Can we enhance soil carbon sequestration with melanised root-associated fungi?

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Abstract
Maintaining or increase levels of soil organic carbon (SOC) is necessary for ensuring ongoing agricultural productivity. Longevity of SOC is dependent on (1) the composition of carbon molecules within the SOC pool, and (2) protection of SOC from degradation through chemical and/or physical protection (e.g. physical protection of SOC from aerobic degradation within micro-aggregates). Melanised root-associated fungi (MRAF) may increase and stabilise SOC through: (1) the transformation of less stable carbon compounds (e.g. polysaccharides, plant-based carbon) to more stable carbon compounds (e.g. chitin, melanin), and (2) the formation and stabilisation of soil aggregates, and resultantly stabilisation of SOC within aggregates. The aim of this experiment was to test whether MRAF increase SOC within a carbon-poor soil. A glasshouse, pot experiment was set up with *Gossypium hirsutum* (Sicot 74683F) as the host. Pots were inoculated with one of two Control treatments (sterilised millet or wheat seed), or one of four fungal isolates (48B, 274A, C004.2 of NV016.17). Total SOC and SOC within each aggregate fraction was not significantly different between treatments; and total SOC didn't significantly change over time. Whilst total SOC remained unchanged, the proportion of SOC within the micro-aggregate fraction was significantly greater 75 days after seedling germination compared with initial soil. Changes in the location of SOC suggest the presence of an active microbial community, transforming and stabilising pre-existing carbon compounds, throughout the experimental period. Variation in SOC between pots may be due to variation in the growth, colonisation and activity of target and non-target microorganisms. Understanding the growth and activity of the soil microbial community may enable better SOC management strategies to be developed.

Keywords
Aggregate size, root-endophytes, climate-change, cotton.

Introduction
The loss of soil organic carbon (SOC) due to intensive agricultural practices is notable. Within the temperate zone, up to 60% of the SOC pool has been lost due to increased agricultural land use; in the tropics, depletion of the SOC pool may be 75% (Lal 2004). Within the Australian cotton industry, a comparison of SOC between native vegetation and a cotton field with a 25 year history of cotton-cotton-wheat rotations suggested SOC losses of approximately 60% (Cattle and Field 2013). A reduction in SOC not only has implications for climate change mitigation but also for continued agricultural productivity. Severe loss of SOC represents the degradation of soil quality (e.g. structure and nutrient availability) and, resultantly, a reduction in plant productivity (Lal 2004). Maintaining or improving levels of SOC is clearly a priority.

SOC levels are dependent on the balance between the addition and depletion of carbon. Strategies to increase SOC rely on increasing carbon inputs (e.g. stubble retention), and decreasing SOC loss resulting from either wind erosion, leaching and run-off, or microbial degradation. Rates of microbial degradation of SOC is controlled by two main factors: (1) the chemical structure of the carbon molecule itself, and (2) the physical or chemical around the carbon molecule (e.g. carbon located within soil aggregates are protected, to a degree, from aerobic degradation) (Jastrow et al. 2007). Highly stable SOC consists of complex carbon molecules which are located within soil aggregates.

Soil fungi help to stabilise SOC through: (1) the decomposition and incorporation of less stable carbon molecules (e.g. polysaccharides and plant-derived carbon) into hyphal biomass (containing complex carbon molecules like chitin and melanin), (2) the deposition of carbon into soil micro-aggregates via hyphal growth, and (3) the stabilisation of soil aggregates by hyphae and, potentially, mucilage secretion (Jastrow et al. 2007; Six et al. 2004). Addition, and stabilisation, of SOC may also occur through the activity of endophytic fungi, through their use of plant photosynthates for hyphal growth (Jastrow et al. 2007). Manipulation of composition and activity of the fungal community to favour SOC stabilisation may provide a solution for SOC management.

Melanised root-associated fungi (MRAF) are root-associated endophytic fungi, characterised by melanised hyphae. MRAF may increase the proportion of stable, long-lived SOC through one or both of two mechanisms: (1) the deposition of stable carbon molecules (melanin) within soil micro-aggregates, and (2) through the formation and stabilisation of soil aggregates. Previous work by Mukasa Mugerwa and McGee (2016) demonstrated that some MRAF have the capacity to increase total SOC and micro-aggregate associated SOC, in a carbon-rich Alfisol. The aim of this experiment was to test whether MRAF increase SOC within a carbon-poor (<1%) soil.

Methods

Root-associated fungi were collected from the Sydney Basin (Mukasa Mugerwa et al. 2013) and from the Australian Cotton Research Institute (ACRI: Narrabri NSW) according to the methods outlined by Mukasa Mugerwa et al. (2013). Isolated fungi with darkly coloured hyphae were considered to be MRAF. Four fungal isolates were selected for this study: two Sydney Basin MRAF isolates (274A & 48B), one ACRI MRAF isolate (C004.2), and one coloured but non-melanised root-associated fungi from ACRI (NV016.17). The two MRAF Sydney Basin isolates (274A & 48B) were obtained from Mukasa Mugerwa. Both isolates are known to increase soil carbon and aggregation in an Alfisol, with *Trifolium subterraneum* as a host plant (Mukasa Mugerwa et al. 2013; Mukasa Mugerwa and McGee 2016). Fungal inoculum was prepared using millet (C004.2 & 274A) or wheat seed (NV016.17 & 48B) (Mukasa Mugerwa et al. 2013).

Pots were set up using a modification of the split-pot system (Figure 1). The pots were filled with twice pasteurised, and sieved (<4mm), soil containing one of the 6 inoculum treatments (Control - millet, C004.2, 274A, Control - wheat, NV016.17 and 48B) (n=6). The fungal chambers were filled with un-inoculated, twice pasteurised soil. The soil used for this experiment was a 3:2 mixture of sand and field soil, obtained from ACRI. The initial carbon content of the soil was 0.3 %. The field soil at ACRI is a high shrink-swell medium grey clay overlying brown clay and is classified as a fine, thermic, montmorillonitic Typic Haplustert (Soil Survey Staff 2010). Three untreated cotton seeds (Sicot 74683F) were planted in the middle of each pot. After germination, seedlings were thinned to a single plant. Pots were watered with to saturation every 2-3 days.

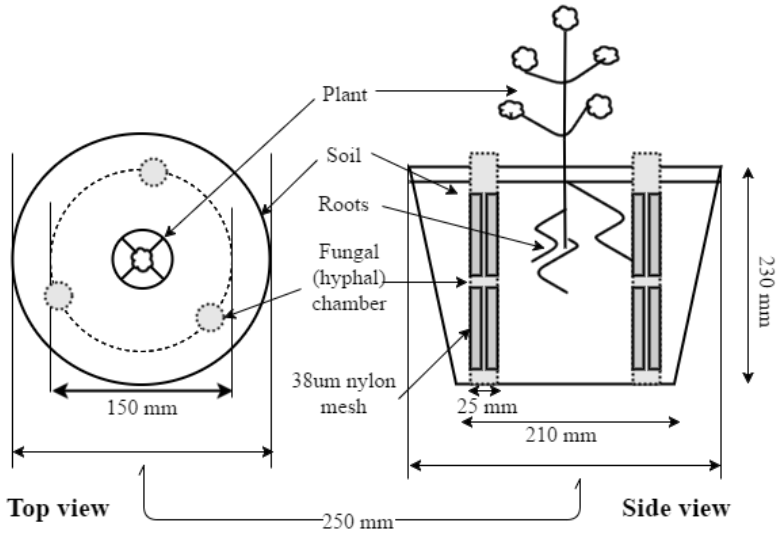

Figure 1. Schematic diagram of the set-up for each pot. Fungal inoculum containing different MRAF treatments were mixed into soil before potting. Hyphal chambers were filled with twice pasteurised soil containing no inoculum. The nylon mesh covering the hyphal chambers prevented plant roots from entering into the fungal chamber but allowed hyphae to penetrate.

Soil was harvested from one of the hyphal chambers 75 days after seedling germination. The soil was then dried at 40°C and two subsamples were subsequently taken from each sample. The first was analysed for total organic carbon (TOC). The second (approximately 25 g) was passed through a 4-mm sieve and separated into four different aggregate classes using a dry sieving technique (Zhang et al. 2013). The four aggregate classes were: large macro-aggregates (> 2 mm diameter), macro-aggregates (2 - 0.84 mm), small macro-aggregates (0.84 - 0.25 mm diameter) and micro-aggregates and silt and clay fractions (< 0.25 mm) (Zhang et al. 2013). The mean weight diameter (MWD) of each sample was calculated as described by (Mukasa Mugerwa and McGee 2016). Soil from each of the aggregate classes was then analysed for TOC.

Differences in soil aggregation, total SOC, and organic carbon within each soil fractions, between each of the treatments was compared using a one-way ANOVA. Pairwise comparisons were made using Tukey's HSD. All statistical analyses were undertaken using R (R Core Team 2014).

Results and Discussion

Total TOC did not significantly change over time or between treatments (Table 1). Total TOC, and TOC in each of the soil fractions, did not vary between fungal treatments (Table 1). However, micro-aggregate TOC in all of the fungal treatments, except C004.2, was significantly higher 75 days after seedling germination compared to initial micro-aggregate TOC (Table 1 and Figure 2). That is, that that a balance in total SOC was maintained over time in all treatments, but that more SOC was found in micro-aggregates than in the macro-aggregate fractions (Table 1 and Figure 2). This increase in micro-aggregate SOC is likely to have resulted from the transformation and stabilisation of carbon compounds by microbial activity (Jastrow et al. 2007). The increase in micro-aggregate SOC, over time, suggests an increasing complexity of carbon compounds and increased stability of SOC within this system over time.

Table 1. Average soil organic carbon (%) in each of the soil fractions obtained by dry-sieving of soil from fungal chambers and Mean Weight Diameter (MWD) (mm) of soil. Samples were taken prior to inoculation (Initial) and 75 days after cotton germination (all other treatments) (n=6). Different subscript letters indicate significant (p<0.05) differences between treatments.

Treatment	Soil Organic Carbon (%)					MWD (mm)
	Total	> 2 mm fraction	2 - 0.84 mm fraction	0.84 - 0.25 mm fraction	< 0.25 mm fraction	
Initial	0.31 ±0.02	0.70 ± 0.05 $_{a}$	0.38 ± 0.01	0.24 ± 0.01 $_{a}$	0.48 ± 0.01 $_{a}$	0.469 ± 0.017
Control (millet)	0.36 ± 0.01	0.41 ± 0.01 $_{b}$	0.32 ± 0.02	0.27 ± 0.00 $_{b}$	0.70 ± 0.01 $_{b}$	0.580 ± 0.175
274A	0.33 ± 0.01	0.44 ± 0.05 $_{b}$	0.34 ± 0.02	0.27 ± 0.01 $_{ab}$	0.69 ± 0.01 $_{b}$	0.572 ± 0.098
C004.2	0.35 ± 0.01	0.47 ± 0.02 $_{b}$	0.35 ± 0.02	0.27 ± 0.00 $_{b}$	0.69 ± 0.01 $_{b}$	0.634 ± 0.132
Control (wheat)	0.35 ± 0.01	0.50 ± 0.05 $_{b}$	0.34 ± 0.02	0.27 ± 0.00 $_{b}$	0.68 ± 0.01 $_{b}$	0.515 ± 0.106
48B	0.36 ± 0.01	0.42 ± 0.02 $_{b}$	0.31 ± 0.02	0.26 ± 0.01 $_{ab}$	0.68 ± 0.02 $_{b}$	0.545 ± 0.099
NV016.17	0.40 ± 0.05	0.43 ± 0.02 $_{b}$	0.33 ± 0.01	0.26 ± 0.01 $_{ab}$	0.70 ± 0.02 $_{b}$	0.592 ± 0.096

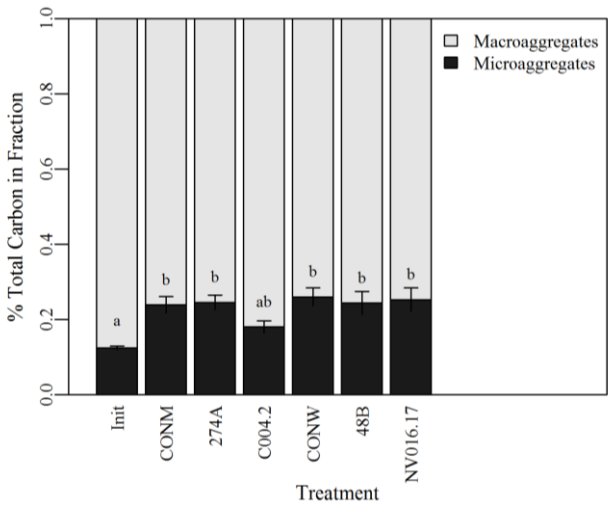

Figure 2. Percentage of total soil organic carbon stored in micro-aggregate (< 0.25 mm fraction) and macro-aggregate (> 0.25 mm fraction) fractions prior to inoculation (Init) and 75 days after cotton germination (all other treatments) (n=6). Different letters indicate significant (p < 0.05) differences between treatments.

The results of this experiment differed to those found by Mukasa Mugerwa and McGee (2016), who observed increases in total SOC by some Sydney Basin MRAF isolates, including 48B and 274A. The differences between these experiments, may result from differences in the experimental set up (e.g. the use of twice pasteurised vs autoclaved soil, differences in host plant, differences in experimental soil) (Mukasa Mugerwa and McGee 2016). Under these differing conditions, the colonisation, growth and activity of the fungi within the host plant and soil may vary. A better understanding the effect of varying environmental parameters on fungal growth and activity is needed if we are to better optimise SOC sequestration by native microbial communities.

The lack of differences between treatments in this experiment was unexpected, especially given the increases in micro-aggregate SOC across all treatments. Transformation, movement and stabilisation of SOC between the different soil aggregate fractions is primarily driven by microbial activity (Jastrow et al. 2007). Whilst soil was twice pasteurised, the experiment was set up using an open-pot system. Colonisation of all pots, including control (Control - millet, and Control - wheat) treatments, by non-target fungi was likely to have occurred (a fact later confirmed by microscopic observation of soil samples taken from the fungal chambers of each pot). Furthermore, the growth, host-plant colonisation, and activity of fungi in soil can be highly variable. Variation in total SOC within treatments may be explained by the variability in the growth and activity of target and non-target organisms. A comparison between SOC with fungal growth might help to clarify the role of fungi on SOC dynamics.

Conclusion

The SOC pool is dynamic. Measures of total SOC, whilst providing a broad indicator of soil health, are unable to display changes in the composition and location of SOC. Measuring changes in the composition of SOC across aggregate fractions is likely to provide a more useful indicator of SOC longevity and long-term soil health. Changes within the SOC pool are partially mediated by microbial activity. A better understanding of the effects of changing environmental parameters on microbial growth might help us better understand SOC dynamics; and is likely to be a key step in developing and enhancing SOC management practices.

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