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

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1 **Title:** Does the chemical nature of soil carbon drive the structure and functioning of soil

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3

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19 **Abstract**

20 The transformation of organic amendments (OA) in soil is in large part performed by soil

21 microbial communities. These processes are strongly affected by the carbon composition of

22 the OAs. We examined microbial community responses to three types of OA: green waste,

23 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.

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.

1 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 agroecosystems 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 agricutural 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.

18 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

1 phenol oxidases, act more generally on phenolic-containing compounds including lignin,
2 humus and polyphenols (Sinsabaugh, 2010). Thus, quantification of soil enzyme activities
3 provides important mechanistic and functional knowledge of SOM transformation processes.

4 Due to the heterogeneity in the sources of organic inputs from one ecosystem to
5 another, SOM composition can be highly variable. The composition of SOM affects its
6 residence time as it influences biological stabilisation, physical protection and the energy
7 required for SOM breakdown (Ekschmitt et al., 2005; King, 2011; Kögel-Knabner et al.,
8 2008; Schmidt et al., 2011). For example, as the decomposition of SOM progresses, the
9 relative proportion of aromatic structures in SOM has been reported to increase (Gressel et
10 al., 1996; Pedersen et al., 2011), as less stable structures are degraded. Phenolic compounds
11 have been correlated with the antioxidant capacity soils that neutralises free radicals, and
12 therefore, protects organic matter from oxidation (Rimmer and Abbott, 2011). Aromatic
13 compounds have also been implicated in hydrophobic protection of SOM (Spaccini and
14 Picollo, 2012). The complexity of SOM composition is especially relevant in the context of
15 OA transformations given the tremendous chemical heterogeneity and complexity of OA.
16 This in turn can also strongly affect the fate of the OA in the soil.

17 Many OA undergo some form of 'pre-prosessing' prior to application to the soil; for
18 example, organic matter is often composted to eliminate viable propagules of pests and
19 pathogens, and further stabilise the organic matter it contains. Similarly, pyroloysis, that is
20 the elevated thermal decomposition of organic matter in the absence of oxygen, is also
21 increasingly being used as a means of stabilising OA prior to their addition to the soil. Given
22 the differences in the nature of these different processes, they are expected to affect the
23 chemical nature of the resulting OA. For example, we would expect that OA derived from
24 pyrolysis processes to have a greater proportions of aromatics than their parent material
25 (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 contaning compounds in the soil, brought about by adding organic amendment and soil microbial community composition and activity, were simultaneously quantifed (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.

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

8

9 **2. Materials and Methods**

10 Site, Experimental Design and Sampling

11 We set up a microcosm-based incubation experiment using two soils. The Cranbourne
12 soil (Cr) was collected from a horticultural farm in Cranbourne, Australia (38°11' S 149°19'
13 E). It is a semiaquic Podosol of loamy sand texture with a pH of 7.79 (H_2O), a C:N ratio of
14 13 and contains 1.3 % organic matter. The Werribee soil (We) was collected from a
15 horticultural farm at Werribee, Australia (37°53' S, 144°40' E). It is strongly dispersive
16 (basaltic) red Sodosol of a slightly sodic light clay texture with a pH 7.79 (H_2O), a C/N ratio
17 of 9.1 and contains 3.9 % organic matter. Both soils were collected from 0-10 cm depths, air
18 dried and sieved to 2 mm. To 300 g of each soil, OA were added separately as raw green
19 waste (Gw), composted green waste (Co) and green waste biochar (Ch) and thoroughly
20 mixed in at rates that aimed to increase total soil C by 1%. This series of OA were selected as
21 they were expected to have increasingly more stabilised carbon and nutrient pools. For details
22 on the composting and pyrolysis, see supplementary information. Soils without OA were
23 used as controls in the experiment. The soil microcosms were maintained at moisture
24 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.

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 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 acquired using a ramped-amplitude cross polarization (CP-ramp) pulse sequence, in which the ^{1}H spin lock power was varied linearly during the contact time. A 1-ms contact time and a 1-s recycle delay were used and 30,000 transients were collected for each spectrum. All spectra were processed with a 50 Hz Lorentzian line broadening. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm.

All spectral processing was completed using Bruker TopSpin 3 software. Empty rotor background signals were subtracted and the resultant spectra were integrated across the following chemical shift limits to provide estimates of broad carbon types: 0-45 ppm (alkyl C), 45-60 ppm (N-alkyl C), 60-110 ppm (O-alkyl C), 110-145 ppm (Aryl C), 145-165 ppm (O-aryl-C),and 165-215 ppm (Carbonyl C). Signal intensity found in spinning side bands was allocated back to their parent resonances according to the calculations presented by(Baldock and Smernik, 2002)

Microbial activity

We assayed the potential activity of four enzymes in the soil samples, here-on referred to as microbial activity. β-glucosidase (BGL), phosphatase (PHOS) and polyphenol oxidase (PPO) activity were determined according to methods modified from Allison and Jastrow (2006). Peroxidase (POX) activity was assayed using a method modified from Frey *et al.* (2000) and Johnsen and Jacobsen (2008). We incubated 0.5 ml of homogenised soil slurry (1 g soil in 50 ml sterile H_2O) with 0.5 ml of substrate solution (see Table 1 for substrates and incubation times). 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (100 mM, pH 7) was used to make up substrate solutions for all assays except for the POX assay, for which acetate buffer was used (100 mM, pH 5). We used buffer of pH 7 where possible to match our soil pH. All assays included a background soil control and a substrate control. A background soil control contained soil slurry and buffer. A substrate blank contained substrate solution and sterile H_2O. Absorbance was measured at 405 nm except for the POX assay, which was measured at 450 nm. Microbial N activity was measured using potentially mineralisable nitrogen (PMN) as a proxy measure in 5 g aliquots of soil using anaerobic incubation (Waring and Bremner, 1964). The ammonium (NH_4^+) was extracted with 2 M KCl and measured colorimetrically following Forster (1995).

PLFA Analysis

We extracted PLFA following the procedures of Bossio and Scow (1998) with slight modification. Lipids were extracted from 4 g of lyophilised soil using 15.6 ml citrate buffer (0.15 M, pH 4.0): $CHCl_3$: methanol (0.8:1:2 v/v/v) mixture. Samples were shaken for 1 h at room temperature then centrifuged for 10 mins at 1900 × *g*. The supernatant was transferred into a clean glass tube. A further 11.7 ml citrate: $CHCl_3$: methanol (0.9:1:2 v/v/v) mixture

was added to the soil pellet. The samples were shaken and centrifuged, and the supernatant was isolated and combined with the first supernatant. A further 13.3 ml of citrate: $CHCl_3$ (0.9:1 v/v) mixture was added to the pooled supernatant. Samples were left overnight for phase separation, after which the aqueous layer was removed and the $CHCl_3$ layer evaporated under a stream of N_2. Each sample was redissolved in 2 ml of $CHCl_3$ and transferred to solid phase extraction cartridges for separation of lipid classes. An aliquot of 3 ml of $CHCl_3$ was added followed by 2 aliquots of 5 ml of acetone. These extracts were discarded. The phospholipid fraction was collected by extracting the cartridges with 5 ml of methanol; the methanol was then evaporated under N_2. For methanolysis, the phospholipid fraction was incubated at 37 °C for 20 mins with 1 ml of a 1:1 mixture of methanol and toluene and 1 ml of methanolic KOH (0.2 M). The samples were neutralised with 0.3 ml acetic acid (1 M) and 2 ml of ultrapure H_2O. Two extractions were carried out with a mixture of 2 ml hexane: $CHCl_3$ (4:1 v/v) and the organic phases combined. The organic layer was collected and evaporated again under a stream of N_2. Each sample was resuspended in 200 μl of hexane containing methyl decanoate (0.005 mg/ ml) and analysed using gas chromatography. The chromatography was conducted with a 30 m (5%-phenyl)-methylpolysiloxane column (Varian CP 3800), using He as acarrier gas, an FID detector, and a temperature program of 120 °C initial temperature, ramped to 220 °C at 4 °C/min, ramped to 325 °C at 20 °C/min, and held 325 °C for 8 mins. Bacterial phospholipid markers of interest were i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, a17:0, 17:0cy, 17:0, and 19:0cy (cf with Frostegard and Baath 1996, and references therein). Linoleic acid (18:2ω6,9) was used as an indicator of fungal biomass (Frostegard and Baath 1996). Concentrations of fatty acid less than 0.1 ppm were treated as 0 and only fatty acids detected in > 4% of treatment were included in the analysis; as a result a total of 21 PLFAs were included.

Calculations and Statistical Analysis

Standardised data were used for multivariate regression tree (MRT), non-metric multidimensional scaling (NMDS), redundancy analysis (RDA) and cluster analysis. The PLFA was standardised by dividing values of each individual fatty acid by the total PLFA. Microbial activities were standardised by chi-square transformation using decostand() function in *vegan* package. The MRT was carried out to examine the relationship between C forms and soil microbial community composition or microbial activities. This analysis involves a response (i.e. the composition or activity data set) and an explanatory data set as detailed in Legendre and Legendre (2005). It forms clusters of sites by repeated splitting of the response data set based on minimising dissimilarity of sites within each cluster. Selection of the best tree was based on cross validation and selecting the smallest tree within one standard error of the best following the method described by De'ath (2002). Unconstrained cluster analysis was comparable to MRT analysis and indicated that the explanatory variables accounted for most of the observed variations. NMDS analysis is an ordination method that plots dissimilar objects far apart in ordination space and similar objects close to one another. The NMDS was carried out to examine unconstrained patterns in microbial activity. For microbial community PLFA composition, cluster analysis was performed. A Bray-Curtis dissimilarity index was calculated using transformed PLFA data followed by Ward's minimum variance clustering. The PLFA clustering was then overlaid onto the ordination plot of the microbial activity as a minimum spanning tree. RDA was then carried out by constraining the microbial community composition or activity to ^{13}C-NMR data as explanatory variables. Where the MRT and RDA analyses disagreed (in terms of variations explained), this informed us of (i) the presence of interactions between the C forms which was not captured in RDA analysis since interactions were not included in the RDA analysis, and/ or (ii) that the relationship between the C forms and microbial activity or composition

was not linear as RDA solution lies in linear space. All analyses were carried out in R 2.15.2 (R Core Team 2012) using vegan (Oksanen et al., 2012) for RDA and NMDS, mvpart (Therneau et al., 2012) and MVPARTwrap (Ouellette and Legendre, 2013) for MRT.

3. Results

3.1 Linking microbial activity to microbial community composition

Patterns in soil microbial community composition and microbial activity could be explained by the type of OA added to the soil (Fig. 1). Specifically, the community composition was more similar between the two soils amended with the same OA than to their respective unamended soils; this is illustrated in the plot where a line connects the treatments based on the similarity in microbial community composition. For example, microbial composition in CrGw and WeGw were very similar, and microbial composition in Co or Ch amended soils were similarly strongly influenced by the OA. As for microbial activity, the type of amendment also separates the amended from the unamended soils; this is reflected in the relative location of the points in the ordination space, with those treatments closer to one another on the plot being more similar than to those further away in the plot. For example, microbial activity in CrGw and WeGw were more similar to each other than to their respective unamended soils. On the other hand, microbial activity in CrCo was more similar to that of CrCh, while microbial activity in WeCo was more similar to that of WeCh.

3.2 Linking microbial activity and microbial community composition to soil carbon forms

To further explore the relationship of carbon composition with soil microbial community composition and activity, we analysed our data using multivariate regression trees

(MRT) and redundancy analysis (RDA). These analyses indicated that the relative content of alkyl-C, O-aryl-C, aryl-C and carbonyl-C explained most of the variation in soil microbial community composition and activity (Fig. 2). Furthermore, the differences in soil microbial community composition and activity due to the application of OA are associated with the relative importance of different C forms and the interaction and/or association among the C forms (Figs. 2A, 2C, see Supplementary table S1 for C composition).

MRT analysis revealed that microbial activity could be separated into two main groups based on the relative abundance of carbonyl-C in the soil (46 % of variation in microbial activities was explained by this split, Fig. 2A). Specifically, the microbial activities in WeCo and WeGw were associated with higher relative carbonyl-C content (> 15.14 %), compared to all other treatments. Further splitting of the remaining groups with lower relative carbonyl content (< 15.14 %) was delineated by aryl-C and alkyl-C. The MRT explained 79 % of the total variation in microbial activity, of which 61 % was explained by the first two splits in the tree. Thus, relative carbonyl-C content was the best predictor of soil microbial activity. Analysis of the data by MRT also allowed us to determine what and how much difference in the microbial activity was explained by the C forms. For example, PMN accounted for 58 % of the variance in microbial activities, and 46 % of this variance was explained by the tree, and 36 % of this variance was explained by the first split (Table 2). POX and PPO largely determine the second split (aryl >/< 16.65), which explained 15 % of the variance in microbial activity. Interestingly, total variance in BGL (4 %) and PHOS (7 %) was low and the tree managed to explain most of it (2 % and 6 %, respectively). Additionally, the MRT identifies potential interactions and/or associations among the C forms that result in the patterns observed for microbial activity. This is because each split of the MRT partitions data into independent subsets that are then further analysed independently (De'ath, 2002). For

example, the interaction between carbonyl-C with aryl-C and alkyl-C produces differences in microbial activities.

RDA analysis of C forms and microbial activity largely showed similar patterns to those found in MRT analysis. For example, as in the MRT analysis, the microbial activity in the WeGw and WeCo treatments were closely, and positively associated with relative carbonyl content. We found that the relative content of carbonyl-C, aryl-C, O-aryl-C, alkyl-C explained 57 % of total variation in microbial activity, of which 55.6 % was explained by axis 1 and 2 (Fig. 2B). PPO activity was closely associated with O-aryl-C and aryl-C while BGL, PHOS, POX and PMN were more closely associated with carbonyl-C. Additionally, POX activity was also closely associated with alkyl-C.

The soil microbial composition was separated into two main groups by MRT analysis according to the aryl-C content, with higher relative content of aryl-C associated with the Ch and Co amended samples (Fig. 2C). Further splitting of the Ch and Co amended soil was determined by the relative contents of carbonyl-C, whereas unamended and Gw amended soils were further separated by O-aryl content. MRT explained 86 % of the total variation in soil microbial community composition, of which 76 % was explained by the first four splits. The variances of bacterial cy19:0 and fungi 18:2ω6 together comprise 60 % of the total variance in microbial community composition; 50 % of this variance was explained by the tree (Table 3). Bacterial cy19:0 dominated the first split (aryl >/< 17.0) while fungi 18:2ω6 dominated the fourth split (aryl >/< 16.6). The MRT also identifies potential interactions between the C forms that result in the patterns observed for microbial composition. For example, the interaction between aryl-C with carbonyl-C were most important for distinguishing microbial composition in Co and Ch amended soils while the interaction between aryl and O-aryl were more important for distinguishing microbial composition in Gw amended soils from unamended soils.

1 RDA indicated that similar to microbial activity, carbonyl-C, aryl-C, O-aryl-C, alkyl-
2 C explained a large portion (46 %) of the total variation in the soil microbial composition
3 (Fig. 2D). The microbial composition of Ch amended soils was associated with higher O-
4 aryl-C and aryl-C compared to other treatments. While the soil microbial community
5 composition of CrCo was similar to Ch amended soils in its association with higher O-aryl-C
6 and aryl-C compared to other treatments, the microbial composition of WeCo was more
7 similar to its unamended soil and both were associated with higher carbonyl-C and alkyl-C
8 compositions.

9

10 **4. Discussion**

11 The patterns in soil microbial community composition and microbial activity
12 observed here could be explained by the type of amendment added to the soil. Both RDA and
13 MRT analysis of the data agree that soil carbon forms explain a large amount of the variation
14 in the soil microbial activity and composition (46 – 86 %). Based on the MRT, soil carbon
15 forms had a larger influence on the microbial composition than the activity, as observed in
16 the differences in variations explained. These results reinforce the important link between the
17 nature of carbon in the soil, and the structure and function of soil microbial communities, in
18 support of our hypothesis. Importantly, the patterns of the soil microbial community
19 composition and activity responded differently to the soil carbon composition, highlighting
20 the importance of the need to consider microbial community structure and function
21 simultaneously rather than in isolation. Taken together, these results highlight the complexity
22 of the relationship between SOM composition, soil microbial community composition, and
23 soil functions, while providing new insights into the factors governing the fate of OA in
24 agricultural soils.

Analysis of the data using MRT allowed us to use data on soil carbon composition to split the complex microbial compositional data in a manner that explained the majority of variation. For example, the first split in the soil microbial composition was driven by aryl-C and was largely due to the bacterial cy19:0 PLFA marker. Bacterial cy19:0 is associated mainly with Gram-negative bacteria (Zelles, 1997). These bacteria are generally known to utilise mainly simple C forms (Holding, 1960) yet they were found in this study to be more abundant in soils with higher relative aryl-C content. Aryl-C and O-aryl-C are aromatic compounds that are found in lignin, humic substances and biochars. They are associated with stabilised OM (Lehmann et al., 2011). But if we consider these results in the context of the observed microbial activities, then it appeared that these bacteria are present under conditions of higher PPO activity. Either these bacteria produce PPO to access the required carbon and nutrients, or they are riding very successfully on other microbes that do produce PPO.

In contrast, the fourth split in the tree for microbial composition was driven by aryl-C and was dominated by the fungal PLFA marker 18:2ω6,9. At this split, CrGw was distinguished from WeGw. The CrGw sample, which contained only marginally more aryl-C than WeGw, also had a greater proportion of fungal PLFA marker 18:2ω6,9. Given that ^{13}C-NMR typically underestimates aryl-C and O-aryl-C (Smernik and Oades, 2000a, b), the differences in aryl-C content of the two may be greater than observed and this may explain why such marginal differences in aryl-C content leads to very different fungal PLFA marker 18:2ω6,9 composition. This is still surprising given the previously established relationship between fungi and recalcitrant SOM (De Boer et al., 2005; Garrett, 1951). Fungi are important producers of PPO and POX enzymes which degrade more recalcitrant aromatic compounds (Kirk and Farrell, 1987; Sinsabaugh, 2010). We had expected Co and Ch amended soils to have greater amounts of the fungal PLFA marker 18:2ω6,9; however, this was not the case, and is worthy of further investigation. Additionally, both Gw amended soils

1 have relatively similar microbial activities. They are positively correlated with hydrolytic
2 enzyme activities (BGL, PHOS) and carbonyl-C contents. Carbonyl-C is found in proteins
3 and organic acids and they are important labile carbon forms. By combining these
4 observations, we hypothesise that (i) bacteria may be playing a more important role in the
5 degradation of recalcitrant C forms in these soils, and (ii) the dominant fungi composition in
6 these Gw amended soils are copiotrophs.

7 Our results also indicated that presence of aryl-C favours the production of PPO (i.e.
8 positive correlation), resulting in production of O-aryl-C. Such a combination of increasing
9 PPO with aryl-C or O-aryl-C is expected of Co and Ch amended soils, and indeed our
10 observations agreed with it. This is corroborated by our observations that soils amended with
11 Co and Ch had higher metabolic quotient and therefore greater respiration per unit biomass
12 compared to soils amended with Gw (supplementary Fig. S2). In contrast, soil amended with
13 Gw had high initial respiration rates and low metabolic quotient (supplementary Fig. S2).
14 Raw green waste, consisting mainly of O-alkyl-C, N-alkyl-C and alkyl-C contains a large
15 pool of labile C. O-alkyl-C is found mainly in carbohydrates and lignin with smaller
16 quantities in proteins and lipids (Baldock et al., 2004; Baumann et al., 2009). N-alkyl-C is
17 found in proteins and lignin (Baldock et al., 2004). Alkyl-C is mainly found in lipids, waxes,
18 cutins, suberins and lignin but is also found in proteins (Baldock et al., 2004; Baumann et al.,
19 2009). The initial high respiration rates and low metabolic quotient provide support for the
20 rapid consumption of labile C forms in the Gw amended soils by copiotrophs, which may
21 explain the similarity of the microbial community composition of the Gw amended soils to
22 the unamended soils 12 weeks after the addition of the OA. The subsequent succession of
23 microbial community upon exhaustion of the easily assimilable C reflects the biotic legacy of
24 the unamended soils.

As discussed above, the patterns of soil microbial community composition and activity clearly responded differently to the soil carbon forms. There are several possible explanations for these observed differences in the relationship between soil microbial composition and function to soil C forms. First, it may indicate that similar communities may not necessarily have similar functions. Strickland *et al.* (2009) found that harsher environment filters generate communities with more similar composition but dissimilar functional capabilities. Second, the weak relationship between composition and function may well reflect the temporal differences in both responses. Third, ^{13}C-NMR captures the bulk soil C forms and is not able to capture the subtle changes in C form in a small fraction of the soil organic matter that matters the most to microbial function. Moreover, not all drivers of microbial community structure and function are related to carbon. For example, we found that other macronutrients, N and P, were also very important in shaping the microbial communities (Ng *et al.,* in review). Cusack et al (2011) also found an interaction between mineral N deposition and the indigenous carbon reserves of two different soils, which caused a divergence in the microbial community structure and function after the N addition. In that case, N-addition to lowland forest soil with more labile carbon caused increases in bacterial dominance and hydrolytic enzyme activities, whilst N addition to highland forest soil with more stable C favoured fungal communities and oxidative enzyme activities (Cusack et al., 2011). Finally, the differences in the RDA and MRT results highlight the importance of interactions and/or associations among C forms leading to the observed patterns in soil microbial community composition and function; this further justifies the use of RDA and MRT together. Other studies on litter decomposition have found that there is a non-additive, synergistic mixture effect to decomposition rates when distinct litters decompose in a mix as opposed to in isolation (Hättenschwiler et al., 2011). One possible explanation for this is that

greater heterogeneity of C forms also leads to greater interaction among the C forms. This warrants further investigation.

As the Cranbourne and Werribee soils have very different physicochemical properties, the patterns in microbial community composition and microbial activities here indicate a strong influence of the carbon composition of the OA. Both soils clearly showed that with the addition of 1 % carbon to the soils, changes in their carbon composition occurs with changes in the microbial community composition and function 12 weeks after the application of OA. However, the magnitude of this effect is mediated by the soil physicochemical properties. In the sandy Cranbourne soil, Gw amendment resulted in the soil microbial composition being a lot more similar to its Werribee counterpart amended with the same OA than to its unamended soil. The same was not true for the amended Werribee soil, which is a clayey soil. The microbial composition in Gw amended Werribee soil reflects both the amendment and its unamended soil. However, it is worthy to note that the 1 % carbon addition represented a bigger proportion increase in soil carbon for Cranbourne soil than Werribee soil. These differences in physicochemical properties of the soils may explain the variation in the treatments that were not explained by carbon forms. We would expect that over time, the relative importance of different environmental factors in determining structure and function of soil microbial community varies; such temporal factors are likely to be important, and therefore, deserve further attention.

5. Conclusions

The results of our study indicate that there is a very strong relationship between carbon composition, as determined by ^{13}C-NMR analysis, soil microbial community composition and microbial activity. The addition of OA changes the soil C composition and

alters the soil microbial community composition and its activity. Compost amended soils were most dissimilar from unamended soils for both microbial community composition and activity. A large proportion of these variations are explained by the relative content of alkyl-C, O-aryl-C, aryl-C and carbonyl-C. The differences in the patterns of soil microbial composition and microbial activity were associated with different C forms appearing as the most influential explanatory variable and the interaction between these C forms.

The results of this study demonstrate the direct relationship between soil carbon composition with soil microbial community structure and function. Such studies combining molecular approaches with functional measures will allow us to better assess effects of OA in agroecosystems and to examine SOM responses to management practices. Building on such a predictive approach to examine the relationship between carbon forms, microbial community structure and function will lead us towards not only understanding the relationship between the soil living and non-living components, but may enable us to predict outcomes for the use of organic amendments in the future based on the chemical composition of the applied organic matter.

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1 Tables

2 Table 1. Enzyme substrates and assay time.

3 Table 2. Variance of soil microbial activity explained by the tree analysis

4 Table 3. Variance of soil microbial PLFA composition explained by the tree analysis.

5 Figures

6 Fig. 1 NMDS plot (showing mean ± se) of microbial activity overlaid with PLFA
7 composition, whereby the minimum spanning tree (line) shows the actual connection between
8 the microbial community composition in one treatment to that in another treatment. We =
9 Werribee soil, Cr = Cranbourne soil, Gw = green waste, Co = composted green waste, Ch =
10 charred green waste (e.g. CrGw refers to Cranbourne soil amended with green waste). See
11 supplementary table S2 for mean ± standard error values of microbial activity.

12 Fig. 2 Multivariate regression tree and redundancy analysis for the microbial community
13 based on (A, B) microbial activities and (C, D) PLFA at 12 weeks. (A) The variation
14 explained at each split of the MRT is shown as a percentage in parentheses in the body of
15 each branch. Cross-validation error = 0.407, standard error of the tree = 0.098. (B) RDA
16 correlation plot showing variance in microbial activities explained by C forms (C) The
17 variation explained at each split of the MRT is shown as a percentage in parentheses in the
18 body of each branch. Cross-validation error = 0.237, standard error of the tree = 0.069. (D)
19 RDA correlation plot showing variance in PLFA composition explained by C forms. We =
20 Werribee soil, Cr = Cranbourne soil, Gw = green waste, Co = composted green waste, Ch =
21 charred greenwaste (e.g. CrGw refers to Cranbourne soil amended with green waste). BGL =
22 β − glucosidase, PHOS = phosphatase, PMN = potentially mineralisable nitrogen, PPO =
23 polyphenol oxidase, POX = peroxidase