Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities?

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ABSTRACT

The transformation of organic amendments (OA) in soil is in large part performed by soil microbial communities. These processes are strongly affected by the carbon composition of the OAs. We examined microbial community responses to three types of OA: green waste, composted green waste and pyrolysed green waste added to two contrasting agricultural soils. We investigated the relationship between the soil carbon composition (as determined by ^{13}C-solid state NMR), microbial community composition (as determined by phospholipid fatty acid analysis) and microbial activity (as determined by soil enzyme assays). We found that alkyl-C, O-aryl-C, aryl-C and carbonyl-C were able to explain most of the variations ($\geq 50\%$) in soil microbial community composition and activity. Aryl-C content (reflecting relatively stable carbon forms) strongly influenced microbial composition, while carbonyl-C content (reflecting relatively labile carbon forms) strongly influenced the microbial activity. Our results confirm that there is a tight relationship between carbon composition and soil microbial community composition and function. Results are discussed in the context of examining the relationship between carbon forms, microbial community composition and activity following the addition of different OAs to the soil.

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1. Introduction

There is more carbon in the soil than in all terrestrial plants and the atmosphere combined. Thus soils play a major role in regulating the global carbon cycle (Kleber, 2010; Lal, 2010). Carbon is present in the soil in many different forms and compounds that vary greatly in their chemical nature (Baldock et al., 2004). These differences in carbon composition play a major role in microbial-mediated soil carbon cycling. If we are to increase soil carbon stocks as a means of mitigating climate change, we must not only consider the amount of carbon present in the soil, but also the forms in which it is present, and the impact this has upon soil microbes involved in soil carbon cycling. With the great diversity of microbes and complexity

of carbon forms present in soil, this is a challenging issue that is yet to be fully resolved.

The conversion of natural lands to agricultural production has led to a significant reduction in soil carbon levels (Lal, 2011). With 38% of the earth's terrestrial surface being used for agricultural production (FAOSTAT, 2010), the potential to mitigate rising atmospheric CO_2 levels through soil carbon sequestration in agro-ecosystems is large; the global soil carbon sequestration potential of agricultural lands has been estimated at 2.1 billion tons C/yr (Lal, 2010). The addition of organic amendments (OA) to soil has been suggested as an option for supplying nutrients to support agricultural production, while increasing soil carbon levels (Quilty and Cattle, 2011). Furthermore, where OA are derived from waste streams, such as municipal green waste or animal manure, there is the added benefit of recycling of nutrients and carbon that would otherwise be 'lost'. Despite being rich in carbon and nutrients, where the addition of OA to the soil yields an increase in soil carbon, usually only a small percentage ($<20\%$) of this carbon is incorporated into the stable soil organic matter (SOM) pool (Sanderman et al., 2010). In some cases, the addition of OA to the

soil has actually resulted in a reduction in soil carbon levels due to priming effects (Fontaine et al., 2004). If we are to realize the full potential of OA to increase soil carbon levels while providing nutrients to plants, we must understand the processes that drive OA transformations in soil.

Soil organic matter transformation is primarily carried out by soil microbes, via processes including decomposition, polymerisation, protection and immobilisation (Jastrow et al., 2007; King, 2011; Sinsabaugh, 2010). These processes are mediated via the production of enzymes by soil microbes. Consequently, soil microbes are considered proximate controllers of soil organic matter (SOM) transformations. Given the chemical complexity of SOM, a wide range of soil enzymes are involved in organic matter transformation. For example, hydrolytic enzymes, such as β-glucosidase and phosphatases, catalyse specific steps in mineralisation of carbon and phosphorus (Shi, 2010), whereas oxidative enzymes, such as phenol oxidases, act more generally on phenolic-containing compounds including lignin, humus and polyphenols (Sinsabaugh, 2010). Thus, quantification of soil enzyme activities provides important mechanistic and functional knowledge of SOM transformation processes.

Due to the heterogeneity in the sources of organic inputs from one ecosystem to another, SOM composition can be highly variable. The composition of SOM affects its residence time as it influences biological stabilisation, physical protection and the energy required for SOM breakdown (Ekschmitt et al., 2005; King, 2011; Kögel-Knabner et al., 2008; Schmidt et al., 2011). For example, as the decomposition of SOM progresses, the relative proportion of aromatic structures in SOM has been reported to increase (Gressel et al., 1996; Pedersen et al., 2011), as less stable structures are degraded. Phenolic compounds have been correlated with the antioxidant capacity soils that neutralises free radicals, and therefore, protects organic matter from oxidation (Rimmer and Abbott, 2011). Aromatic compounds have also been implicated in hydrophobic protection of SOM (Spaccini and Picollo, 2012). The complexity of SOM composition is especially relevant in the context of OA transformations given the tremendous chemical heterogeneity and complexity of OA. This in turn can also strongly affect the fate of the OA in the soil.

Many OA undergo some form of 'pre-processing' prior to application to the soil; for example, organic matter is often composted to eliminate viable propagules of pests and pathogens, and further stabilise the organic matter it contains. Similarly, pyrolysis, that is the elevated thermal decomposition of organic matter in the absence of oxygen, is also increasingly being used as a means of stabilising OA prior to their addition to the soil. Given the differences in the nature of these different processes, they are expected to affect the chemical nature of the resulting OA. For example, we would expect that OA derived from pyrolysis processes to have a greater proportions of aromatics than their parent material (Lehmann et al., 2011). In turn, such changes in the properties of the OA are likely to have direct effects on the soil microbial community composition and activity, and indirect effects through changes in the interaction of the OA with the abiotic environment (Bastida et al., 2008; Cross and Sohi, 2011; Pérez-Piqueres et al., 2006; Singh et al., 2012). Therefore, we contend that if we are to understand the fate of OA in the soil, we need to take into consideration OA, how it changes SOM chemistry and how this affects microbial metabolism (Cebrian, 1999; Manzoni et al., 2008; Moorhead and Sinsabaugh, 2006; Schimel and Weintraub, 2003).

One approach to studying the relationship between organic matter chemistry and microbial metabolism is to amend soil with known or labelled substrates, alone or in combination (e.g. Orwin et al., 2006). While this approach can yield detailed information about the turnover of specific compounds or groups of compounds, it can be difficult to use such results to make inferences about the behaviour of complex SOM pools. An alternative approach is to describe the chemical nature of soil carbon pools and OA inputs in the soil environment, e.g. by using solid-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR). As ^{13}C NMR provides overall carbon characterisation of the SOM, it is a powerful tool to link changes in the nature of SOM to soil microbial community composition and/or functions. A number of studies have linked carbon forms to microbial community composition (e.g. Pascault et al., 2010), or carbon forms and microbial activity (e.g. Alarcón-Gutiérrez et al., 2008; Flavel and Murphy, 2006; Pane et al., 2013). However, to our knowledge, there have been few studies of OA transformation, where changes in the chemical nature of carbon containing compounds in the soil, brought about by adding organic amendment and soil microbial community composition and activity, were simultaneously quantified (e.g. Moorhead and Sinsabaugh, 2006; Šnajdr et al., 2011; Wickings et al., 2012). If OA are to become a reliable soil carbon input, such knowledge will be essential.

Here, we report findings of an experiment in which we added three OA sourced from the same municipal green waste but treated to produce three materials of different chemical stability — raw green waste, composted green waste and pyrolysed green waste (biochar) — to two contrasting soils. We then measured differences in the soil carbon composition, the soil microbial community composition and activity after 12 weeks of incubation. Specifically, we hypothesise that the changes in chemical nature of soil carbon as a result of the addition of OA would be reflected in the soil microbial structure and activity.

2. Materials and methods

2.1. Site, experimental design and sampling

We set up a microcosm-based incubation experiment using two soils. The Cranbourne soil (Cr) was collected from a horticultural farm in Cranbourne, Australia (38°11′ S 149°19′ E). It is a semiaquic Podosol of loamy sand texture with a pH of 7.79 (H_2O), a C:N ratio of 13 and contains 1.3% organic matter. The Werribee soil (We) was collected from a horticultural farm at Werribee, Australia (37°53′ S, 144°40′ E). It is strongly dispersive (basaltic) red Sodosol of a slightly sodic light clay texture with a pH 7.79 (H_2O), a C/N ratio of 9.1 and contains 3.9% organic matter. Both soils were collected from 0 to 10 cm depths, air dried and sieved to 2 mm. To 300 g of each soil, OA were added separately as raw green waste (Gw), composted green waste (Co) and green waste biochar (Ch) and thoroughly mixed in at rates that aimed to increase total soil C by 1%. This series of OA were selected as they were expected to have increasingly more stabilised carbon and nutrient pools. For details on the composting and pyrolysis, see Supplementary information. Soils without OA were used as controls in the experiment. The soil microcosms were maintained at moisture between −30 and −40 kPa, and incubated at 25 °C in the dark. Each treatment was replicated four times, and sampled at 12 weeks. The soil from each replicate was then divided into three sub-samples to be stored at 4 °C for microbial activity assays, at −20 °C for phospholipid fatty acid (PLFA) analysis, and air-dried for chemical analysis.

2.2. Carbon characterisation by ^{13}C NMR

Sub-samples from the four replicates of each treatment were combined for ^{13}C NMR analysis. Solid-state ^{13}C cross polarization (CP) NMR spectra were acquired with magic angle spinning (MAS) at a ^{13}C frequency of 50.33 MHz on a Bruker 200 Avance spectrometer. Samples were packed in a 7 mm diameter cylindrical zirconia rotor with Kel-F end-caps, and spun at 5 kHz. Spectra were