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Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil

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ABSTRACT

Organic matter (OM) decomposition and breakdown of crop residues are directly linked to carbon (C) sequestration in agricultural soils as a portion of the decomposed C becomes assimilated into stable microbial biomass. Microbial decomposition of OM may vary with quality of OM, addition of nutrients and functional types of microbes. While the role of fungi and bacteria in OM decomposition has received considerable attention, the succession and co-occurrence patterns of these communities during decomposition remain unexplored. Using 454 pyrosequencing and network analysis of bacterial 16S rRNA and fungal ITS genes in a time-course microcosm experiment, this study shows a positive effect of nutrient addition on overall microbial biomass and abundance of bacteria and fungi. Abundance of different bacterial and fungal groups changed up to 300-folds under straw- and nutrient amended treatments while the rate of decomposition remained similar, indicating a possible functional redundancy. Moreover, addition of nutrients significantly altered the co-occurrence patterns of fungal and bacterial communities, and these patterns were resource-driven and not phylogeny-driven. Richness, evenness and diversity decreased and were negatively associated with decomposition rate. *Acidobacteria*, *Frateuria* and *Gemmatimonas* in bacteria and *Chaetomium*, *Cephalotheca* and *Fusarium* in fungi were found as the keystone taxa. These taxa showed strong positive associations with decomposition, indicating their importance in C turnover. Overall, we show that addition of nutrients reduces diversity but favours the keystone taxa, and thereby increases microbial biomass.

1. Introduction

Soils comprise the largest sink of terrestrial C, 2500 Gt at 1 m depth, and approximately 25% of the soil C has been lost to the atmosphere due to intensive agricultural production (Lal, 2004). Enhancing the potential of agricultural soils to sequester C has significant implications for reducing atmospheric CO_2 and also for alleviating soil degradation and nutrient depletion. The pivotal role of soil microbiota in C sequestration is now well-acknowledged (Dungait et al., 2012; Schimel and Schaeffer, 2012; Trivedi et al., 2013; Wieder et al., 2013) with recent studies reporting that fine-fraction C, i.e., stable OM, is likely of microbial origin (Liang and Balser, 2011; Schmidt et al., 2011). The amount of microbially derived organic C in soil is determined by this balance of mineralization and assimilation i.e. microbial carbon use efficiency (Allison et al., 2010; Wieder et al., 2013), which may depend on community structure and composition and/or organic matter quality (Fontaine et al., 2003; Six et al., 2006).

The quality of straw-stubble is a very relevant issue for stubble management in agricultural soils as it can affect the rate of its decomposition (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2003). For example, the energy content of straw-stubble may change with straw-age (i.e. with time from harvest), which may determine its breakdown and further incorporation into soils organic matter. This is particularly important because for soil microorganisms, the ability to breakdown 'fresh' organic matter compared to 'old' organic matter may change with functional types (Fontaine et al., 2003). For example, it has been suggested that residue decomposition may be initially dominated by *r*-strategists (copiotrophs, preferring fresh organic matter and higher nutrient

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content) in response to fresh OM and nutrients whereas *K*-strategists (oligotrophs, able to feed on degraded organic matter and lower nutrient content) may dominate the system when nutrients are used (Fontaine et al., 2003; Six et al., 2006; Trivedi et al., 2013). Microbial ecosystems are dominated by fungi and bacteria (van der Heijden et al., 2008) and as such their role in OM dynamics has received considerable attention (Bardgett and McAlister, 1999; Bardgett et al., 1996; Mau et al., 2014; Six et al., 2006). The succession of bacteria and fungi has been proposed as a driver of OM dynamics (Fontaine et al., 2003; Six et al., 2006). Despite this the specific role of fungal communities in OM dynamics is particularly unexplored with poorer representation of fungi in high-throughput sequencing studies (Trivedi et al., 2013). Recent studies have demonstrated that addition of non-C nutrients may enhance C sequestration by increasing microbial biomass (Kirkby et al., 2014, 2013; Richardson et al., 2014), which makes it particularly important to know what this increased biomass comprises and how nutrient addition may alter fungal and bacterial community succession.

Microbial populations vary in their substrate preferences and strategies of nutrient-acquisition (Goldfarb et al., 2011), whereby some microbes may have higher acquisition ability and thus an advantage in the community. Based on their nutrient-preference, microorganisms may arrange in trophic groups and functionally distinct niches, allowing them to co-exist (Schimel and Schaeffer, 2012). Exploring these microbe–microbe interactions involved in OM decomposition can reveal important information, such as exchange of C compounds, competition for nutrients, exchange of electron acceptors and predation or parasitism (Scow, 1997). This makes co-occurrence and modular patterns in microbial ecosystems particularly important. Network analysis of co-occurrence, as measured by correlations between abundances of microbial taxa, can unravel complex microbial communities and offer meaningful information beyond sample-level comparison (Barberán et al., 2012; Gilbert et al., 2012). Essentially, microbial network analysis can reveal how some species occur together in niches and how they interact with environmental parameters (Fuhrman, 2009). Moreover, it can identify the 'keystone' taxa that have the largest influence in communities (Vick-Majors et al., 2014; Banerjee et al., 2015). While SOM decomposition is generally perceived as a "broad process" involving a wide array of microorganisms (Schimel and Schaeffer, 2012), the relative importance of specific microbial groups may vary with environmental conditions, such that some groups may play a more important functional role than others. While inclusion of microbial biomass has resulted in more improved models of soil C storage (Wieder et al., 2013), insight into microbial drivers, beyond biomass level estimates, remains to be explored. Identifying these keystone groups may be critical for predictive understanding of OM decomposition with subsequent potential for microbial-mediated C sequestration when crop residues are returned to soil.

The aim of this study was to explore the interplay of fungal and bacterial communities during OM decomposition in an arable soil. Specifically, we investigated i) how the abundance, diversity and co-occurrence of fungal and bacterial communities changed during OM decomposition both in response to nutrient-addition and straw of different age; ii) what the key microbial players were involved in OM decomposition in an arable soil under continuous cropping; and iii) whether higher decomposition rates were associated with higher diversity or the abundance of keystone taxa. We hypothesized that addition of nutrients enhances organic matter decomposition and microbial biomass in an arable soil under continuous cropping.

2. Materials and methods

2.1. Soil and straw sampling

Soil samples were collected from a long-term experimental site at Harden, NSW, Australia (148.37E, 34.56S) and is described by Kirkegaard et al. (1994). The site has an elevation of 490 m with a 3% slope. The soil at the research site is described as a Red Chromosol (Isbell, 2002) and the surface texture (0–10 cm) is sandy loam (15% clay, 10% silt and 75% sand) with a pH of 6.1 and total C of 1.3%. The site receives equi-seasonal rainfall with a long-term annual mean of 610 mm and has been cropped annually with wheat (*Triticum aestivum*) rotated with canola (*Brassica napus*) or lupin (*Lupinus angustifolius*) since 1990. Approximately, 50 kg of surface (0–15 cm) soil was collected from five discrete locations at the site. Soils were air-dried and homogenized to pass through a 2 mm sieve. Standing wheaten (*T. aestivum*) straw was also collected over a time period of 0, 1, 5 and 9 months after harvest in December 2013. The average monthly rainfall (cumulative) in those months was 49.6 mm, 100.3 mm, 284.5 mm and 502.2 mm, respectively (Bureau of Metereology, AU Government). The mean maximum temperature was 29.1 °C, 30.4 °C, 17.0 °C and 17.4 °C, respectively (Bureau of Metereology, AU Government). Straw of differing age was collected from the field as it was hypothesised that this would provide residue with differing potential for decomposition based on extent of weathering and nutrient composition. The energy content of different straw samples was analysed using a Parr calorimeter (Parr Instrument Company, Moline, IL). The internode regions of the straw stems were oven dried and puck-milled to use in the microcosm experiments.

2.2. Experimental design

For each age-class of straw, four treatments were set up as i) soil only, ii) soil + nutrient, iii) soil + straw, iv) soil + straw + nutrients, resulting in a total of 16 treatments. Each treatment had three replicates. Microcosms were prepared with 52 g of dry soil in 120 ml plastic containers (Sarstedt AG and Co., Numbrecht, Germany). In treatments with added straw, 1.03 g of finely chopped straw was added at a field application rate of 10 tonnes per hectare (to 7.5 cm soil depth), which is realistic of maximum rates of residue incorporation under field conditions in southern Australia (Kirkby et al., 2013). For treatments with nutrient addition, a solution containing 1.1 N, 0.24 P and 0.17 S was added, which was equivalent to field rates of 11 kg N, 2.4 kg P and 1.7 kg S per hectare. This nutrient solution was prepared at a rate that was calculated to match the stoichiometry and C:N:P:S ratio (10,000:833:200:143) commonly found in stable soil organic carbon (Kirkby et al., 2011). Nutrient solutions (or water controls) were added as a 2 ml aliquot to each specific microcosm. Water was then added to attain a final saturation of 80% field capacity. Microcosm were placed separately in 1 L airtight Mason jars and 5 ml of distilled water was added to the bottom of the jars to prevent drying during incubation. An open scintillation vial containing 10 ml of 1 M sodium hydroxide (NaOH) was also placed in each jar to absorb carbon dioxide (CO_2) respired during each of the incubation periods. Three extra jars containing NaOH vials only and water were incubated as blanks. The microcosms were incubated in dark at 30 °C for 50 days.

Three replicates of soil microcosms for each treatment were sampled after 0, 4, 7, 14, 28 and 50 days. At each sampling time CO_2 vials were changed, microcosms were weighed and soil moisture was re-adjusted to retain 80% field capacity. From each replicate microcosm, a sub-sample of soil (25 g) was collected at each sampling point for measurement of microbial biomass and microbial community analyses, which were stored at 4 °C and −80 °C,