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# Development and stabilisation of soil structure via interactions between organic matter, arbuscular mycorrhizal fungi and plant roots

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#### 1. Introduction

#### ABSTRACT

A clearer understanding of the mechanisms that underpin the development and stabilisation of soil structure would enable a more predictable restoration of degraded soil. A hierarchical model of soil aggregation (HM) is posited that predicts soils to be self-organising systems, mediated via interactions and feedbacks between their mineral constituents, organic matter and biotic activity, which serve to create and stabilise soil structure. To determine the contribution of these latter constituents, combinations of organic matter (compost), living plant roots (three perennial species: two woody, one grass) and a community of arbuscular mycorrhizal (AM) fungi where added to a massive mine spoil in a controlled pot experiment. It was hypothesized that the absence of any of these three components would retard the development of stable soil structure, as assessed through the development of porosity, changes in bulk density, soil water retention characteristics and water-stable aggregation following a 6 month incubation period. The concentration and content of soil organic carbon (SOC), nitrogen, cation exchange capacity and pH were also determined. All three factors, organic matter, living plant roots and AM fungi were required for the development of stable soil structure, but in complex ways. Overall, the data indicate that in the presence of adequate organic matter, plant roots are key contributors to the development of soil structure which is further stabilized by AM fungi.

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Soils provide essential ecosystem services (Daily et al., 1997; Wall et al., 2012), many of which are related to the content of soil organic carbon (Schmidt et al., 2011). Past and present anthropogenic activities have degraded soils (Lal et al., 1989). Degradation arises when surface layers are eroded, organic carbon is lost from the soil or the structure of soil is compromised (e.g. Charman and Murphy, 2007). Various management practices aim to reverse soil degradation. For example, the use of cover crops and conservation tillage aim to reverse mild degradation (Lal, 2002), the installation of remedial earth works and land use changes aim to address moderate degradation (Tongway, 1990) and more intensive interventions aim to restore severely degraded soils, such as soils associated with open-cut mining (Bradshaw, 1987). Management practices have had variable success (Bradshaw, 1987), with unpredictable changes to soil structure and reserves of organic carbon (Govaerts et al., 2009), and as such, restoration of severely degraded soil remains problematic.

The structure of soil is long been known to be influenced by the soil physical, chemical and biological properties (e.g. Emerson, 1959a,b; Griffiths, 1965; Griffiths and Jones, 1965), and that these properties interact to form stable aggregates in soil (Edwards and Bremner, 1967). Soil aggregation, described using a hierarchical model (HM) has provided a framework to associate various aggregate sizes with differing binding agents (Tisdall and Oades, 1982; Oades, 1984). Experimentally the HM has been demonstrated through the physical liberation, and subsequent dispersion, of soil aggregates with incremental energy increases (Field et al., 2006). The smallest fraction size consists of mineral and organic particles  $<2 \mu m$  diameter that are held together by physico-chemical forces. These particles in turn are bonded by inorganic and organic materials into aggregates <20 µm diameter, which are highly persistent and resist ultrasonic dispersion (Field et al., 2006). Fine microaggregates are in turn bonded into



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increasingly persistent microaggregates <250  $\mu$ m but are more susceptible to ultrasonic dispersion (Edwards and Bremner, 1967; Field and Minasny, 1999). At the microaggregate scale the biological agents (Griffiths and Jones, 1965; Hayes, 2009) that may contribute to aggregate development include adhesive bonding by saprotrophic true (Caesar-Tonthat, 2002; Daynes et al., 2012) and AM fungi (Rillig et al., 2010), bacteria (Martin, 1946) and plant root exudates (Watt et al., 1993). Persistent microaggregates and organic debris are bound into less stable macroaggregates >250  $\mu$ m by more transient factors, such as enmeshment by fungal hyphae and the fine roots of plants (Miller and Jastrow, 1990).

The HM implies that soils become self-organizing systems (Crawford et al., 1997; Ritz and Young, 2004; Young and Crawford, 2004) through aggregating processes, provided that appropriate combinations of physical, chemical and biological properties are present (Feeney et al., 2006; Crawford et al., 2011). The HM can therefore be used as the basis for an experimental design to test the self-organising process through the development and stabilisation of soil structure, after the addition of selected chemical and biological materials. This should contribute to a mechanistic approach to predict a reliable means to restore severely degraded soils. To achieve this, mixtures of organic matter, AM fungi and growing plant roots were introduced into mine spoil that is known to have negligible organic matter, limited microbiota and lacks aggregation. We hypothesised that these additions would result in self-organising processes resulting in the development of stable aggregates that contribute to an improved soil structure. Soil structure was assessed by quantifying the changes in soil water retention characteristics, water-stability of aggregates, carbon concentration and total carbon, cation exchange capacity (CEC), pH, and plant biomass and height (Fig. 1). Furthermore, we hypothesised that the absence of any individual factor would retard selforganising processes reducing the development and/or stabilisation of soil structure.

#### 2. Materials and methods

#### 2.1. Experimental materials and design

Spoil (overburden) collected from the R horizon from an opencut coalmine at Mount Owen, New South Wales (NSW), Australia (Lat. -32.388, Lon 151.114) was utilised as the soil parent material (Table 1). The spoil (<710 µm diameter) was sterilised to remove any contaminant arbuscular mycorrhizal (AM) fungi by autoclaving 3 times for 2 h over a 72 h period, with cooling between each autoclave run. Indigenous soil microbes were collected by passing sterile water through fresh spoil. The spoil washings, which contained microbes, were inoculated to the autoclaved spoil.

'Complex' organic matter was provided in compost produced from bulk household garbage via the Bedminster Process (Tardy and Beck, 1996) at the SITA Environmental Solutions Advanced Resource Recovery Facility, Raymond Terrace, NSW, Australia. Compost (<2 mm) was sterilised and inoculated with indigenous compost microbes as above. Air-dry compost (0, 6, 12 or 18% w/w) was thoroughly mixed through the spoil. Within each compost treatment equal mass of spoil alone, or the compost spoil mixture, was added to each pot (Fig. 1), gently packed to the same density and watered. The surface of the soil was 2 cm below the lip at the start of the experiment.

Dodonaea viscosa (L.) Jacq. was germinated in water at 65 °C for 200 min, and then soaked overnight in water 22 °C (modified from Floyd, 1966). A single seedling each of *D. viscosa, Acacia decora* Rchb. (both perennial woody plants) and *Lolium perenne* L. (perennial grass) were sown in each planted pot. The eight isolates of AM fungi utilised in the study were isolated from a wide variety of highly disturbed sites in the Sydney basin and agricultural soils in central NSW, Australia. The AM fungi were cultured from single spores and identified using morphological criteria, and are held in the University of Sydney culture collection: *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe Isolate NBR 4.1 and BUR11A, *Glomus* sp. Isolate PP1, *Glomus* sp. Isolate PM1.2, *Glomus intraradices* N.C.

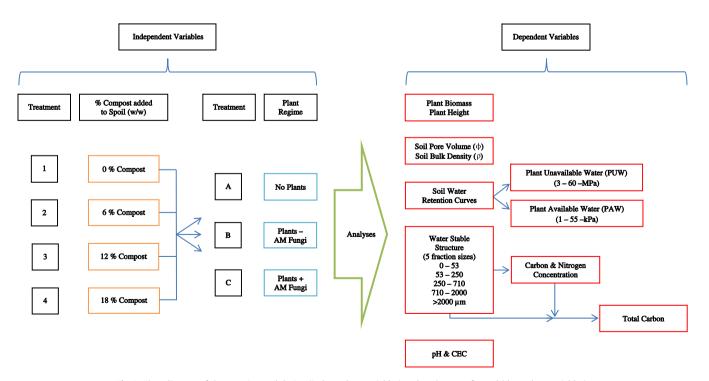


Fig. 1. Flow diagram of the experimental design (independent variables) and analyses performed (dependent variables).

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