# The impact of biosolids application on organic carbon and carbon dioxide fluxes in soil

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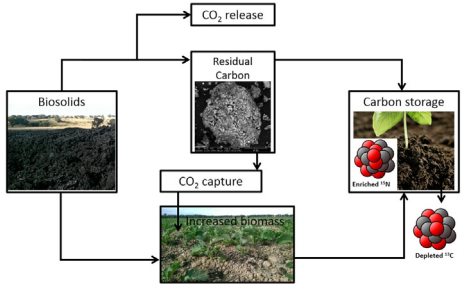
## HIGHLIGHTS

- Increased non-labile and labile soil organic carbon under biosolids amended soils.
- Depleted $\delta^{13}C$ in biosolids amended soils showed the residual carbon contribution to soils.
- Application of biosolids caused enriched $\delta^{15}N$ in soils.
- Enhanced $CO_2$ emission observed under biosolids land application.
- Storing biosolids carbon in soils for a longer period is a challenge.

## GRAPHICAL ABSTRACT

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## ABSTRACT

A field study was conducted on two texturally different soils to determine the influences of biosolids application on selected soil chemical properties and carbon dioxide fluxes. Two sites, located in Manildra (clay loam) and Grenfell (sandy loam), in Australia, were treated at a single level of 70 Mg $ha^{-1}$ biosolids. Soil samples were analyzed for SOC fractions, including total organic carbon (TOC), labile, and non-labile carbon contents. The natural abundances of soil $\delta^{13}C$ and $\delta^{15}N$ were measured as isotopic tracers to fingerprint carbon derived from biosolids. An automated soil respirometer was used to measure in-situ diurnal $CO_2$ fluxes, soil moisture, and temperature. Application of biosolids increased the surface (0 −15 cm) soil TOC by > 45% at both sites, which was attributed to the direct contribution from residual carbon in the biosolids and also from the increased biomass production. At both sites application of biosolids increased the non-labile carbon fraction that is stable against microbial decomposition, which indicated the soil carbon sequestration potential of biosolids. Soils amended with biosolids showed depleted $\delta^{13}C$, and enriched $\delta^{15}N$ indicating the accumulation of biosolids residual carbon in soils. The in-

Soil $\delta^{13}C$
Soil $\delta^{15}N$

situ respirometer data demonstrated enhanced $CO_2$ fluxes at the sites treated with biosolids, indicating limited carbon sequestration potential. However, addition of biosolids on both the clay loam and sandy loam soils found to be effective in building SOC than reducing it. Soil temperature and $CO_2$ fluxes, indicating that temperature was more important for microbial degradation of carbon in biosolids than soil moisture.



## 1. Introduction

Sustainable strategies for overcoming anthropogenic climate change are needed. Increasing carbon storage capacity (i.e., carbon sequestration) in soils can increase sustainability. For the first time, the 21st Conference of the Parties to the United Nations (UN) Framework Convention on Climate Change (COP21) held in 2015 emphasised the importance of soil organic carbon (SOC) in mitigating climate change. At this conference, the French Agriculture Ministry proposed idea of "4 per Thousand", which has the goal of enhancing SOC at the 0–40 cm depth in world soils at the rate of 0.4% per year (Four per thosand, 2015). This strategy identifies soil as a sink for atmospheric $CO_2$ through SOC sequestration which is promoted by using recommended carbon management practices (RCMP) including conservation agriculture, mulch farming, agroforestry, and addition of biochar, manure, compost, and biosolids (Lal, 2016). Globally, it has been suggested that, through the adaption of RCMP, the annual rate for SOC sequestration could be 0.4–1.2 gigatons of carbon or 5–15% of the global fossil-fuel emission (Lal, 2004). Among these practices, application of biosolids has increasingly been used to improve soil health and increase soil carbon sequestration (Brown et al., 2011; Cogger et al., 2013).

Biosolids are a stabilised organic solid derived from sewage treatment processes. Currently, around $10 \times 10^7$ tons $year^{-1}$ of biosolids are generated worldwide, and it is projected that $17.5 \times 10^7$ tons $year^{-1}$ will be generated in 2050 (Wijesekara et al., 2016). Because they are a good source of organic matter and essential nutrients, biosolids are used to enhance the physical, chemical, and biological properties of soils, thereby improving soil health. Over the past four decades, they have been used extensively in agriculture, forestry, land reclamation and revegetation (Sopper, 1992; Tian et al., 2006; Kajitvichyanukul et al., 2008; Lamb et al., 2012; Brown et al., 2014; Torri et al., 2014). They provide ecosystem services such as food and energy production, water purification, nutrient cycling, and carbon sequestration, which represent the indirect benefits of applying them to land (Larney and Angers, 2012; Bolan et al., 2013; Chowdhury et al., 2016). Advances in treatment and management of sewage wastewater have resulted in a steady decline in metal content and pathogens in biosolids and a reduction of air pollution through greenhouse gases (GHGs) and volatile gases abatement.

Addition of biosolids to soil can enhance SOC sequestration through direct input of organic matter and increased crop rhizodeposition (Table 1). Experimental results relating to carbon in soil treated with biosolids are variable and depend on factors such as the experimental method and set-up, the climatic region, and the properties of the soil and biosolids. Supporting the positive linear relationship between rate and carbon accumulation, in many case studies a significantly higher soil carbon sequestration has been reported with high application rates of biosolids (Tian et al., 2009; Gardner et al., 2010). Leaching and mineralization of organic matter can cause depletion of the carbon content in soils (Toribio and Romanya, 2006; Schwab et al., 2007). Decreased SOC has been reported following cessation of biosolids land application (Li et al., 2013). Microbial degradation of biosolids has been identified as a major cause limiting carbon sequestration in soils, because it releases GHGs. Microbial induced GHG emissions occur during the life cycle of biosolids from their stockpiles to land application. Direct emission of GHGs from biosolids stockpiles has been examined in Melbourne, Australia (Majumder et al., 2014). In this study, the youngest (<1 years), those aged between 1 and 3 years, and the oldest (>3 years) released 60, 29, and ~10 kg of $CO_2$ equivalents per Mg (one megagram (Mg) equals a metric ton) of biosolids per year, respectively. Enhanced GHGs have been reported in many case studies (Ros et al., 2006; Mar Montiel-Rozas et al., 2015; Pitombo et al., 2015). Different authors have put forth contrasting conclusions to explain the higher $CO_2$ fluxes in the biosolids-soil environment. For example, a long-term study showed that addition of biosolids changed the soil biota community composition, thereby resulting in a higher basal respiration and metabolic quotient (Ros et al., 2006). Pitombo et al. (2015) concluded that soil $CO_2$ flux was highly responsive to soil temperature in treatments with biosolids, while Mar Montiel-Rozas et al. (2015) showed that soil moisture content negatively affected carbon escape from biosolids treated soils. However, the relationship between the application of biosolids and carbon retention or $CO_2$ fluxes, specifically focussing soil texture, has not been studied in detail. Therefore, the objectives of this study were (a) to estimate the $CO_2$ flux, and (b) to quantify the SOC contents, and its fractions in two texturally different agricultural soils, as influenced by application of biosolids.

## 2. Materials and methods

### 2.1. Site description

The experimental field sites were located in the Central West part of New South Wales, Australia. The Manildra (33° 9′ 44. 2512″ S and 148° 40′ 36.2064″ E) and Grenfell (33° 56′ 31. 5924″ S and 148° 2' 15.72" E) sites featured a hot, dry summer and cool winter, with the mean annual maximum and minimum temperatures of ~22.5 and ~9.5 °C, respectively and mean annual rainfall of ~624 mm. The soils in the Manildra and Grenfell sites are classified as Red Chromosol (i.e., also known as red brown earths or red podzolic soils) with a clay loam texture, and Brown Chromosol with a sandy loam texture, respectively, according to the Australian Soil Classification (Isbell, 2002). The properties of soils at the 0–15 cm depth, and site information are given in Table 2.

### 2.2. Experimental design

The two experimental sites were limited to two treatments: biosolids amended and unamended (control). At both sites, a single level of 70 Mg $ha^{-1}$ biosolids was applied by the horizontal disc rear discharge spreaders and incorporated into surface soils (i.e., to the 15 cm depth) using chisel ploughs. The biosolids were produced through aerobic and anaerobic digestion processes at two sewage

**Table 1**
Selected references on biosolids application to various vegetation sites in terms of soil carbon sequestration.

| Country | Biosolids application rate (Mg ha$^{-1}$) | Tested plant | Soil carbon storage | References |
|---|---|---|---|---|
| Australia | 25 | Mustard | 6.41 (control 3.11) Mg SOC ha$^{-1}$ year$^{-1}$ | (Bolan et al., 2013) |
| | 50 | | 7.54 Mg SOC ha$^{-1}$ year$^{-1}$ | |
| | | | 0.24–0.36 Mg Biomass dry matter Mg$^{-1}$ Biosolids | |
| | 25 | Sunflower | 3.81 (control 2.48) Mg SOC ha$^{-1}$ year$^{-1}$ | |
| | 50 | | 4.80 Mg C ha$^{-1}$ year$^{-1}$ | |
| | | | 0.15–0.23 Mg Biomass dry matter Mg$^{-1}$ Biosolids | |
| Argentina | 7 | Pine | 49.88 Mg Biomass dry matter ha$^{-1}$ over 3 years | (Torri and Lavado, 2012) |
| | 14 | | 239.24 Mg Biomass dry matter ha$^{-1}$ over 3 years | |
| | 7 | Eucalyptus | 508.91 Mg Biomass dry matter ha$^{-1}$ over 3 years | |
| | 14 | | 1316.82 Mg Biomass dry matter ha$^{-1}$ over 3 years | |
| Canada | 50 | A grass legume mix | 120% increased SOC over 13 months (compared to fertilizer control) | (Gardner et al., 2010) |
| | | | 0.31 (fertilizer control 0.026) Mg C ha$^{-1}$ year$^{-1}$ over 13 months | |
| | 250 | | 500% increased SOC over 13 months (compared to fertilizer control) | |
| | | | 0.44 (fertilizer control 0.026) Mg C ha$^{-1}$ year$^{-1}$ over 13 months | |
| United States | 2.5 to 30 | Perennial grasses | 82% increased SOC over 2 years at 30 Mg ha$^{-1}$ Biosolids application | (Ippolito et al., 2010) |
| | | | 60% increased SOC over 14 years at 30 Mg ha$^{-1}$ Biosolids application | |
| | | | Greater live vegetation, and an increased perennial grasses | |
| United States | 4.2 kg/m$^2$ | Corn, Wheat, Soybean | 10.3 (control 3.59) kg SOC m$^{-2}$ over 34 years | (Tian et al., 2015) |
| | | | 32.5% of total crop residue-C (control 11.8%) in soils | |

**Table 2**
Soil (i.e., 0–15 cm depth) and biosolids properties, of the control experimental plots at Manildra and Grenfell. The numbers after "±" are the standard deviations (n = 3).

| | Manildra | Grenfell | Biosolids* | Biosolids** |
|---|---|---|---|---|
| $pH_{1:5\ soil:\ water}$ | 6.165 (0.092) | 7.625 (0.120) | 6.4 (0.093) | 6.7 (0.151) |
| EC$^{\#}$ μS cm$^{-1}$ | 275.55 (7.778) | 93.50 (15.42) | 8.7 (0.042) | 7.9 (0.053) |
| Total carbon % | 2.377 (0.087) | 1.2771 (0.233) | 45.306 (0.255) | 35.199 (0.208) |
| DOC (mg kg$^{-1}$) | 63.28 (4.24) | 40.50 (3.87) | 2815.32 (44.54) | 5635.45 (153.44) |
| Total nitrogen % | 0.1654 (0.013) | 0.0856 (0.015) | 4.280 (0.069) | 4.340 (0.113) |
| Total sulphur % | 0.0199 (0.006) | 0.0125 (0.003) | 1.17 (0.085) | 1.59 (0.078) |
| $\delta^{13}C$ ‰ | −24.26 (0.14) | −24.45 (0.13) | −25.68 (0.38) | −25.97 (0.53) |
| $\delta^{15}N$ ‰ | 4.11 (0.29) | 6.26 (0.18) | 8.30 (0.74) | 14.88 (0.36) |
| Textural class | clay loam | sandy loam | – | – |
| Crop type | canola sorghum | canola | – | – |

*and ** denote biosolids that were used in Manilda, and Grenfell, respectively. # unit for biosolids EC is mS cm$^{-1}$.

treatment stations in Sydney, Australia. The properties of biosolids are given in Table 2. Five plots at the two sites combined were established in January 2017 with four cropping systems. The crops at the Manildra site as follows: Plot 1 – M: canola (*Brassica napus*), Plot 2 – M: fodder sorghum (Sorghum spp.) and wheat (*Triticum aestivum* L.), Control – M (i.e., Plot 3, M stands for Manildra). The Grenfell site had a canola (*Brassica napus*) paddock with two plots: Plot – G (i.e., Plot 4) and, Control – G (i.e., Plot 5, G stands for Grenfell). The land management practices in the both sites are shown in Fig. 1a–b.

### 2.3. Soil sampling and analyses

Prior to the application of biosolids, a baseline soil sampling was completed in January 2017 at both sites. Soil samples were collected manually to a depth of 15 cm. The biosolids treated soil sampling was conducted in March, April, May, and June of 2017 using a random grid sampling design. From the biosolids treated and control plots, fifteen and four soil samples were collected, respectively and a composite sample was made for the analysis of different parameters. The <2 mm fraction of oven dried (i.e., at 36 °C) soil samples was used for the chemical analysis. Soil $pH_{1:5\ soil:\ water}$ and electrical conductivity (EC) were analysed using a pH/EC meter (LAQUA PC1100, Kyoto, Japan). Total carbon (TC), total nitrogen (TN), and total sulphur (TS) of both soil and biosolids were estimated by a CNS analyzer (LECO: 630-300-200, TruMac CNS, MI, USA) using ~ 0.25 g of sample mixed with ~0.10 g of an ignition accelerator (LECO Com-Cat 502-321, St. Joseph, MI, USA) prior to ignition. A soil reference material (LECO 502-308, St. Joseph, MI, USA) was used as the standard for carbon determination. In the soil solution, the dissolved organic carbon (DOC) was estimated by a TOC analyzer (Shimadzu: TOC-L CSH, Kyoto, Japan). In this, ~3 g of field moist soil was shaken with 30 mL of Mili-Q water (1:10 w/v soil-to-solution ratio) for 30 min in polypropylene tubes on an end-over-end shaker at 30 rpm. The soil extracts were then centrifuged at 3000 rpm (Sigma 3–30 KS, Osterode, Germany) for 20 min and filtered through 0.45 μm syringe filter units before instrumental analyses was performed. Potassium hydrogen phthalate (NACALAI TESQUE Inc., 44935-52, Kyoto, Japan) was used as a standard in the TOC analysis.

### 2.4. Soil carbon fractionation and stocks

The modified Walkley-Black method was used to quantify the labile and non-labile carbon fraction in soils (Chan et al., 2001). Soil samples collected in March were used to determine carbon fractionation. Therefore, biosolids were applied to Plot 1 – M and Plot – G sites two weeks, and Plot 2 – M two months prior to sampling taking place and soil carbon fractionation being performed. In brief, increasing oxidizing conditions through a series of acid-aqueous solutions (i.e., 12 N, 18 N, and 24 N of $H_2SO_4$) were used to estimate the carbon fractions. The labile carbon fraction was estimated by oxidizing soils with 12, 18, and 24 N $H_2SO_4$ solutions. The difference in oxidisable carbon fraction with 24 N $H_2SO_4$ and TOC content from the LECO combustion method was considered as the non-labile carbon fraction.

The soil carbon stocks were calculated based on Equation (1).

$$Mc = \frac{DS\rho C}{100} \quad (1)$$

where Mc is the soil carbon stock in Mg ha$^{-1}$, D is the surface soil layer depth (m), S is the surface area of a hectare (10,000 m$^2$ ha$^{-1}$), ρ is the bulk density (Mg m$^{-3}$), and C is the SOC concentration (%)

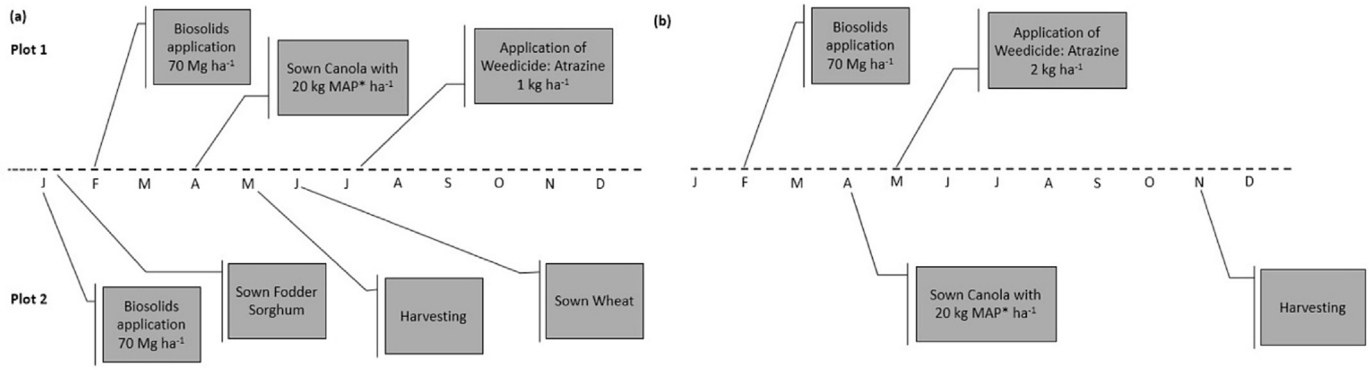


**Fig. 1.** Land management practices at; a) Manildra, and b) Grenfell sites *MAP: Mono Ammonium Phosphate.

(Tian et al., 2009). In our study, no attempt was made to distinguish inorganic carbon and organic carbon, because the $CaCO_3$ content of soils from both sites was negligible. Therefore, TC content was considered to be equal to TOC.

### 2.5. Soil microbial enzyme activity analyses

The field moist soil samples were stored at 4 °C until microbial enzyme analyses were performed. Because the assay of dehydrogenase activity (DHA) is considered to be related to the overall microbial activity or as an index of general activity of soil microorganisms, DHA was assessed using the method described in Klein et al. (1971). For this, 1 g of field-moist soil was incubated for 24 h at 28 °C with a solution containing 0.2 mL of 3% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) and 0.5 mL 1% (w/v) glucose. The amount of triphenyl formazan formed was measured spectrophotometrically at 485 nm.

### 2.6. Soil $\delta^{13}C$ and $\delta^{15}N$ study

Natural abundances of $\delta^{13}C$ and $\delta^{15}N$ in soils are used as the in-situ tracers to determine carbon and nitrogen turnover rates in soil, thereby providing insights to their functional role in soil environment (Mary et al., 1992; Wang et al., 2005). Therefore, soil $\delta^{13}C$ and $\delta^{15}N$ isotopes abundances were examined to fingerprint carbon and nitrogen derived from biosolids. The <125 μm soil and biosolids fractions were analysed for $\delta^{13}C$ and $\delta^{15}N$ using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFloIV to a ThermoFinnigan DeltaVPLUS using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS) at James Cook University's Advanced Analytical Centre (Cairns, Australia). Precisions (S.D.) on internal standards were better than ± 0.1‰ and ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

The $^{13}C/^{12}C$ isotopic ratio was expressed in the form of δ per thousand, relative to the international VPDB (Vienna Pee Dee Belemnite) standard (Equation (2)).

$$\delta^{13}C = \left[\left(\frac{^{13}C}{^{12}C}Sample \Big/ \frac{^{13}C}{^{12}C}Standard - 1\right)\right] \times 1000 \quad (2)$$

where, $^{13}C/^{12}C$ sample is $^{13}C/^{12}C$ in the sample and $^{13}C/^{12}C$ standard is $^{13}C/^{12}C$ in the standard. Relative to the air reference standard scale, $\delta^{15}N$ was expressed as (Equation (3)):

$$\delta^{15}N = \left[\left(\left(\frac{^{15}N}{^{14}N}Sample\right) - \left(\frac{^{15}N}{^{14}N}Standard\right) \Big/ \frac{^{15}N}{^{14}N}Sample\right)\right] \times 1000 \quad (3)$$

### 2.7. Soil respiration measurement and analyses

An automated soil respirometer (LI-8100A, LI-COR Inc., Lincoln, NE, USA) was used to measure diurnal $CO_2$ fluxes. The areas of biosolids treated and control plots at both the Manildra and Grenfell sites were 100 m × 100 m and 25 m × 25 m, respectively. Each site had twelve $CO_2$ flux monitoring points (i.e., soil respiration collars) that included eight for the biosolids treated plots and four for the control plots. Moisture (GS1, Decagon Devises, Inc WA, USA) and temperature (6000-09TC Omega, NJ, USA) probes, which were coupled with the automated soil respirometer, were used along with $CO_2$ flux measurements. The SoilFluxPro™ software from LI-COR Inc., Lincoln, NE, USA was used to view and analyse data files for chamber measurements generated by the soil respirometer. The soil $CO_2$ fluxes were calculated on the basis of a linear increase in chamber $CO_2$ concentrations over time.

### 2.8. Statistical analysis

Statistical analysis was performed using IBM SPSS statistics version 24.0.0.1 (IBM Corp., Armonk, NY, USA). In order to evaluate the correlation between soil temperature and moisture for soil $CO_2$ fluxes, one-way analysis of variance (ANOVA) was used to determine the significance of differences ($P < 0.05$) between variables.

## 3. Results and discussion

### 3.1. Soil carbon fractionation

Data from all sites showed that application of biosolids increased the soil TOC, which is attributed to the direct contribution from residual carbon and also increases in biomass production. At Manildra, average TOC of 4.94 ± 0.9 and 5.4 ± 0.1% were recorded for the biosolids amended Plots 1 (i.e., canola paddock) and 2 (i.e., fodder sorghum and wheat paddocks), respectively indicating the exogenous organic carbon increase over the control plots (Fig. 2). At Grenfell, the recorded average TOC for biosolids treated soil was 2.16 ± 0.15%, indicating ~45% increment over the control soil. On average, the labile fractions of SOC constituted ~27% of TOC for all

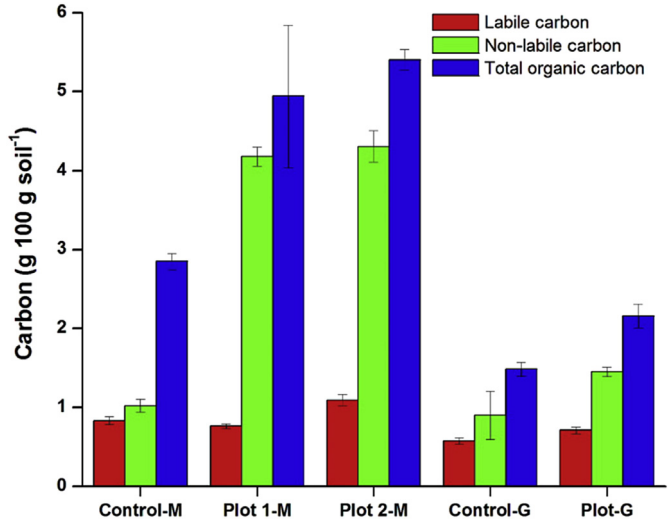


**Fig. 2.** Effect of biosolids land application on SOC fractions. Soil samples were collected in March and used to determine carbon fractionation. Mean values ± standard deviation are shown (n = 3). Control − M, Plot 1 − M, and Plot 2 − M denote plots in Manildra, while Control − G, and Plot − G denote plots in Grenfell.

the plots. The Grenfell site had the highest labile C proportion (36%) while the Manildra sites showed the highest non-labile C percentage (67%) to the TOC (Fig. 2). Enhanced DOC contents were recorded for all the biosolids amended plots, indicating a fraction of bioavailable carbon storage in soils (Fig. S1). In March, the average DOC contents of biosolids amended plots at Manildra were 102.07 and 57.07 mg $kg^{-1}$ for Plot 1 and Plot 2, respectively. At Grenfell biosolids amended soils illustrated an average DOC of 60.53 mg $kg^{-1}$ whilst the control soil was recorded as having 40.5 mg $kg^{-1}$. Among the plots, the highest DOC content change was recorded at the Plot 1 − M, indicating 120% compared to control soils in June. Biosolids influenced labile fraction can be used as a sensitive indicator for the changes in SOC dynamics with respect to the management practices (Oliveira et al., 2016). The data indicated that application of biosolids at both sites increased the non-labile carbon fraction which is stable against microbial decomposition. Chiu and Tian (2011) revealed an increased stability of soil organic matter and alkyl-dominant in humic acids carbon in biosolids amended soils.

### 3.2. Carbon stocks

Application of biosolids increased soil carbon stocks at both sites. In biosolids treated plots at Manildra, soil carbon stocks showed a ~28% C increment over the control in March. Three months later, a decline (~10%) of carbon stocks was observed in Plot 1, possibly indicating the decomposition of carbon in fresh biosolids and soil carbon (Fig. 3a) (Fontaine et al., 2004). At Grenfell, treatments with biosolids showed ~15% increment of soil carbon at the beginning (Fig. 3b). Similar to Plot 1 at Manildra, a slight decrease of soil carbon was observed at the Grenfell site over time. When compared to the Grenfell plots, the Manildra plots retained higher amounts of biosolids carbon. This observation can be attributed to the soil texture. Soil texture plays an important role in carbon stabilization in soils (Lal, 2004). The Manildra soil possesses a clay loam texture, which can associate with SOC and form stable clay-sized organo-mineral complexes (Post and Kwon, 2000). Sandy or sandy to loam soils, which are found at Grenfell, do not hold SOC for long periods due to their low protective capacity with their low clay content (Chan et al., 2003).

### 3.3. Soil microbial enzyme activity

Application of biosolids had a positive effect on soil microbial activity, as observed by the dehydrogenase activity (Fig. 4). The addition of large amounts of substrate (i.e., organic matter) can increase soil microbial activities, because soil organic matter as the primary energy source of microbes (Killham, 1994). Dehydrogenase activity increased in biosolids-amended soils at both sites compared to the control. When compared to the Manildra plots, the Grenfell sites had the highest increase of dehydrogenase activity. Such an observation was attributed to the higher contents of DOC observed in the biosolids utilised at the Grenfell plots (Table 2). High dehydrogenase activities after addition of biosolids to soil have been reported in previous studies (Mora et al., 2005; Mingorance et al., 2014). An increase in dehydrogenase activity attributes to enhanced microbial activities, thereby resulting in loss of carbon from soil as carbon dioxide or methane. Nevertheless, it is important to illustrate the microbial biomass contribution (i.e., through increased microbial community) to the net carbon storage in soils.

### 3.4. Soil $\delta^{13}C$ and $\delta^{15}N$ study

Fig. 5a shows the changes of soil $\delta^{13}C$ values that reflect the contribution of carbon in biosolids to soil. Soil $\delta^{13}C$ was similar in both control sites with ~ − 24.4‰, and biosolids amended plots for − 25.6‰. However, the biosolids amended plots showed a depletion of $\delta^{13}C$ with 1.4‰ in soils compared to the control plot in Manildra. In the case of Grenfell site, the biosolids treated plot showed a less depletion of $\delta^{13}C$ with 1.1‰ compared to the control (i.e., Control - G). Depleted $\delta^{13}C$ in biosolids amended soil have been observed in previous studies (Fernandes et al., 2005; Tian et al., 2015).

Fig. 5b shows the alterations in $\delta^{15}N$ abundance in biosolids amended soils. The data for Manildra site showed 4.3 and 7.3‰ $\delta^{15}N$ for control, and biosolids amended soils, respectively. Compared with the Manildra soils, biosolids amended soils in Grenfell showed a lower enrichment of $\delta^{15}N$ with 1.9‰ over the control soils. Overall, biosolids-amended soil in the both sites showed an enrichment of $\delta^{15}N$, indicating the short time effect of biosolids input to soil. Comparable evidences for $\delta^{15}N$ in biosolids amended soil have been reported in a previous study (Wang et al., 2005).

Abundance of $\delta^{13}C$ in soils reflects the historical $C_3/C_4$ vegetation, degree of organic matter decomposition, and land management practises that include application of biosolids (Alves et al., 2004; Fernandes et al., 2005; Parat et al., 2007). Because of the physical and biochemical factors related to $\delta^{13}C$ discrimination in the photosynthesis of $C_3$ plants (i.e., paddy), they show a less abundance to $\delta^{13}C$. Soils which have been exclusively cropped with $C_3$ plants show more negative $\delta^{13}C$ value (i.e., ~ − 27‰), while soils under vegetation comprised with $C_4$ plants (i.e., corn) for a long period show relatively a high abundance or a less negative value (~−10 to −14‰) for $\delta^{13}C$ in their organic matter (Mary et al., 1992; Staddon, 2004; Singh and Cowie, 2014). Because of the preferential degradation of labile fractions during biosolids stabilization processes, the enrichment of lignin derived organic carbon causes more depleted or more negative $\delta^{13}C$ (Tian et al., 2015). Therefore, when biosolids are mixed with soils, the abundance of $\delta^{13}C$ in soil is changed or become more negative.

Isotope fractionation of N occurs when N undergoes a physical, chemical, or biological process, because there is usually a slight preference for either the lighter or the heavier isotope associated with the process. The nature of chemical bonds related to different isotopic species is a main factor which determines their natural

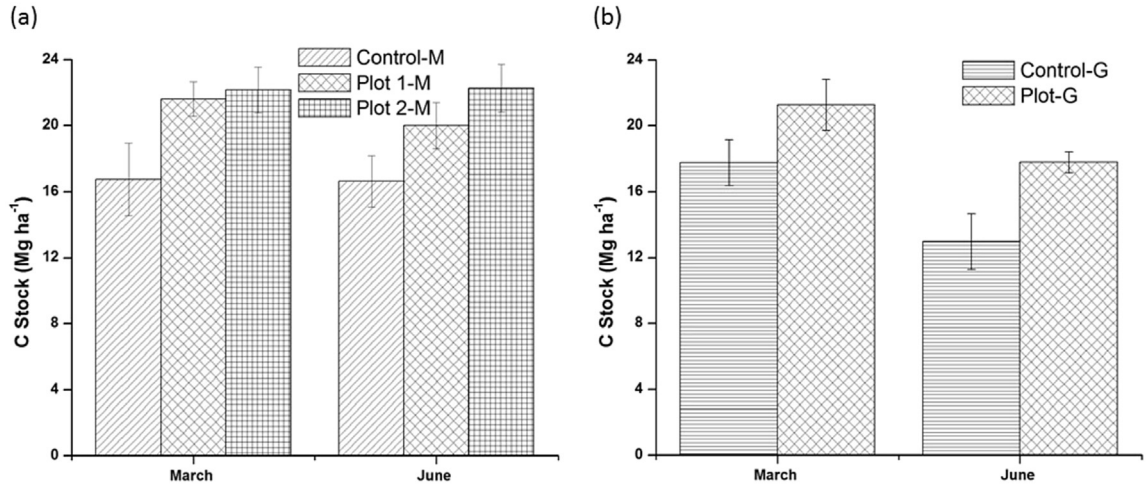


**Fig. 3.** Total C stocks (mean ± standard deviation, n = 3) per hectare in the total profile (0–15 cm) at; a) Manildra, and b) Grenfell. Control – M, Plot 1 – M, and Plot 2 – M denote plots in Manildra, while Control – G, and Plot – G denote plots in Grenfell.

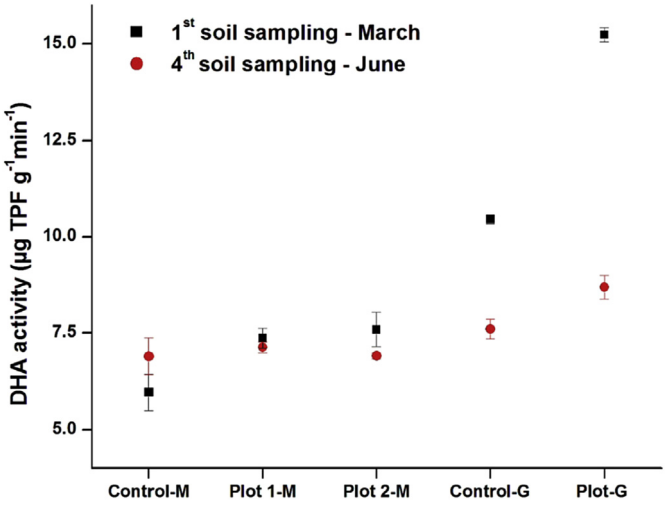


**Fig. 4.** Dehydrogenase (DHA) activity (µg TPF $g^{-1}$ $min^{-1}$) dependence with biosolids land application (mean ± standard deviation, n = 3). Control – M, Plot 1 – M, and Plot 2 – M denote plots in Manildra, while Control – G, and Plot – G denote plots in Grenfell.

abundance. In N, chemical bonds between the lighter isotopes ($^{14}N$) are broken easily (i.e., because of the lower energy requirements) than the stronger bonds between the heavy isotopes ($^{15}N$), thereby the lighter isotopes react more readily (Dawson and Brooks, 2001). This causes to enrich light isotopes in products, and heavy isotopes in the residual reactants. For example, generation of biosolids involves a number of biochemical processes, which causes enrichment in $\delta^{15}N$, and N limited forest soil in depleted $\delta^{15}N$ abundance (Wang et al., 2005). Therefore, abundance of $\delta^{15}N$ in biosolids can be used as a tracer to understand the fate and transport of N in soil-biosolids environment.

### 3.5. Soil $CO_2$ flux

Soil $CO_2$ flux rates, were measured over four months, are shown in Fig. 6. The data from both sites showed that application of biosolids increased the $CO_2$ flux, indicating microbial degradation input from biosolids, and possibly native organic compounds in the soil. At Manildra, the highest flux was recorded in April, because of the higher soil temperature. The lowest flux at Manildra was in June, due to the lower soil temperature and moisture contents. At Manildra, average $CO_2$ fluxes of 330.6 ± 17.8 and

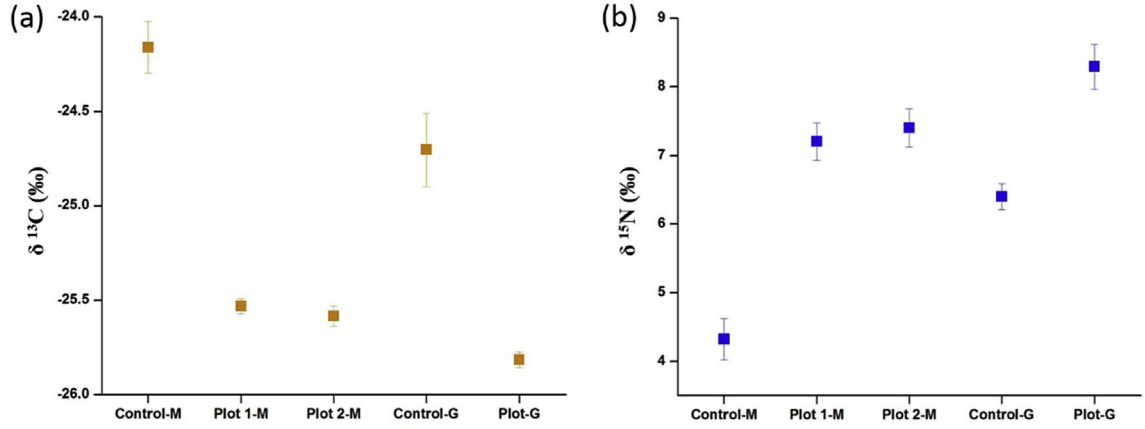


**Fig. 5.** Changes of soil isotopic tracers; a) $\delta^{13}C$, b) $\delta^{15}N$ abundance at layer depth of 0–15 cm, in control and biosolids treated soils, collected in June 2017 (mean ± standard deviation, n = 3). Control – M, Plot 1 – M, and Plot 2 – M denote plots in Manildra, while Control – G, and Plot – G denote plots in Grenfell.

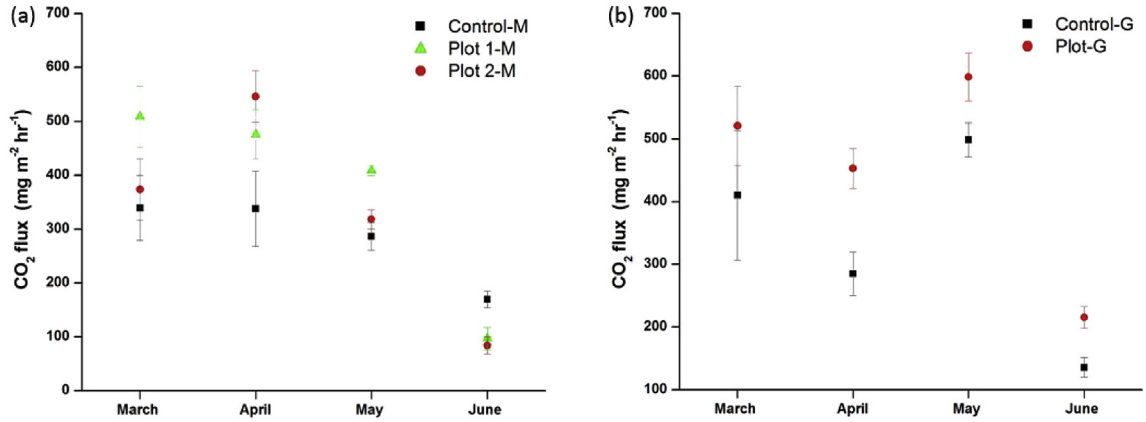


**Fig. 6.** Soil respiration rate measured for each plot during March–June, 2017 (mean ± standard deviation, n = 8 for biosolids treated, and n = 4 for control plots). Control − M, Plot 1 − M, and Plot 2 − M denote plots in Manildra, while Control − G, and Plot − G denote plots in Grenfell.

372.5 ± 18.2 mg $m^{-2}$ $hr^{-1}$ were recorded for the biosolids amended Plots 1 and 2, respectively (Fig. 6a) with an increase of 17–25% compared to the control soils. At Grenfell, the average $CO_2$ flux for biosolids treated soil was 447.1 ± 37.6 mg $m^{-2}$ $hr^{-1}$, indicating an 80% increment over the control soils (Fig. 6b). The high content of DOC in the biosolids used in Plot − G could have attributed to enhanced $CO_2$ fluxes when compared to other plots, thereby indicating the mineralization of bioavailable carbon in soils (Ibrahim et al., 2015). Additionally, it is evident that soil texture may also have an effect on $CO_2$ flux in soil. This is evident in comparisons made between the sandy loam soils of Grenfell and the clay loam soils of Manildra. The Grenfell soil demonstrated a higher $CO_2$ flux than the Manildra soil which was attributed to the sandy loams low ability to hold carbon than clay loam soils (Chan et al., 2003). Therefore, these observations reflect that, compared to the control soils, a significant amount of $CO_2$ escaped to the atmosphere and carbon was lost from the biosolids treated soils. This pattern of soil $CO_2$ fluxes reflects the decay of carbon from soils soon after application of biosolids. To enhance soil carbon sequestration, the carbon in the biosolids needs to be stabilised before the biosolids

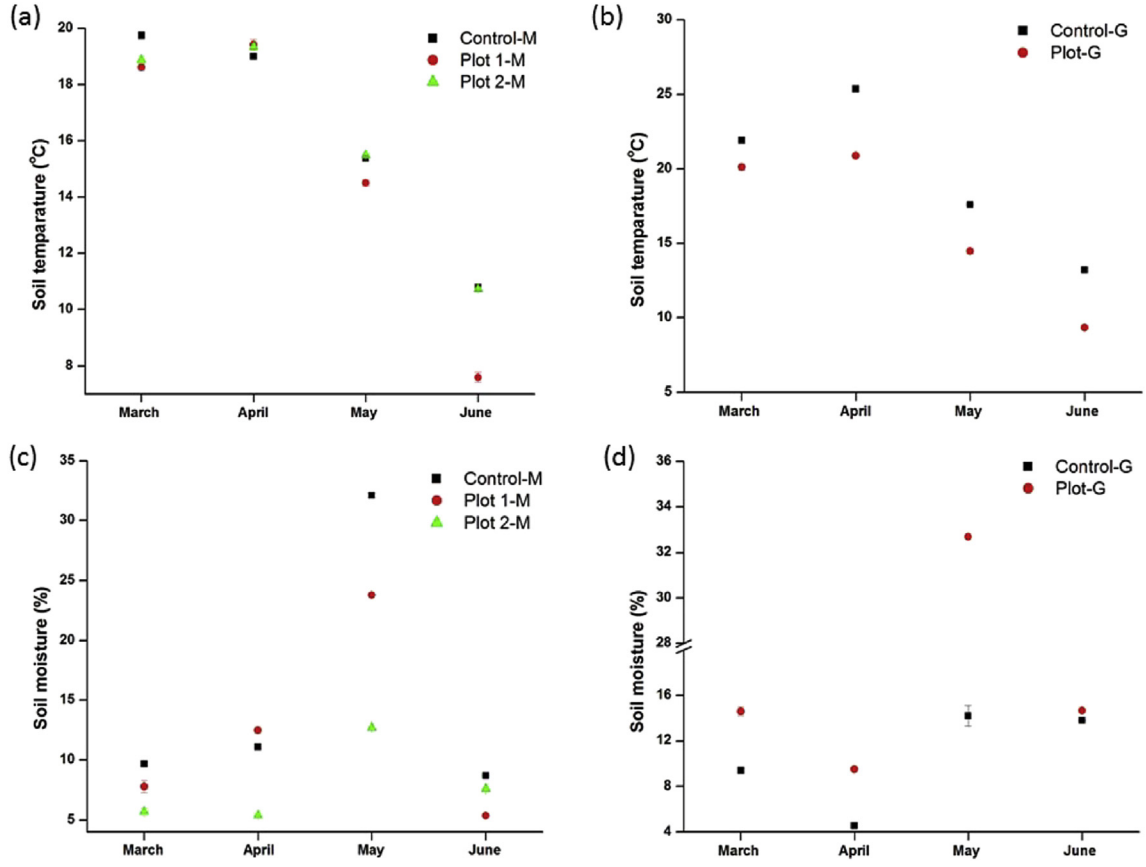


**Fig. 7.** Soil temperature and moisture measured for each plot during March–June, 2017 (mean ± standard deviation, n = 8 for biosolids treated, and n = 4 for control plots). Control − M, Plot 1 − M, and Plot 2 − M denote plots in Manildra, while Control − G, and Plot − G denote plots in Grenfell.

are applied to land. Stabilization of biosolids using alkaline materials (e.g., lime, fluidized bed boiler ash, and red mud) through co-composting can be used to improve the stability of carbon in biosolids (Chowdhury et al., 2016). In our study, we observed a significant ($p < 0.01$) direct relationship between $CO_2$ fluxes and temperature, which indicated the enhanced degradation of biosolids is influenced by microbial activity (Fig. 7a–d; Table S1). However, it has been noted that drought and high temperatures cause a decrease in microbial activity (Schloter et al., 2003). The relationship between soil $CO_2$ fluxes and soil moisture content was observed to be not-significant for all plots in our study (Fig. S2-4).

## 4. Conclusions

This study compared the changes in the concentration and quality of SOC in clay and sandy loam cropped soils after adding biosolids. The results showed increases in SOC fractions, in particular the non-labile carbon fraction, and in microbial populations, as shown by dehydrogenase activity after application of biosolids. Tracers of $\delta^{13}C$ and $\delta^{15}N$ in biosolids amended soils were measured, and we concluded that their abundance indicates biosolids-derived carbon and nitrogen redistribution in soils. Enhanced $CO_2$ emissions were observed from field sites that were treated with biosolids, thereby limiting its carbon sequestration potential. Soil temperature was a major factor that influenced microbial degradation of biosolids. We conclude that storing biosolids-derived carbon in soils for long period will be a challenge. However, biosolids application is likely to improve soil health, thereby resulting in carbon sequestration through increased biomass production.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2017.09.090.

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