Geoderma 315 (2018) 96–103

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Relationship between microbial composition and substrate use efficiency in a tropical soil

Mark T.L. Bonner[a,*], Luke P. Shoo[b], Richard Brackin[a], Susanne Schmidt[a]

[a] School of Agriculture and Food Sciences, University of Queensland, QLD 4072, Australia
[b] School of Biological Sciences, University of Queensland, QLD 4072, Australia

ARTICLE INFO

Handling Editor: Jan Willem Van Groenigen

Keywords:
Fungi
Bacteria
Carbon sequestration
Selective inhibitors
Microcosms
Litter decomposition

ABSTRACT

Soil organic matter (SOM) is crucial to soil health, supporting most of the soil properties that benefit plant growth and ecosystem services including carbon sequestration, nutrient recycling and water infiltration. Recent study is exposing the soil microbial community as not only decomposing SOM, but also providing the primary source of chemicals for its formation. All else equal, SOM formation is theoretically greatest when microbes maximise enzyme efficiency (ratio of enzyme activity to carbon loss from respiration) and biomass efficiency (biomass gain per unit substrate added). Our study examines the relationship between microbial composition and metrics of these two efficiencies. We hypothesised that both will increase with higher ratios of soil fungi to bacteria. We manipulated microbial composition through sustained use of selective microbial inhibitors in microcosms with tropical soil, alongside variation of litter quality and diversity and the presence or absence of a simulated root exudate. Both litter and inhibitor treatments significantly changed soil microbial composition and function, and enzyme efficiency and biomass efficiency were both higher in microbial communities with more fungi, supporting our hypothesis. Structural equation modelling suggested that the observed efficiency changes did indeed occur in part *via* changes in microbial composition after accounting for direct effects of treatments. Taken together the results provide some support for the hypothesis that soil fungi benefit SOM formation.

1. Introduction

Soil organic matter (SOM) is of global significance as a terrestrial carbon store, containing ~1500 Gt of organic carbon (compared with ~600 Gt in vegetation). SOM is declining in many soils subjected to agricultural use (Wei et al., 2014) which contributes to greenhouse gas emissions and jeopardises soil function. Of particular concern are highly weathered tropical soils where SOM is responsible for much of the soils' physical, chemical and biological integrity (Raich, 1983). Despite its importance, mechanistic understanding of how SOM accumulates remains contentious, hindering progress on devising effective measures for re-introducing SOM into degraded soils.

The case is increasingly clear that soil microbial biomass and exudates form the dominant precursors of SOM (Cotrufo et al., 2013; Grandy and Neff, 2008; Kallenbach et al., 2016; Miltner et al., 2012, 2009). There is an emerging view that microbial ecology and SOM turnover are tightly linked (Lehmann and Kleber, 2015) and that stronger emphasis ought to be placed on carbon (C) fluxes compared with stocks (de Vries and Caruso, 2016). This has given rise to well-founded models of SOM formation such as the Microbial Efficiency-Matrix Stabilisation (MEMS) framework and its extension (Castellano et al., 2015; Cotrufo et al., 2013), which predict higher SOM formation potential with i) higher microbial biomass generated per substrate input and ii) higher microbial efficiency of substrate turnover. Substrate type is thus likely to have a strong and direct influence on SOM formation potential due to differential uptake and cycling efficiencies (Cotrufo et al., 2013). Another, more poorly characterised determinant of substrate use efficiency is microbial community composition. If certain microbial species are intrinsically more efficient at converting substrate into microbial biomass and residues, SOM formation may vary considerably even with the same substrate.

As decomposers, heterotrophic soil bacteria and fungi functionally overlap, with the majority of species synthesising a wide range of enzymes for catalysing hydrolysis and oxidation of leaf and root litter, root exudates, and senesced microbes and animals (de Vries and Caruso, 2016). This process releases low molecular weight molecules used in turn by soil microbes and plants for energy gain and nutrition. Multicellular soil fungi are likely to be more efficient than most bacteria at attaining energy and nutrition in the heterogeneous soil environment because mycelial growth can ameliorate poor localised stoichiometry

* Corresponding author.
E-mail address: mark.bonner@uqconnect.edu.au (M.T.L. Bonner).

https://doi.org/10.1016/j.geoderma.2017.11.026
Received 9 October 2017; Received in revised form 13 November 2017; Accepted 20 November 2017

resulting from the spatial separation of water, nutrients and carbon, e.g. between rhizosphere, O-horizon and bulk soil (Guhr et al., 2015; Holland and Coleman, 1987; Šnajdr et al., 2011; Strickland and Rousk, 2010). Conceptually, mycelial growth can be hypothesised to increase the trophic return on *exo*-enzyme investment, considering that decay products of exo-enzyme exudation are more likely to return to the synthesising individual that enmeshes a target substrate in a mycelium (multicellular fungus) than to one that is merely adjacent to a target substrate (unicellular bacterium). In addition, higher C to nutrient (N, P, S) ratios in fungal biomass compared with bacterial biomass (Kirkby et al., 2011) potentially enable fungi to accumulate more biomass with the same nutrient supply. There is thus a mechanistic basis for hypothesising that the broad microbial compositional metric of fungi:-bacteria biomass ratio (F/B) will correlate with whole community function, perhaps in a manner that increases SOM accumulation potential, due to potentially higher C-use efficiency, more chemically recalcitrant necromass and stronger promotion of soil aggregation (Six et al., 2006; Strickland and Rousk, 2010), but currently this contention has limited empirical support (Strickland and Rousk, 2010; Thiet et al., 2006).

The tropics account for ≈ 40% of ice-free landmass and tropical soils may be particularly vulnerable to SOM loss due to weathered, low activity clays and high SOM turnover (Don et al., 2011; Feller and Beare, 1997; Sanchez et al., 1992). Stabilisation of C in tropical soils may be especially dependent on microbial function and composition due to fewer environmental limitations to decomposition (Six et al., 2002), but manipulative microbial experiments on tropical soils are comparatively rare. The present experiment examines microbial drivers of soil C dynamics with treatments that are relevant to the tropical context.

Soil microcosms provide a powerful means to test hypotheses with manipulative treatments, but are nevertheless a compromise for studying microbial C cycling, perhaps most significantly due to the absence of a rhizosphere and associated root exudates that have the potential to dramatically change C cycling (Fontaine et al., 2011; Keiluweit et al., 2015). We hypothesised that higher F/B would be associated with i) higher microbial biomass formation per substrate input, and ii) higher microbial efficiency of substrate turnover (enzyme efficiency). We manipulated the ratio of fungi to bacteria through sustained use of selective inhibitors in soil microcosms, alongside variation of litter quality and diversity and the presence or absence of a simulated root exudate. Previously, inhibitors have typically been used over short periods (i.e. less than a day) to estimate fungal and bacterial contributions to respiration following the addition of glucose (Anderson and Domsch, 1973; Bååth and Anderson, 2003). We follow methodological work by Badalucco et al. (1994) who found complete and targeted inhibition by these biocides no longer occurs when they are used over time periods longer than two days due to partial microbial acclimation; we aim instead for microbial compositional shift over time. A novel aspect of our experiment is therefore the sustained application of selective microbial inhibitors (broad spectrum antibiotics that act selectively on prokaryotes or eukaryotes, respectively) over a medium-term incubation of 27 days to manipulate the soil fungal/bacterial ratio.

2. Materials and methods

2.1. Experimental set-up

The experimental soil, a clay-rich (49%) acidic (pH 4.3) Rhodic Ferralsol (11% SOC, 0.8% nitrogen, 9 mg kg^{-1} Colwell-extractable phosphorus), was collected from the Atherton tableland at the Thiaki Creek Restoration Experiment site in north Queensland, Australia (S17.4302, E145.5140). The soil was chosen as representative of acidic soils (pH ranging from 3.5 to 5.5) that are typical of the humid tropics (Sanchez et al., 1992), and for its high organic matter content and thereby high microbial biomass (Fierer et al., 2009). Between 36 and 40 g of soil was placed unsieved into microcosms constructed out of 50 mL centrifuge tubes (Inselsbacher et al., 2009) and incubated at 27 °C and 90% air humidity, in the dark (Clayson Incubator, Clayson Laboratory Apparatus Pty Ltd., Narangba, QLD, Australia). After four days of acclimation at 60% water holding capacity (maintained for the duration of the experiment), microcosms were subjected to a three-stratum experimental design (Table 1) involving two inhibitor treatments (fungal inhibitor cycloheximide, bacterial inhibitor streptomycin, no inhibitor control), three litter treatments (pasture grass *Brachiaria decumbens* Stapf, *Eucalyptus grandis* Hill ex Maiden, rainforest litter from remnant forest within the study area), and simulated exudate treatment (dissolved sucrose addition, no-sucrose added control), with six replicates of each treatment combination. The inhibitor compounds cycloheximide and streptomycin were acquired from Sigma Aldrich Co., Australia (product codes C7698 and S6501 respectively). Respiration rates were monitored in the microcosms over 27 days. This period was chosen to minimise the influence of an artificial soil environment without a rhizosphere while providing sufficient time for microbial communities to recompose. The litter treatments provided tropically relevant contrasts of chemical recalcitrance (pasture grass compared with *Eucalyptus* and mixed rainforest litters) and diversity (mixed rainforest litter compared with grass or *Eucalyptus*). *Eucalyptus* plantations, pasture and rainforest are widespread and often competing land uses in the humid tropics (Chen et al., 2013; Don et al., 2011).

Table 1
Design of experiment performed in this study.

Variable	Value	Levels
Exudate	None Sucrose	2
Litter	Pasture grass *Eucalyptus* Mixed rainforest	3
Inhibition	None Streptomycin Cycloheximide	3
	Total number of microcosms:	2 × 3 × 3 treatments × 6 replicates = 108

We calibrated the concentrations of the inhibitors (dissolved in distilled water) to deliver an initial dose of approximately 1 mg streptomycin (bacterial inhibitor) and 2 mg cycloheximide (fungal inhibitor) per gram of dry soil, a rate at which the inhibitors have been deemed effective (Anderson and Domsch, 1973), followed by ongoing daily additions amounting to circa 0.33 mg g^{-1} streptomycin and 0.66 mg g^{-1} cycloheximide to maintain inhibition throughout the experiment.

Litter treatments involved addition of 200 mg of pasture grass *Brachiaria decumbens* (42% C, 1.7% nitrogen), *Eucalyptus grandis* (50% C, 1.3% nitrogen) or mixed rainforest litter (49% C, 1.4% nitrogen) to the soil surface of each microcosm at the start of the experiment, which is equivalent to 2.7 t ha^{-1}, or roughly four months of rainforest litterfall (Parsons et al., 2014). Prior to addition, the litter was oven-dried at 55 °C for 72 h and homogenised to 1–2 mm fragments (Retsch ZM200 centrifugal mill). The quantity of added litter was standardised by total dry mass in order to balance differences in added C, nitrogen and litter volume. The pasture grass and mixed rainforest litter were collected from within the study area from long-term pasture and remnant rainforest. Grass litter was cut from living grass, whereas rainforest litter showing minimal signs of oxidation and decomposition was collected from the forest floor. *Eucalyptus* litter was gathered from beneath a solitary tree growing on the St Lucia campus of The University of Queensland (S27.4986, E153.0125) in the same manner as the rainforest litter. C/N ratios for litter treatments were 24.5, 38.9, 34.7 for grass, *Eucalyptus* and mixed rainforest respectively (Flash 2000 Organic Elemental Analyser, Thermo-Fisher Scientific, Scoresby, VIC, Australia). Lignin content of leaf litters was not assessed for this study, but