# ACCEPTED VERSION

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**Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter**

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**Title:** Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter

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## Abstract

The transformation of organic matter amendments in the soil is regulated by soil microbial communities. We examined the utility of ecological and functional stoichiometry theories to explain microbial transformation of organic amendments in the soil and examined the key relationships between soil microbial community composition, biomass and activity with resource elemental composition (soil and organic input) and nutrient availability. Using two contrasting soils amended with raw green waste, its compost or biochar, we found that microbial PLFA composition was distinct for each soil and organic amendment. Microbial activity was strongly influenced by organic amendment. Further, we observed that changes in the soil stoichiometry with inputs were accompanied by changes in total PLFA and bacteria: fungal ratio, but the relationships between them were inconsistent and changed over time. Microbial activities involved in C, N and P cycling were generally correlated, but the relationship between hydrolase β-glucosidase (BGL) and microbial N and P activities was stronger and more consistent than that between oxidases (phenol oxidase PPO, peroxidase POX) and microbial N and P activities. These microbial activity relationships translated to a consistent relationship between log(BGL):log(nutrient) and soil C:nutrient but a weaker and inconsistent relationship between log(PPO+POX):log(nutrient) and soil C:nutrient. Our analyses indicate that microbial composition can be different, but stoichiometric invariance of microbial activity constrained microbial community response to organic input.

## 1. INTRODUCTION

In a time of dwindling global fertilizer resources (Cordell et al., 2009) and rising food demand, there is growing interest in the development of an agricultural paradigm based around sustainable biologically regulated nutrient supply systems. The addition of organic amendments, such as nutrient rich plant residues and composts, to soils can deliver nutrients and organic matter that improve soil structure and plant productivity (Hargreaves et al., 2008). In Victoria, Australia, municipal waste comprises approximately 1.6 million tonnes or 36 % of solid waste going to landfill in 2002 (Nolan-ITU, 2002). The use of such waste as organic amendment allows large quantities of waste to be diverted from landfills. In recent years, incorporation of such organic amendment in its stabilised form is emerging as management option due to its added potential for long-term carbon sequestration (Lal, 2011; Lehmann, 2007). If widespread use of organic amendments to supply nutrients for plant productivity is to become a viable option to offset the use of inorganic fertilisers in agriculture, and potentially improve soil carbon content, their use must be predicated upon a complete understanding of the soil ecological processes that govern their fate in soil (Jackson et al., 2008).

Soils contain arguably the most diverse terrestrial communities on the planet (Wardle, 2006), and vary considerably on scales ranging from the microsite ($\alpha$-diversity) to landscape ($\gamma$-diversity) (Ettema and Wardle, 2002). While the importance of soil microbial communities in organic matter decomposition is well recognised, the factors that regulate microbial-mediated carbon transformation and nutrient release are still being debated (e.g. Dungait et al., 2012; Schmidt et al., 2011). If we are to discover the factors regulating the fate of organic amendment in the soil, a theoretical framework that effectively deals with the tremendous structural and functional complexity in soils must guide our work.

Ecological stoichiometry, that is, the balance of chemical elements and the concept of inflexible elemental ratios (particularly of C, N and P), has been proposed to explain ecosystem processes (Sterner and Elser, 2002). In a study on plant litter – microbe system, C:nutrient ratios of the soluble litter fraction have been linked to shifts in microbial community composition (Fanin et al., 2013). The ratios of C:N:organic P:S in the humus fraction of soil organic matter was reported to be constant for a wide range of soils from Australia and elsewhere under different land uses (Kirkby et al., 2011). These results suggest that while microbial community composition varies from ecosystem to ecosystem, and in response to organic amendment addition, the overall stoichiometry of soil organic matter proceeds toward a constant equilibrium ratio.

Soil microbial communities are regulated by both the supply of energy and nutrients (especially N and P) (Hessen et al., 2004). In Ng *et al.* (2014), we found that carbon composition of the organic input explained at least 50% of the variation in microbial community composition and activity in soils treated with different organic inputs. While previous studies have shown that the chemical nature of the organic amendment affects microbial community composition and activity; few studies have specifically examined the underlying mechanisms that regulate these responses (Bowles et al., 2014; Fanin et al., 2013; Güsewell and Gessner, 2009; Kallenbach and Grandy, 2011). Microbial demand and use of resources is driven by the elemental stoichiometry of their biomass, but is regulated by the elemental stoichiometry of the resources (Cleveland and Liptzin, 2007; Sterner and Elser, 2002). The balance of these competing constraints is hypothesised to be captured by the functional stoichiometry of the microbial community (Sinsabaugh et al., 2009); that is, the ratio of the activity of microbial processes involved in the cycling of C, N and P (i.e. C:N:P activity ratios). Specific ecoenzyme activities (e.g. β-glucosidase, amidase, peroxidase, and phosphatase) provides a reliable measure of functional stoichiometry which integrates the

stoichiometric and metabolic theories of ecology, and links microbial metabolic efficiency to microbial biomass and the elemental composition of their food resources (Allen and Gillooly, 2009; Sinsabaugh et al., 2009; Sinsabaugh et al., 2008). Past studies have generally examined functional stoichiometry at landscape and global scales (Sinsabaugh et al., 2009; Sinsabaugh et al., 2008; Sinsabaugh and Shah, 2011). These studies have identified a constrained functional stoichiometry in response to climatic and edaphic variables. While studies have shown broad global patterns in functional stoichiometry, the relevance of this theoretical framework to farm scale variations due to differences in management inputs has not yet been explored.

In this study, we explore the utility of ecological and functional stoichiometry theories to understand the microbial transformation of three organic amendments differing in elemental stoichiometry as a result of composting and pyrolysis processes, upon addition to soil. Our goal is to examine the key relationships between soil microbial community composition, biomass and activity, with resource elemental composition and nutrient availability. To do so, we examined the effects of green waste (raw, composted or pyrolysed) addition on nutrient availability and soil microbial community composition and activity in two contrasting soils. We measured the activity of two oxidases (peroxidase and phenol oxidase) that are known for their role in stable organic matter breakdown, and the more commonly measured hydrolase, β-glucosidase. We also measured potentially mineralisable nitrogen and alkaline phosphatase activity as proxies for/indicators of N and P cycling. We hypothesised that the functional stoichiometry of the microbial community would correlate well with input stoichiometry initially, and as the added organic amendments decomposed, the functional stoichiometry would evolve to better reflect the overall soil stoichiometry. We expect this relationship to be consistent across ecosystems, i.e. the distinct soil microbial communities in both soils experience similar stoichiometric constraints.

## 2. MATERIALS AND METHODS

### *2.1 Experimental set-up*

This paper is the second arising from an experiment in which we assessed the fate of green waste-derived OA in soils. Whereas the first paper dealt with the fate and cycling of C present in the soil in different forms (Ng et al., 2014), this paper focuses on the soil N and P cycling. A microcosm-based incubation study was conducted. Organic matter derived from municipal green waste was added to two soils, in either its raw state, or following composting or pyrolysis of the green waste (see Ng et al. (2014) on preparation of the organic amendments).

The Cranbourne soil, which was collected from a horticultural farm in Cranbourne, Australia (38°11' S 149°19' E), was a semiaquic Podosol, loamy sand, pH 7.79 ($H_2O$); C:N:P (73.8:10.5:1); organic matter (1.3 %); water holding capacity (20.8 %). The Werribee soil, which was collected from a horticultural farm in Werribee, Australia (37°53' S, 144°40' E), was a strongly dispersive (basaltic) red Sodosol (Isbell 1996), slightly sodic light clay topsoil, pH 7.79 ($H_2O$), C:N:P (26.5:2.5:1); organic matter (3.9 %); water holding capacity (49.5 %). Both soils were collected from the top 10 cm soil layer, air dried and sieved to 2 mm. The organic amendments added to the soils were a raw green waste or its composted or pyrolysed forms, which are referred to as green waste, compost and biochar, hereafter (see supplementary table S1 for properties). For details on the composting and pyrolysis, see Ng *et al.* (2014). Before they were applied, the green waste and compost were sieved to 12.5 mm. The green waste was additionally passed through a garden mulcher before sieving. The biochar did not require further pre-processing prior to application due to its small particle size. The organic amendments were added to 300 g of either soil; these application rates correspond to an increase in total soil C of ca. 1%. Unamended controls were also included.

The soils were wet up to between 30 and 40 kPa and incubated at 25 °C. Soil moisture content was maintained during incubation by the addition of water after weighing the soils every 4 to 7 days. Each treatment was replicated four times and sampled at 4 weeks and 12 weeks. Samples were air dried for chemical analysis, kept at 4 °C for enzyme analysis or -20 °C for all other analyses.

*2.2 Microbial analysis*

Soil microbial structure was assessed by phospholipid fatty acid (PLFA) analysis. PLFA was extracted using a method modified from Bligh and Dyer (1959) with addition of citrate buffer (Nielsen & Petersen 2000) followed by alkaline methanolysis of phospholipids (Bossio and Scow, 1998). PLFA determination was done using a Varian CP 38/00 gas chromatograph fitted with 5 % phenyl:95 % methylsiloxane column (Varian, Walnut Creek CA, USA). The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7, i17:0, a17:0, 17:0cy, 17:0, and 19:0cy were chosen as bacterial biomarkers and linoleic acid (18:2ω6,9) was chosen as the biomarker for decomposer fungi (see Frostegård and Bååth, 1996 and references therein). We did not include 18:1ω9 as a fungal marker in our analysis, as this marker is also found in plants and bacteria and it is a poor indicator of fungi in agricultural soils (Frostegård et al., 2011). Concentrations of fatty acids less than 0.1 ppm were treated as 0 and only fatty acids detected in > 4% of treatments were included in the analysis which included 21 PLFAs. Total PLFA was used as a relative measure of soil microbial biomass (Frostegård and Bååth, 1996).

β-glucosidase (BGL) and phosphatase (PHOS) are hydrolases involved in cellulose degradation and mineralisation of organic phosphorus respectively (Shi, 2010). Phenol oxidase (POX) and peroxidase (PPO) are oxidases associated with the degradation of recalcitrant carbon forms (Sinsabaugh, 2010). The activities of BGL, PHOS and PPO were

determined according to methods modified from Allison and Jastrow (2006). POX was determined according to Frey et al. (2000) and Johnsen and Jacobsen (2008). Briefly, we incubated 0.5 ml of soil slurry (1 g soil in 50 ml sterile $H_2O$) with 0.5 ml of substrate solution for 2 hr (BGL and PHOS), 1 hr (PPO) or 10 mins (POX). We used a buffer of pH 7 (3-(N-morpholino)propanesulfonic acid or MOPS, 100 mM) where possible to match our soil pH, except for POX assay for which acetate buffer (pH 5, 100 mM) was used to make up the substrate solution. *p*NP-β-D-glucopyranoside (5 mM), *p*NP – phosphate (5 mM), pyrogallol/EDTA (50 mM/ 50 mM) and 3,3',5,5'- tetramethylbenzidine (TMB; 1 mM) were substrates for BGL, PHOS, PPO and POX respectively. A background soil control and a substrate control were prepared for all enzymes. Colorimetric measurement of reaction products was made at absorbance 405 nm except for POX which was measured at 450 nm. The PPO standard was obtained by measuring the absorbance of completely oxidised products of known amounts of pyrogallol with commercial mushroom tyroxinase (1mg/ mL). A standard curve of absorbance versus *p*-nitrophenol was used for BGL and PHOS. The extinction coefficient was obtained from the manufacturer for TMB. Changes in N activity were measured using potentially mineralisable nitrogen (PMN) as a proxy measure in 5 g soil using an anaerobic incubation at 37 °C for 7 days as described by Waring and Bremner (1964). The ammonium ($NH_4^+$) was extracted with 4 M KCl and measured colorimetrically following Forster (1995) modified for 96-well microplate. Two reagent blanks and immediate $NH_4^+$ extracts were made using 2 M KCl.

*2.3 Chemical analysis of soils and organic amendments*

The organic amendment, unamended and amended soil samples were also analysed for a suite of chemical properties. Mineral N ($NO_3$, $NH_4$) and $CaCl_2$-P (which is a measure of

labile P fraction) were determined as described in methods 7C2, and 9F1 of Rayment and Higginson (1992). A high-frequency induction furnace (LECO Pty Ltd) was used to measure total soil C and N. Total soil P was determined by perchloric acid digestion before analysis by inductively coupled plasma atomic emission spectroscopy (Spectro Analytical Instruments Pty Ltd, Kleve, Germany).

*2.4 Statistical analysis*

C:nutrient stoichiometry was calculated using %N, %P and %C of soil dry weight. Input stoichiometry is determined from the organic amendment at the start of the experiment. Non-metric multidimensional scaling (NMDS), an unconstrained ordination analysis (Legendre and Legendre, 2012), was performed to assess general trends in microbial community composition and soil function with organic amendment and over time. Redundancy analysis (RDA), a constrained ordination analysis (Legendre and Legendre, 2012), was used to identify the environmental variables that explain the trends in microbial PLFA composition and activity (i.e. PHOS, PMN, BGL, PPO and POX). The ordination plots display similar objects close to one another while dissimilar objects are farther apart. For multivariate analysis, all data were standardized accordingly as follows: PLFA data was log (n+1) transformed and standardised by chord transformation, microbial activity was standardised by chord transformation and environmental variables (i.e. soil $NH_4^+$, soil $NO_3^-$, soil C:N ratio, soil C:P ratio, initial C content upon addition of organic amendment or of unamended soil, in the case of control) were standardised to zero mean and unit variance. For the NMDS, Bray-Curtis distance matrix was calculated using the above standardised data and NMDS performed using metaMDS() function in *vegan* package. The general trends observed in NMDS agree with patterns observed in the RDA. NMDS is presented in the main text and

the RDA is provided in the supplements. NMDS and RDA were plotted as 95 % confidence ellipses using the component scores for each of the four replicates.

Kendall τ is a nonparametric rank correlation method to quantify the relationship between two variables (Legendre and Legendre, 2012). Kendall τ correlations were calculated using log (n+1) transformed data for the amended and unamended soils' C:nutrient ratios at sampling, input (organic amendment) C:nutrient ratios and functional C:nutrient ratios. In the calculation of average ratios for functional stoichiometry, unrepresentable (NaN) and infinite (Inf) values that appeared at 12 weeks due to undetectable activity level were excluded. Two-way analysis of variance (ANOVA) was carried out for total PLFA and bacterial: fungal ratio to examine the effect of organic amendment and soil. Data was checked for homogeneity of variance and normality. Where the assumptions were violated, data was transformed and compared to the untransformed data. As the results were similar with both transformed and untransformed data, we have retained all analysis using the untransformed data. Where significant interaction between soil and organic amendment were found, we compared differences between organic amendments within each soil using least significant difference test with *p*-values adjusted using bonferroni.

Data analysis was carried out on R 2.15.1 (R Core Team 2012) using vegan package (Oksanen et al., 2012) for ordinations and agricolae package (Mendiburu, 2012) for Kendall correlations.

## 3. RESULTS

### *3.1 Stoichiometry of the resources*

The input C:N ratio was highest in biochar (20.9) followed by green waste (14.2) and compost (10.7). The input C:P ratio was highest in green waste (88.5) followed by biochar (63.3) and compost (53.6). The initial C:N ratios of the Cranbourne and Werribee soils were 7.0 and 10.6 respectively; and the initial C:P ratios of the Cranbourne and Werribee soils were 73.8 and 26.5. The total soil C, N and P were positively correlated at 4 and 12 weeks (Table 1A, Fig. 1). Patterns in the total soil C, N and P were similar in both soils, i.e. compost amended soils had the highest total C, total N and total P at 4 and 12 weeks. Green waste and biochar amended soils also had higher total C, total N and total P than unamended soils at both time points. Soil stoichiometry was inconsistently correlated with input stoichiometry (Table 1B). There was a positive correlation between soil C:N with the input C:N ratio only at 12 weeks and soil C:P ratio with input C:P ratio only at 4 weeks.

### *3.2 Soil microbial community composition, size and structural responses to amendments*

Soil microbial biomass, measured as total PLFA, differed markedly among the soils and organic amendment at both time points (Fig 2A, 2C, see supplements for ANOVA table). The Werribee soil generally had higher microbial biomass than the Cranbourne soil, irrespective of sampling time or organic amendment. Whereas the addition of biochar to the soils resulted in similar or even lower total PLFA than their respective unamended soils, the addition of green waste and compost resulted in significant increases in total PLFA, particularly at 4 weeks.

The bacterial:fungal ratio also changed with organic amendment. For each soil, the bacterial:fungal ratio was consistently highest in unamended soils, and lowest in green waste amended soils, at both time points (Figure 2B, 2D). Compost and biochar amended Werribee soil had similar bacterial:fungal ratios, whereas the biochar amended Cranbourne soil had higher bacterial:fungal ratio than the compost amended Cranbourne soil at both time points.

Based on NMDS, the variation between microbial compositions in the Cranbourne soil with different amendments was greater than the variation between microbial compositions among Werribee soils with different organic amendment at 4 weeks (Fig 3A). The non-overlapping confidence ellipses in the ordination plot indicated that the microbial composition was unique with each treatment. At 12 weeks, microbial composition in compost and biochar amended Werribee soils diverged from unamended and green waste amended Werribee soils. In contrast, in Cranbourne soils, the microbial composition of biochar amended soil was more similar to the unamended soil and compost amended soil remained least similar to other treatments.

*3.3 Relationships between microbial structure, resource stoichiometry and mineral nutrients*

Total PLFA was negatively correlated with soil C:N ratio at both 4 and 12 weeks, and negatively correlated with soil C:P ratio only at 12 weeks (Table 1C). Total PLFA was not correlated with input C:nutrient at any time point. Total PLFA was also positively correlated with soil $NH_4^+$ at both time points but the relationship with $NO_3^-$ was inconsistent (Table 1C). The bacterial:fungal ratio was negatively correlated to soil C:N ratio at 12 weeks and positively correlated to input C:nutrient ratios at 4 weeks (Table 1C). The bacterial:fungal ratio was positively correlated with $NH_4^+$ at both time points but showed no relationship with $NO_3^-$ (Table 1C).

The shifts in the soil microbial composition with addition of organic input, at both time points, were partly explained by soil type, soil C:N ratio, soil C:P ratio, initial C content, soil $NH_4^+$ and soil $NO_3^-$ (Supplementary Fig. 1A). Based on RDA, 46.7% of total variance in the microbial composition was accounted for by these variables.

*3.4 Soil microbial activity responses to amendments*

The microbial C, N and P activities were generally positively correlated at 4 and 12 weeks except for the insignificant relationship of the oxidases (PPO and POX) with PHOS and PMN at 4 weeks (Table 1D, Fig. 4, see supplementary Fig. 3 for 3D plots). At 4 weeks, each of the soils showed similar patterns where green waste amended soils had the highest hydrolase C activity, followed by compost or biochar and unamended soils. The pattern for oxidase C activity was the opposite for amended soils, where compost or biochar amended soils had higher oxidase C activity than green waste amended or unamended soils.

Based on NMDS, overall microbial activity in the green waste amended soils was least similar to unamended soils, whereas overall microbial activity in compost and biochar amended soils were more similar to unamended soils at 4 weeks (Fig. 3B). At 12 weeks, overall microbial activity became more distinct among the differently treated soils in each soil. At the same time, overall microbial activity in green waste amended soils became more like that of the unamended soils as compared to the activity at 4 weeks, while overall microbial activity in compost amended soils remained similar to that at 4 weeks (Fig 3B).

We analysed the mean ratio of microbial C:nutrient activities of controls and treatments combined. The ratio of log(BGL):log(PMN) averaged 0.18 ± 0.03 (se); the corresponding log(BGL):log(PHOS) averaged 0.38 ± 0.07. The ratio of log(PPO+POX):

log(PMN) averaged 2.09 ± 0.24; the corresponding log(PPO+POX):log(PHOS) averaged 13.4 ± 3.76. By 12 weeks, hydrolase C activity has declined in all amended soils, with the greatest decline found in green waste amended soils. At the same time, oxidase C activity has increased in green waste amended soils but oxidase C activity remained about the same in compost or biochar amended soils. Correspondingly, the ratio of log(BGL):log(PMN) averaged 0.11 ± 0.03(se); the log(BGL):log(PHOS) averaged 0.24 ± 0.06. The ratio of log(PPO+POX):log(PMN) averaged 4.47 ± 1.13; the corresponding log(PPO+POX):log(PHOS) averaged 12.8 ± 5.70.

*3.5 Relationships between functional stoichiometry, resource stoichiometry and mineral nutrients*

The ratios of log(BGL):log(PMN) at both sampling times were negatively correlated with soil C:N ratios and the ratios of log(BGL):log(PHOS) were negatively correlated with soil C:P ratios at both sampling times (Table 1B). There were also some weaker positive correlations between functional C:nutrient ratios to soil C:nutrient ratios when oxidase C activity (PPO+POX) was examined instead of hydrolase C activity (BGL). Functional C:nutrient ratios correlated only with input C:nutrient ratio for the ratios of log(PPO+POX):log(PMN) at 4 weeks.

Based on RDA, the variations in microbial activity among the treatments were largely due to differences in PMN and PPO activity at 4 and 12 weeks, in both soil types (Supplementary Fig. 1B). At both times, soils amended with green waste were generally characterised by higher PMN and lower PPO activity as compared to unamended soil or soil amended with compost or biochar, which were generally characterised by higher levels of PPO and lower PMN (Supplementary Fig 1B). RDA identified soil $NO_3^-$, soil $NH_4^+$, initial C

content, soil C:P ratio and soil type as important for explaining 46.9% of total variance in microbial activity among treatments (Supplementary Fig. 1B). Although the addition of all of the organic amendments to both soils generally resulted in increase in available nutrients, the increase due to compost was generally high and sustained over time compared to green waste and biochar in both the Werribee and Cranbourne soils (see supplementary table S1).

## 4. DISCUSSION

In this study, we explored the utility of ecological and functional stoichiometry theories for understanding soil microbial transformation of organic amendments. We also examined key relationships between soil microbial community composition, biomass and activity with resource elemental composition and nutrient availability. We found that the addition of organic amendment lead to a distinct microbial composition with similar constant ratios of C:N:P activities. We observed generally consistent negative correlations between the functional ratio of log(BGL):log(nutrient) and soil C:nutrient, and inconsistent weak positive correlations between the functional ratio of log(PPO+POX):log(nutrient) and soil C:nutrient at both sampling times. The constancy of the functional stoichiometry indicates that microbial composition can be different but there is a limit to their response. We found that functional stoichiometry correlates well with soil stoichiometry and poorly with input stoichiometry. These findings were further supported with results of a redundancy analysis that identified variations in initial C content, mineral N, ratios of soil C:nutrient and site as drivers of variations in microbial community composition and activity under different organic amendments.

*4.1 Relationship between microbial community composition, structure and resource stoichiometry*

By analysing PLFA profiles of the treated soils, we found that microbial community composition and structure were distinct with different organic amendments and these variations in composition and structure with organic amendment persisted over time. The bacteria:fungal ratio was positively correlated to input C:nutrient ratio at 4 weeks and negatively correlated to soil C:N ratio at 12 weeks, suggesting a turnover in the microbial community composition as the resource base changed. For example, there were shifts in the bacteria:fungal ratio in the compost amended soils from 4 to 12 weeks but this shift in bacteria:fungal ratio occurred in opposite directions for Cranbourne and Werribee soils. It is possible that these shifts in relative abundances are associated with competitive interactions between the different microbial guilds present in each soil (Fontaine et al., 2003; Moorhead and Sinsabaugh, 2006). It is also important to consider the changes in microbial community composition due to inclusion of microbes originating from the organic amendment. Based on NMDS analysis, we found that the PLFA composition of the compost or raw green waste amended soils clusters with the PLFA composition of the original input, particularly at 4 weeks (data not shown). Additionally, the negative relationship between bacteria:fungal ratio and soil C:N ratio at 12 weeks generally agrees with previous findings that soils with higher C:N ratio tend to become increasingly dominated by fungi (Fierer et al., 2009; Waring et al., 2013). As bacteria generally require more N per unit biomass and have lower carbon use efficiency than fungi at higher resource C:nutrient stoichiometry, stoichiometric and physiological constraints may favour fungi over bacteria in soils with higher C:N (De Deyn et al., 2008; Keiblinger et al., 2010). Given that we have not measured microbial elemental C content, this remains to be confirmed.

Interestingly, we found that resource stoichiometry better accounted for variations in soil microbial community composition than soil microbial activities. Based on the RDA, ratios of soil C:N and soil C:P were important variables in explaining the variations in soil microbial community composition as shown by the length of the vector (see supplementary data). These ratios of soil C:nutrient act on different axes and are associated with the change in microbial composition from 4 to 12 weeks. Resource quality is proposed to affect microbial element use efficiency (Manzoni et al., 2012; Mooshammer et al., 2014), and our results suggest the microbial community element use efficiency is flexible in response to resource dynamics due to shifts in microbial community composition. These results support Fanin and colleagues' (2013) hypothesis that the changes in resource ratio leads to changes in community composition rather than changes in stoichiometry of the same community. This view on the role of resource competition in the organisation of soil microbial community is reminiscent of Hutchinson's (1961) paradox of the plankton and Tilman's (1982) resource competition and resource-ratio theory, which describe how it is possible for many competing species to co-exist. On the other hand, the ratio of soil C:P or soil C:N explained less than one percent of the overall variations in soil microbial activities (data not shown). Additionally, its importance relative to the other explanatory variables (initial C content and mineral N) was lower as seen in the length of the vector (see supplementary data). It is likely that microbial activities are more dynamic and reflects the immediate conditions while microbial composition reflects longer term soil organic matter dynamics (Grandy et al., 2007).

*4.2 Relating functional stoichiometry to resource stoichiometry*

Our measures of microbial C, N and P activities showed similar relationships where the increase in one is generally correlated with the increase in the other. Such relationships

were strong between hydrolase BGL with N and P activities but inconsistent and weaker in the oxidases PPO and POX with N and P activities. The positive correlations observed between the functional ratios of log(PPO+POX):log(nutrient) and soil C:nutrient, and the negative correlations between functional ratio of log(BGL):log(nutrient) and soil C:nutrient indicate that microbes respond to differences in resource quality by shifting their activities accordingly. This coupling of microbial activities makes sense as enzyme production is energetically costly and mechanisms to synchronise optimal enzyme activity is crucial to minimise entropy. These results also indicate that there is great capacity to response to the different organic amendments in both soils, but this response is constrained by stoichiometry, as proposed by Sinsabaugh and colleagues (2009; Sinsabaugh et al., 2008). Based on these results and our findings in Ng et al. (2014), measures of microbial activity may serve to extend functional stoichiometry from nutrient availability to carbon composition as proposed by Sinsabaugh and Shah (2011). Previous studies have found oxidases PPO and POX to be more variable compared to hydrolase BGL (Sinsabaugh et al., 2008; Sinsabaugh and Shah, 2011). Although we found that PPO and POX activity inconsistently associate with that of other hydrolases, it is possible that the sequential breakdown of more recalcitrant C sources by oxidases followed by increased hydrolysis of more labile products leads to a temporal coupling of microbial oxidative and hydrolytic enzyme activities (Moorhead et al., 2013a; Šnajdr et al., 2011). This is a subject for further investigation.

We had expected functional stoichiometry to relate better to the input stoichiometry initially and, as the organic amendment decomposed, that functional stoichiometry would correlate better with soil stoichiometry, reflecting the change in resource for the soil microbial community. However, we found that the functional stoichiometry was more consistently correlated with the stoichiometry of the soil (i.e. soil C: nutrient ratios) than with the input stoichiometry, at both 4 weeks and 12 weeks after addition of the organic

amendment. As functional stoichiometry is a function of resource quality and the elemental requirements of the microbial biomass, these results suggest on one hand that the organic input was not necessarily the primary resource for the soil microbial community 4 weeks after input application. Microbial necromass has been found to be a substantial resource at any given time during litter decomposition (Kaiser et al., 2014). On the other hand, it could also indicate that the elemental requirements of the microbial biomass had changed, which is supported by the observed changes in the microbial community composition.

It has been proposed that that the C storage potential of a system depends on its ability to sequester more nutrients (Fontaine et al., 2004; Kirkby et al., 2011). This is supported by our observation that the amounts of C, N and P in the soils were highly correlated. As the stoichiometry indicates no saturation in the nutrient cycling in this experiment (i.e. there was no apparent levelling off in terms of functional or soil C: nutrient ratios), there is the possibility to further increase C, N and P cycling through increasing the quantity and quality of organic amendments added to the soil (see Ng et al. 2014).

*4.3 Soil microbial biomass dynamics*

The addition of chemically different organic amendments resulted in clear differences to the growth dynamics (measured as total PLFA) of the soil microbial community. Microbial biomass rapidly increased in both soils receiving input of raw green waste, but the population was not sustained over the 12 week incubation period. In contrast, compost amended soil showed a lower, but sustained, increase in total microbial biomass over the 12 weeks, whilst biochar actually inhibited growth in the Werribee soil at 4 weeks. The fact that no correlation was observed between the input stoichiometry and the overall microbial biomass indicates that the size of the microbial biomass is not strongly dependent on the stoichiometry of the

input at 4 or 12 weeks after the incorporation of the organic inputs. It is proposed that microbial growth is limited by C:N ratio of the bioavailable dissolved organic matter, which is derived from a mixture of complex plant-derived and microbial-biomass derived pools (Kaiser et al., 2014). In this study, we found a good correlation between microbial biomass and $NH_4^+$ or $NO_3^-$, which indicated the influence of available nutrients from the overall input-soil mix on microbial biomass.

*4.4 Study limitations and considerations for future work*

Although this study demonstrates the potential for stoichiometry theories to contribute to understanding organic matter dynamics, limitations to both this study and the framework are apparent. First, redundancy analysis indicated that as much as 47% of microbial community composition and activity can be explained by variation in the initial C content, mineral N ($NO_3^-$, $NH_4^+$), site (or soil type) and ratios of soil C:nutrient. However, unmeasured properties of the organic amendment and/or the environment still account for another 50% of the variation. In Ng *et al.* (2014), we also found that carbon composition of the organic amendment and subsequent changes in bulk soil carbon composition due to the organic input are also important drivers of microbial community composition and activity. As shown previously, management practices not related to organic matter stoichiometry also account for a large proportion of the variation in microbial composition (De Vries et al., 2012; Kallenbach and Grandy, 2011) and may be even more dominant in other soils not explored here. Additionally, the spatial scale and time scale of our study is relatively narrow. Future work needs to integrate these aspects to improve predictions and determine their relative importance.

Second, variations in technical and statistical methodologies between published studies may give rise to slightly different or contrasting results, hindering a consistent interpretation. For example, Moorhead et al. (2013b) found that the dynamics of soil mineral nutrients provided little explanation for the dynamics of their system's microbial biomass or enzyme activities, whereas our study suggested the opposite, particularly with regard to $NH_4^+$. We used PMN as a proxy measure of microbial N activity rather than the fluorescence-based β-N-acetyl-glucosaminidase assay used by other authors including Moorhead et al (2013b). PMN is an integral measure of organic-N-cleaving enzymes over the period of one week, rather than an activity "snapshot", and probably contributed to the greater association we observed between microbial activity and nutrients. Our use of a multivariate approach, compared to the individual correlations used by Moorhead and colleagues may have further reinforced these differences. Finally, microbial biomass C was measured by fumigation extraction in Moorhead et al. while we used total PLFA as a measure of microbial biomass. PLFA turnover rate is determined by environmental conditions and therefore interpretation of microbial abundance is complex (Frostegård et al., 2011; Zelles et al., 1992). There is need for research to identify the best approaches and standards developed for ease of cross-study comparisons.

*Conclusions*

In conclusion, we observed that organic amendments can alter the soil microbial community size, composition and structure. Microbial community PLFA composition was distinct for each soil and organic amendment. Microbial activity was also strongly influenced by organic amendment. Furthermore, functional ratios of C:nutrient were generally constant,

indicating that microbial composition can be different but that there are stoichiometric limitations to their responses.

The results presented here highlight the potential for organic amendments to play a major role in sustainable biologically regulated nutrient supply systems, and the potential to monitor these changes using a stoichiometric approach. By linking functional stoichiometry to resource stoichiometry and soil microbial composition, we have gained new insights into key relationships and constraints between regulators of the transformation of organic matter in the soil. Much of the information used to develop enzyme-based models to date was gleaned from multiple studies and result in uncertainty about key relationships (Moorhead et al., 2013b). This study provides a form of "ground truthing" to examine the key relationships between soil microbial community composition, biomass and activity with resource elemental composition and nutrient availability. The results from our study provide indications that some key features of the soil system are robust and can be captured by cross-system analyses. Given the time and spatial scale of our study, similar studies with longer time scale and across larger spatial scales will confirm if this pattern is effectively transferred across hierarchies.


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## Table

Table 1. Kendall τ correlation of (A) soil C, N and P totals; (B) ratios of OA input C:nutrients, soil C:nutrient and C:nutrient activity; (C) ratios of bacteria:fungal ratio and total PLFA with ratios of OA input C:nutrient and soil C:nutrient; (D) microbial C activity (BGL or PPO+POX) in relation with microbial N activity (PMN) and microbial P activity (PHOS)

**Figures**

Fig. 1 Total soil C in relation with total soil N and total soil P for 4 (A) and 12 (B) weeks. Brown = Werribee soil, grey = Cranbourne soil. Units are in g/ 100g. Regression plane serves only for illustration of the relationship among the C,N and P. Correlation between total soil C, total soil N and total soil P analysed using Kendall τ (table 1A).

Fig. 2(A) Total PLFA and (B) bacteria to fungal biomass (mean ± se) for 4 weeks. (C) Total PLFA and (D) bacteria to fungal biomass (mean ± se) for 12 weeks. Numbers in bracket refer to bacteria:fungal ratio for each treatment in (B) and (D). . Significant levels are *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (refer to supplementary table S3 for test statistics).See table 1C for correlation between bacteria:fungal ratio and total PLFA with edaphic properties. Samples refer to We = Werribee unamended soil, Cr = Cranbourne unamended soil, WeGw = Werribee soil + green waste, CrGw = Cranbourne soil + green waste, WeCo = Werribee soil + compost, CrCo = Cranbourne soil + compost, WeCh = Werribee soil + biochar, CrCh = Cranbourne soil + biochar.

Fig. 3 NMDS ordination plot based on (A) microbial PLFA composition and (B) microbial activity. Samples refer to 1 = We, Werribee unamended soil, 2 = Cr, Cranbourne unamended soil, 3 = WeGw, Werribee soil + green waste, 4 = CrGw, Cranbourne soil + green waste, 5 = WeCo, Werribee soil + compost, 6 = CrCo, Cranbourne soil + compost, 7 = WeCh, Werribee soil + biochar, 8 = CrCh, Cranbourne soil + biochar.

Fig. 4 Microbial C activity for hydrolase BGL or oxidase PPO+POX in relation with microbial N activity (PMN) and microbial P activity (PHOS) for 4 and 12 weeks. Correlation between microbial C,N and P activities analysed using Kendall τ (table 1D). See supplementary Fig. S3 for 3D plot equivalents.