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Organic nitrogen cycling microbial communities are abundant in a dry Australian agricultural soil

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ABSTRACT

Some microbial nitrogen (N) cycling processes continue under low soil moisture levels in drought-adapted ecosystems. These processes are of particular importance in winter cropping systems, where N availability during autumn sowing informs fertilizer practices and impacts crop productivity. We evaluated the organic and inorganic N-cycling communities in a key cropping soil (Vertosol), using a controlled-environment incubation study that was designed to model the autumn break in south Australian grain growing regions. Soils from wheat, lucerne, and green manure (disced-in vetch) rotations of the Sustainable Cropping Rotations in Mediterranean Environments trial (Victoria, Australia) were collected during the summer when soil moisture was low. Microbial community structure and functional capacity were measured both before and after wetting (21, 49, and 77 days post-wetting) using terminal restriction fragment length polymorphism measures of bacterial and fungal communities, and quantitative PCR of nitrogen cycling genes. Quantified genes included those associated with organic matter decomposition (laccase, cellobiohydrolase), mineralization of N from organic matter (peptidases) and nitrification (bacterial and archaeal ammonia monooxygenase and nitrite oxidoreductase). In general, the N cycling functional capacity decreased with soil wetting, and there was an apparent shift from organic-N cycling dominance to autotrophic mineral-N cycling dominance. Soil nitrate levels were best predicted by laccase and neutral peptidase genes under drought conditions, but by neutral peptidase and bacterial ammonia monooxygenase genes under moist conditions. Rotation history affected both the structural and functional resilience of the soil microbial communities to changing soil moisture. Discing in green manure (vetch) residues promoted a resilient microbial community, with a high organic-N cycling capacity in dry soils. Although this was a small-scale microcosm study, our results suggest that management strategies could be developed to control microbial organic-N processing during the summer fallow period and thus improve crop-available N levels at sowing.

1. Introduction

Sowing in the grain growing regions of south eastern Australia usually occurs between March and June, after the autumn rainfall break (Pook et al., 2009; Stokes and Howden, 2010). Unlike northern hemisphere cropping systems, grain crops are sown after a typically hot and dry summer. Average summer daytime temperatures in these regions often exceed 30 °C, with a cumulative rainfall of less than 100 mm. Management strategies for this summer fallow period have primarily focused on agronomic measures to retain soil water and soil nitrogen (N) for subsequent crops (Kirkegaard et al., 2014 and references therein), often by controlling weeds (Haskins and McMaster, 2012; Hunt et al., 2013). The amount of N remaining in the system at sowing, however, will also be influenced by interactions between soil microbes and their environment during the fallow period. Previous assumptions that

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microbial activity during this period will be low due to low soil moisture levels are being challenged (Alster et al., 2013; Barnard et al., 2013; Placella and Firestone, 2013), raising the question as to whether management strategies should also consider the active N-cycling microbial communities that are likely to be present.

In drought-adapted ecosystems some functions, including nitrification, may continue at a high level during the dry season (Parker and Schimel, 2011; Sullivan et al., 2012; Sher et al., 2013). Sullivan et al. (2012) found that N-fluxes during the dry season in arid grassland soils in Arizona were either equal to or greater than those in the wet season. The authors suggest that a dominance of archaeal nitrifiers (AOA) over bacterial nitrifiers (AOB) in arid sites may drive this observed nitrification. AOA are generally detected in greater numbers than AOB when soils are sampled during hot, dry periods in Australia (O'Sullivan et al., 2013) and in other comparable drought-adapted environments (Sher et al., 2013). Although AOA found in arid soils grow optimally at higher temperatures (ie. >35 °C; Hatzenpichler, 2012), evidence from Western Australia suggests that as soil dries nitrification becomes limited more quickly than other N-cycling processes, including mineralization (Hoyle and Murphy, 2011). The decomposition of incorporated residues and the subsequent mineralization or release of NH_4^+ appears less affected by seasonal changes in soil moisture (Schomberg et al., 1994; Coppens et al., 2007; Hoyle and Murphy, 2011), allowing mineral N to accumulate in dry soils. If rainfall events do occur when no crops are present, this mineral-N pool may be quickly nitrified and lost from the system. A greater understanding of the microbial communities driving this apparently drought-resilient organic-N cycling is necessary (Cabrera et al., 2005), and is particularly needed to inform management strategies for the Australian summer fallow period.

How soils respond to prolonged drought and subsequent rewetting events will depend on the organic matter levels (Yuste et al., 2011), the length of the drought period (Unger et al., 2010; Meisner et al., 2013), the frequency of the rewetting events (Xiang et al., 2008; Butterly et al., 2009), and, inherently, the nature of the microbial community present when the re-wetting event occurs. Most recent research has focused on microbial or chemistry responses in native grassland soils, with a climate change focus regarding nutrient fluxes (Huygens et al., 2011; Parker and Schimel, 2011; Sullivan et al., 2012; Sher et al., 2013). Although the response of N-cycling microbial communities to extreme changes in moisture is of particular importance in agricultural systems, such information is still limited (for review see Borken and Matzner, 2009). Agronomic management practices, such as crop rotation (Yin et al., 2010) and tillage (Cookson et al., 2008; Hoyle and Murphy, 2011; Souza et al., 2013), are known to impact microbial community composition and thus will also likely influence how drought and rewetting influences N-cycling.

In this controlled environment study, we used soil from a long term field trial in the Wimmera grain growing region of southern Australia, to evaluate the N-cycling functional capacity of dry soils from different agronomic treatments (rotation and residue management). We examined how previous agronomic management impacted the resistance and resilience of these microbial communities to increased soil moisture after the initial pulse of activity (Placella et al., 2012; Blazewicz et al., 2014) stabilized, and how microbe-environment interactions affected soil N pools. We hypothesized that microbes involved in organic-N transformations would be key contributors of N release in dry soils, while microbes involved in mineral-N transformations would be more important after soil re-wetting occurred. Although studies on the N cycle have typically focused on one step, these are not linear independent processes. Products of one step become substrates in another, and any of the downstream products may be re-bound to soil organic matter via immobilisation processes. To assess the inter-related microbial functional capacity of this soil, we used qPCR methods that targeted genes associated with organic matter and residue decomposition (laccase and cellobiohydrolase), mineralization (alkaline and neutral aminopeptidases), and nitrification (bacterial and archaeal amoA and nitrite oxidoreductase).

2. Materials and methods

2.1. Site and treatment characterisation

The soil was a self-mulching grey Vertosol (Isbell, 1996) collected from a long-term (est. 1998) field experiment 'Sustainable Cropping Rotations in Mediterranean Environments' (SCRIME) located at Longerenong, Victoria, Australia (−36.671° S; 142.290° E. 110 M asl). The SCRIME site investigates how rotation, cultivation and stubble management affect soil chemistry, biology, and physics, all in relation to crop yield. Soils were collected from the 0–10 cm depth of three cropping rotations (three replicates per treatment) within SCRIME. The three rotations sampled were 1) continuous wheat (*Triticum aestivum* L.), 2) wheat–barley–purple vetch (*T. aestivum* L. – *Hordeum vulgare* L. – *Vicia benghalensis* L.), and 3) canola–wheat–pea–lucerne–lucerne (*Brassica napus* L. – *T. aestivum* L. – *Pisum sativum* subsp. *arvense* L. – *Medicago sativa* L. – *M. sativa* L.).

In the cropping season prior to sampling the plots were in the last rotation of the above listed sequences and included (1) wheat (2) purple vetch, and (3) lucerne. The different crops were under different management strategies. The wheat plots were under a reduced tillage regime (chisel plough) and were sown with a tyned seeder. The vetch was disced into the soil at flowering (early October). The lucerne was slashed prior to flowering and the residues retained *in situ*. Soils from each of these treatments will be referred to as 'Wheat', 'Green manure' and 'Lucerne', respectively. Soils were collected in the post-harvest summer period when soil moisture was low and passed through a 2-mm sieve. The soils were stored dry, in the dark, and at ambient temperatures until use, in order to minimise pre-study changes to 'summer-adapted' microbial communities.

2.2. Sample preparation and controlled environment study conditions

A 40 g soil sample (2 mm sieved) was lightly packed into incubation containers consisting of a 40 mm diameter × 40 mm high PVC tube, with a mesh bottom (aperture 0.06 mm). Three replicates of each treatment, corresponding to the original field plots, were established for each destructive sampling point. Soils were wet to 60% field capacity with sterile deionised water (dH_2O) (the volume of which was previously determined) and placed in 1 L air-tight incubation chambers. Prior to soil wetting, gravimetric moisture was approximately 0.05 g/g; post soil wetting gravimetric moisture was approximately 0.23 g/g for all samples. A beaker containing 30 ml of water was placed in the incubation chamber to maintain humidity. The chambers were incubated in the dark at 25 °C for eleven weeks. A gas sample was taken periodically through an air-tight septum and analysed for CO_2 using a Servomex 1450 gas analyser (Servomex Pty Ltd, Crowborough, England). The incubation samples were weighed regularly, with dH_2O added as required to maintain the samples at 60% field capacity.

Microbial activity (CO_2 respiration) was monitored and destructive samples (n = 3) were taken at specific time points (0, 21, 49, 77 days) for comprehensive chemical and microbial characterization. Time 0 samples (n = 3) were dry soils, taken just prior to soil wetting. The next sampling date (day 21) was determined by monitoring CO_2, and coincided with the time after which the pulse