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

Ee Ling Ng [a, b, *], Antonio Frank Patti [b], Michael Timothy Rose [a, b], Cassandra Rae Schefe [c, 1], Kevin Wilkinson [c], Timothy Richard Cavagnaro [a, d]

[a] School of Biological Sciences, Monash University, Victoria 3800, Australia
[b] School of Chemistry, Monash University, Victoria 3800, Australia
[c] Department of Environment and Primary Industries, Victoria, Australia
[d] School of Agriculture, Food and Wine, University of Adelaide Waite Campus, Urrbrae, South Australia 5064, Australia

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.

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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 (Lehmann, 2007; Lal, 2011). 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 (α-diversity) to landscape (γ-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. Schmidt et al., 2011; Dungait et al., 2012). 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 (Güsewell and Gessner, 2009; Kallenbach and Grandy, 2011; Fanin et al., 2013; Bowles et al., 2014). 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 (Sterner and Elser, 2002; Cleveland and Liptzin, 2007). 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 (Sinsabaugh et al., 2008; Allen and Gillooly, 2009; Sinsabaugh et al., 2009). Past studies have generally examined functional stoichiometry at landscape and global scales (Sinsabaugh et al., 2008, 2009; 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, 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—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 and 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