



# Chemical, physical and microbial properties and microbial diversity in manufactured soils produced from co-composting green waste and biosolids

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## ABSTRACT

The effects of adding biosolids to a green waste feedstock (100% green waste, 25% v/v biosolids or 50% biosolids) on the properties of composted products were investigated. Following initial composting, 20% soil or 20% fly ash/river sand mix was added to the composts as would be carried out commercially to produce manufactured soil. Temperatures during composting reached 50 °C, or above, for 23 days when biosolids were included as a composting feedstock but temperatures barely reached 40 °C when green waste alone was composted. Addition of biosolids to the feedstock increased total N, EC, extractable NH<sub>4</sub>, NO<sub>3</sub> and P but lowered pH, macroporosity, water holding capacity, microbial biomass C and basal respiration in composts. Additions of soil or ash/sand to the composts greatly increased the available water holding capacity of the materials. Principal component analysis (PCA) of PCR-DGGE 16S rDNA amplicons separated bacterial communities according to addition of soil to the compost. For fungal ITS-RNA amplicons, PCA separated communities based on the addition of biosolids. Bacterial species richness and Shannon's diversity index were greatest for composts where soil had been added but for fungal communities these parameters were greatest in the treatments where 50% biosolids had been included. These results were interpreted in relation to soil having an inoculation effect and biosolids having an acidifying effect thereby favouring a fungal community.

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## 1. Introduction

In most cities in the developed world, green waste (also known as yard waste), which is composed of tree wood and bark, prunings from young trees and shrubs, dead and green leaves and grass clippings, is collected separately from other wastes. It is normally mechanically shredded and then composted, either alone or with other organic wastes. It is then used in products such as garden mulches and composts, organic soil amendments, soilless potting media, and particularly in Australia, manufactured “topsoils” for landscaping purposes. For the latter product, small quantities (e.g. 10–20% v/v) of inorganic additives (e.g. fly ash, sand, soil) are often blended with the compost and it is then used as a topsoil substitute. Little is known regarding the properties of these manufactured soil materials.

Decomposition of green waste during composting is characteristically slow and in order to initiate intense microbial activity, the addition of a more readily decomposable material (e.g. animal manure, grease trap waste) is required (Francou et al., 2008; Belyaeva and Haynes, 2010). Biosolids, which are also produced by municipalities in large quantities, may well be a suitable organic

material since there is, characteristically, intense microbial activity during its composting (Epstein, 2003). Indeed, in order to stabilise and sanitise biosolids, they are often composted before use. This normally involves blending dewatered biosolids with a bulking agent (added to give adequate aeration), and green waste would act as such an agent.

The composting process includes four phases: (i) an initial decomposition phase, (ii) a thermophilic phase of intense microbial decomposition, (iii) a second thermophilic phase, and (iv) a maturation phase. Rapidly multiplying thermophilic bacterial species dominate during the thermophilic phase but once the bulk of the easily decomposable substrate is exhausted, the majority of the remaining material is woody, lignin-dominated material plus stabilised humic material and fungi tend to dominate (Ryckeboer et al., 2003). Although succession of bacterial (Adams and Frostick, 2009; Partanen et al., 2010), fungal (Hansgate et al., 2005; Bonito et al., 2010) and archaeal (Yamamoto et al., 2011) communities during the composting phases for specific wastes has been investigated by a number of workers using molecular techniques (e.g. PCR-DGGE) very little is known about the comparative nature of the microbial communities in matured composts derived from different materials. Klammer et al. (2008) did, however, show that there were clear distinctions between bacterial communities present in matured composts made from biowastes versus sewage

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sludge. The addition of other materials such as fly ash and soil to the matured compost presumably has an inoculation-effect and this is likely to influence the microbial community present.

The aim of this study was to investigate composting intensity and the chemical, physical and microbial properties and bacterial and fungal diversity, in composts made from green waste alone or green waste plus 25% or 50% v/v biosolids. From a practical viewpoint, the 25% biosolids treatment represents greenwaste compost where biosolids have been added to increase composting intensity while 50% biosolids represents composted biosolids where green waste was the bulking agent. Following initial compost production, the products were amended with 20% topsoil, or 10% coal fly ash plus 10% river sand (to produce manufactured soil material) and allowed to mature.

## 2. Materials and methods

### 2.1. Materials and composting

Municipal green waste, originating from the Brisbane City Council, was collected from a pile at Phoenix Power Recyclers, Yatala, Queensland, soon after it had been mechanically shredded. It had a particle size distribution of: >5 mm = 55%, 2–5 mm = 26% and <2 mm = 19%. Recently-deposited fly ash was collected from the fly ash disposal lagoon at Tarong Power Station, 80 km west of Brisbane. Particle size distribution of the ash was: 100–200  $\mu\text{m}$  = 9%, 50–100  $\mu\text{m}$  = 36%, 2–50  $\mu\text{m}$  = 52% and <2  $\mu\text{m}$  = 3%. The fly ash was the same as that used previously (Belyaeva and Haynes, 2009) and has TCLP concentrations of Cu, Zn, Cr, Cd, Pb, and As well below USEPA standards (USEPA, 1992) and a hot water extractable B concentration of only 0.71  $\text{mg kg}^{-1}$ , well below potentially phytotoxic levels of about 30  $\text{mg kg}^{-1}$ . Biosolids were collected from the Oxley Creek Wastewater Treatment Plant (Brisbane). At this plant, the sewage sludge is treated by the Cambi process (155 °C at 4.5 bar) and digested anaerobically prior to dewatering. The A horizon of a soil was excavated from an unfertilized area under grassland vegetation. The soil had a silt loam texture and was classified as a Clastic Rudosol (Isbell, 2002) or in FAO classification as a Eutric Regosol. The soil was air-dried and sieved (<5 mm dia.) prior to use. River sand was sourced from a landscape supplies retailer.

The compost treatments were: (1) 100% green waste (GW), (2) 75% green waste/25% biosolids v/v (25B), and (3) 50% green waste plus 50% biosolids (50B). Two hundred litre samples of the mixtures were placed in 250 l plastic composting bins. The bins were wrapped with two layers of fibreglass insulation blankets (each 6 cm thick) to prevent excessive heat loss during composting (Belyaeva and Haynes, 2009). The experiment was replicated three times. Additional holes were cut in the bottom of bins to provide improved aeration and piles were turned every 7 days in order to ensure adequate  $\text{O}_2$  levels inside piles. Temperature was monitored at a depth of 40 cm inside the piles at 0900 h each day. The water content of piles was maintained at 60–70% of their water holding capacity throughout the experiment and water was added each week if necessary after piles were turned. After 8 weeks of composting each treatment replicate was split into three: (i) 100% compost (control), (ii) 80% compost plus 20% v/v soil (So), and (iii) 80% compost plus 10% river sand and 10% fly ash v/v/v (S/A). The resulting materials were thoroughly mixed and allowed to react and mature for a further 4-week period.

Ten subsamples were taken randomly from within each pile. Subsamples were bulked, homogenised and ground to pass a 5 mm sieve. A part of each sample was stored at 4 °C for microbial and physical analysis and the rest was air-dried and stored for chemical analysis.

### 2.2. Chemical analysis

Electrical conductivity and pH were analysed in a 1:5 (v/v) water extract using a glass electrode (Rayment and Higginson, 1992). Extractable mineral N was extracted with 2 M KCl (1:100 ratio for 1 h) followed by colorimetric analysis of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ -N using a Seal automated discrete analyser. Exchangeable bases were extracted with 1 M ammonium acetate (pH 7) and Ca, Mg, K, and Na in the extracts were analysed by atomic absorption spectrophotometry. Cation exchange capacity was determined with 1 M ammonium acetate at pH 7 (Rayment and Higginson, 1992). Available “Colwell” P was extracted with 0.5 M  $\text{NaHCO}_3$  (pH 8.5) (1:100 w/v for 16 h) (Rayment and Higginson, 1992) and measured colorimetrically by the molybdenum blue method. Organic C and total N content were measured by automated dry combustion using a Carlo Erba C, H, N analyser. Water soluble organic C was measured in an aqueous extract (1:10 w/v for 1 h), after filtration through a 0.22  $\mu\text{m}$  Millipore filter, using a Shimadzu 5000A soluble C/N analyser.

The total content of P, K, Ca, Mg and heavy metals in the green waste was determined after nitric-perchloric acid digestion and that of fly ash after HF/HCl/ $\text{H}_2\text{SO}_4$  digestion (CEM, 1993). Phosphorus in the digested extracts was determined colorimetrically and K, Ca, Mg, Na, Al, and Fe by ICP-AES.

### 2.3. Physical analysis

Bulk density was determined on naturally compacted samples, particle density by the pycnometer method (Blake and Hartge, 1986) and total porosity by difference. Soil water content in samples was determined at –10 and –1500 kPa using a pressure plate apparatus. Pore size distribution was calculated as macropores (>29  $\mu\text{m}$  diameter; air-filled porosity at –10 kPa), mesopores (0.2–29  $\mu\text{m}$  diameter; drained between –10 and –1500 kPa) and micropores (<0.2  $\mu\text{m}$  diameter; water filled pores at –1500 kPa).

### 2.4. Microbial analysis

Microbial biomass C was estimated based on the difference between organic C and N extracted with 0.5 M  $\text{K}_2\text{SO}_4$  from chloroform-fumigated and unfumigated soil samples using a  $K_c$  factor of 0.38 (Vance et al., 1987). Soluble C in the  $\text{K}_2\text{SO}_4$  extracts was analysed as described for the water extracts. Basal respiration was determined by placing 30 g oven dry equivalent of moist soil in a 50-ml beaker and incubating the sample in the dark for 10 days at 25 °C in a 1-l air-tight jar along with 10 ml 1 M NaOH. The  $\text{CO}_2$  evolved was determined by titration (Anderson, 1982). The metabolic quotient was calculated as basal respiration ( $\mu\text{g CO}_2\text{-C h}^{-1}$ ) expressed per mg of microbial biomass C.

Faecal coliforms and *Salmonella* spp. in composts were isolated and enumerated using the Most Probable Number (MPN) technique (USEPA, 1999) and results expressed as colony forming units (CFU) per gram dry compost.

In order to explain some of the changes in microbial diversity found, the fungal to bacterial ratio was estimated for the GW, GW25B and GW50B control samples. This was carried out by using selective inhibition of substrate induced respiration (SIR) by a modification of the technique of Anderson and Domsch (1975) as described by Bailey et al. (2003). Each compost (with glucose added at 10  $\text{mg g}^{-1}$  to stimulate respiration) was treated with (a) no antibiotics, (b) the fungicide captan, (c) the bactericide oxytetracycline hydrochloride and (d) both the fungicide and bactericide. Concentrations of added fungicide and bactericide (which were mixed with talc to aid mixing with the composts) were 1, 2, 4, 6, and 8  $\text{mg g}^{-1}$  soil. The inhibitor additivity ratio and fungal and

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