biomass, *amo*A, mineralization

Introduction
Soil biological functions in southern Australian dryland cropping soils are mainly regulated by soil moisture and the amount of biologically available carbon, and it is therefore critical that a regular addition of carbon sources occurs to maintain the functional capability. In the low rainfall Mediterranean region of southern Australia, fallowing every second or third year in a rotation has remained a common practice to supplement soil moisture and nutrient levels. The low productivity in the traditional crop-fallow systems with multiple cultivations combined with factors such as heavy grazing and wind erosion have resulted in low returns of organic matter to the soils with subsequent limitations to microbial activities and functions (Gupta *et al*. 2009). In these lower fertility soils, with soil organic C of ~0.5%, soil biota under a fallow-crop rotation 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. Crop management practices such as crop rotation, stubble retention and tillage influence the quality, quantity and location of crop residues.

In these Mediterranean environments, exposure to repeated wet-dry events is common during summer and can impact on the resilience of biological activity through physico-chemical stresses on microbial habitat and substrate availability. The stability of soil communities is a key to their continued functional capability when exposed to different external stresses. Stability depends on both resistance (i.e. ability to withstand disturbance) and resilience (i.e. ability to recover after the disturbance), i.e. resistance prevents further decline in ecosystem function and resilience allows its recovery (Figure 1A).

The measurement of a soil property reflects the capacity of soil to function at a particular time, whereas knowledge of a soil's resilience assists in the development of systems or practices that promote the recovery of degraded soils. In addition, a better understanding of the resilience of the system is also useful to understand the role of changing environments and climates on ecosystem function. The measurement of soil resilience involves quantifying short-term changes in specific biological properties (e.g. measures of the activity, diversity and population levels) following an exposure to disturbance or stresses, e.g. chemicals, wet-dry or freeze-thaw cycles (Morely and Coleman 1989; Kuan *et al.* 2007).

In this study we discuss the impact of 5 years of intensive cropping, no-till and optimum fertilizer input systems on microbial activity, diversity and resilience when compared to the traditional fallow-crop rotations on a Belah loam (alfisol) at Paringi, New South Wales, Australia.

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Methods

Soil and site description

A long-term field experiment was established at Paringi (Kerribee station) in New SouthWales (WGS84 lon 142.37, lat -34.28) in 2002 with the aim to investigate the potential of improving rainfed farming systems in terms of productivity, profitability and overall soil biological health. Treatments included a combination of rotations (wheat, canola, fallow and grain legumes), tillage (no-till and conventional cultivation) and fertiliser inputs (district practice and high-input). Each treatment was replicated four times. The climate is a Mediterranean-type, characterised by hot dry summers and a winter-dominant, average annual rainfall of only 260mm. Soil is an alfisol (calcic Calcarosol). Soil chemical properties (0-10cm) at the start of the trial were pH(water) 7.6, organic C 0.68%, total N 0.06%, and clay content 10.6%. Surface soil samples collected prior to sowing (May) in 2007 were analysed for various microbial, biochemical, chemical properties.

Microbial biomass C, N levels and metabolic status of microbiota

Microbial biomass (MB) C and N (chloroform fumigation-direct extraction methods), substrate induced respiration (SIR) and Potential C and N mineralization were measured using methods described by Gupta *et al* (1994).

Catabolic diversity profiling

A measure of the ability of soil microorganisms to use a diverse array of added C substrates gives a profile of catabolic potential for microbial community. 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, Grains RDC report, Australia, unpublished).

Functional gene analysis:

DNA was extracted from subsamples of soils used for catabolic diversity (0.4 g soil samples) using the MoBio UltraClean soil DNA extraction kit (MoBio Laboratories, CA) and used to quantify the abundance of functional genes. Nitrogenase reductase (*nif*H) gene fragments were amplified using primers described by Rösch *et al*. (2002), and the ammonia monooxygenase (*amo*A) gene was amplified using primers described by Stephen *et al*. (1999).

Resilience analysis:

The stability of soil biological communities and processes was estimated using a laboratory based repeated wet-dry cycle assay standardised for Australian soils. Briefly, 120 g (dry wt equivalent) soil was weighed into 250ml polypropylene containers (Sarstedt Australia Pty Ltd) and bulk density adjusted to 1.3g/cc. Soil moisture was adjusted to -30kPa (100% field capacity (FC) or 60% water filled pore space). Duplicate samples were prepared for each field sample; one sample for resilience exposure and the other kept moist throughout the incubation assay. After soil moisture adjustment, all samples were pre-incubated for 5 days at 25 ^{o}C after which some of the samples were exposed to wet-dry (W-D) cycles where as others remained moist. The resilience samples were subjected to a series of 3 drying events (at 40 ^{o}C), 3 days each, interjected with 4 days of wet condition (25 ^{o}C). At the end of W-D cycles, soil moisture was adjusted to FC and incubated at 25 ^{o}C for 28 days. Subsamples at the start of the experiment (T1, at the end of pre-incubation), within 24h after W-D cycles (T2) and at the end of 28d post W-D cycle incubation (T3) were analysed for microbial activity, catabolic diversity and functional gene analyses. Stability parameters (resistance and resilience) were calculated using procedures described by Orwin and Wardle (2004). In order to overcome the interference from temporal changes in microbial activity measurements at different times, data for the W-D exposed samples were normalised against their counterpart samples that were not exposed to stress events.

Results

Average C inputs from above and below-ground crop residues were less than 1.5t C/ha/year. In the continuous crop rotations C residues were retained whereas during the fallow there were no C inputs. Legume crops in these environments generally added lower amounts of C compared with cereal crops. Grain yields of cereal crops were generally higher following fallows compared to the intensive cereal systems; mainly due to the extra stored soil water under fallow treatments. However, fallow-crop rotations only provided grain harvest in alternate years. The positive effect of stored water was highlighted because of the multiple seasons of less than average rainfall received during the trial.

Microbial biomass C ranged between 250 to 400ug C/g soil and accounted for 3-5% of soil organic C levels. In the initial period of the field experiment, there was little difference in MB levels between crop rotations

but after 5 years, soils from continuous crop rotations showed higher MB-C. MB was generally lower in the fallow phase of crop rotation (up to 25% lower than after wheat crops). MB-N ranged between 50-70kg N/ha; levels were highest after legume crops and lowest after the fallow phase. Soil respiration ranged from 25 to 45ug CO_2-C/g/day and was generally higher (>15%) in soils under continuous cereal cropping systems followed by the legume-cereal rotation and lowest in the fallow-crop rotation, in particular during and after the fallow period.

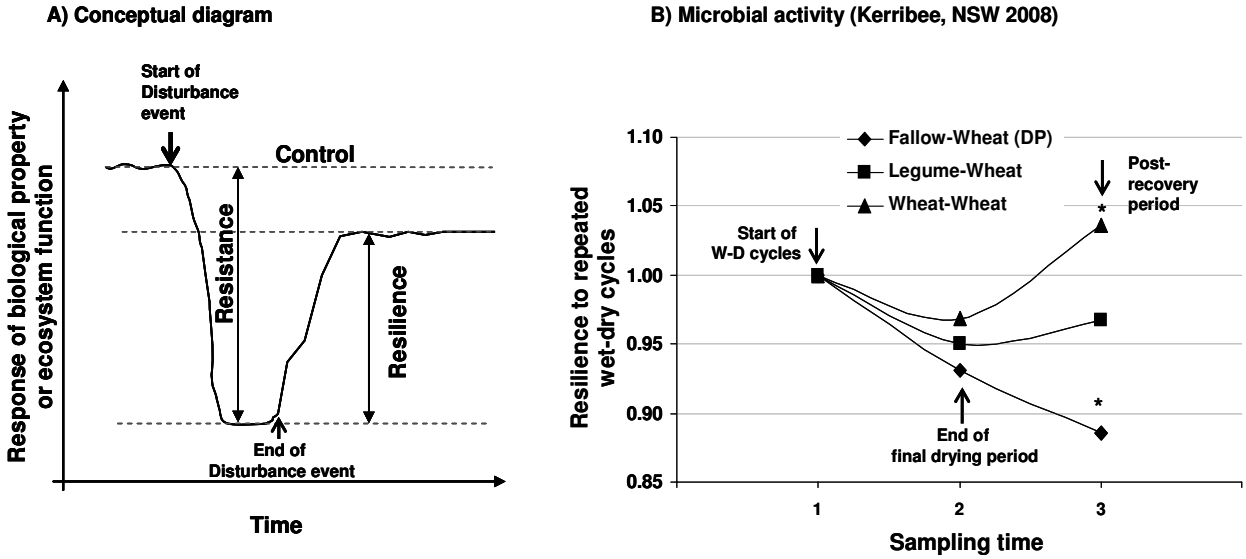

Figure 1. Stability of soil biotic communities - resistance and resilience. (A) The conceptual diagram is based on discussion by Herrick (2000) and Griffith *et al.* (2004). (B) Microbial activity resilience responses to 3 consecutive wet-dry cycles – after 6 years of three farming system practices (rotations and associated tillage practices). Microbial activity values normalised using data from samples that were not exposed to stress events.

Biological resilience (expressed as changes in microbial activity) was found to be lower in fallow-crop rotations than under continuous cropping (Figure 1B). Soil biota under fallow-crop rotations generally experience boom-bust cycles of C availability. The depletion of C rich microsites affects the distribution, diversity and metabolic status of microbial communities and reduces the overall biological resilience. The higher N content in the legume residues results in faster degradation and depletion of C rich microsites.

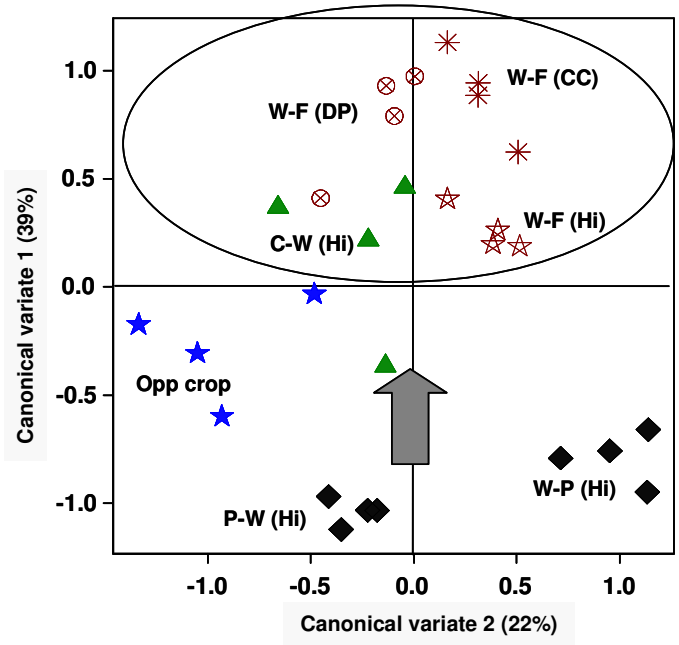

Figure 2. Canonical variate analysis of catabolic diversity profiles for microbial communities in surface soils after 5 years of different cropping systems at Kerribee, NSW, Australia. W=Wheat, F=Fallow, C=Canola, P=Peas, DP=District Practice, Hi=High inputs, Opp crop=Opportunity cropping i.e. crop type is selected based on seasonal conditions at the time of sowing.

Since the carbon availability, both in terms of quantity and quality, is one of the major regulatory factors influencing the biological processes, we measured the catabolic diversity of microbial communities. Community level physiological profiling using multiple C substrate usage showed significant differences between soils from different cropping systems. For example, all the treatments that had a fallow in rotation were different to intensive cropping treatments (Figure 2). Soil communities under the legume-wheat rotation were different from those under continuous cereal and canola. Differences in the quantity and quality of above- and below-ground crop residues for different crops resulted in the differences in catabolic diversity of microbial communities. Soils from continuous cropping systems generally indicated higher levels of N mineralization potential compared to those from fallow-crop rotations. Soils after legume crops exhibited a higher N mineralization potential. In general, soils from fallow-crop rotations had the lowest quantities of functional genes (e.g. *amo*A copy number for fallow-wheat < grain legume-wheat < continuous wheat). Exposure of soils to repeat W-D cycles resulted in a decline in the *amo*A copy number compared to those not subjected to stress, with the highest decline occurred in the legume-wheat rotation. After 5 years of intensive cropping there was no significant change in soil organic C (0.75 to 0.82%) and total N (0.065-0.073%) in the surface soils compared to pre-experimental concentrations. Soils under fallow-crop rotation showed a small decrease in the POM-C and POM-N (3-5%) whereas intensive cropping under no-till exhibited no significant change.

Conclusion

In the southern Australian rainfed region crop management practices that increased the C inputs from above and below-ground crop residues improved soil microbial activity and biological functions involved in C turnover and nutrient mineralization. Crop rotations that include a fallow season reduced the microbial biomass, catabolic and N mineralization potential and quantities of functional genes involved in N cycling. The decline in the biological resilience (expressed as changes in microbial activity) of soils under fallow-crop rotations could be attributed to the boom-bust cycles of C availability. Overall, the results suggest that there is a need for increased inputs of carbon to increase microbial populations, sustain biological functions for longer periods within the season and associated benefits.

Acknowledgements

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