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Natural and Regenerated Saltmarshes Exhibit Similar Soil and Belowground Organic Carbon Stocks, Root Production and Soil Respiration

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Abstract

Saltmarshes provide many valuable ecosystem services including storage of a large amount of 'blue carbon' within their soils. To date, up to 50% of the world's saltmarshes have been lost or severely degraded primarily due to a variety of anthropogenic pressures. Previous efforts have aimed to restore saltmarshes and their ecosystem functions, but the success of these efforts is rarely evaluated. To fill this gap, we used a range of metrics, including organic carbon stocks, root production, soil respiration and microbial communities to compare natural and a 20-year restoration effort in saltmarsh habitats within the Sydney Olympic Park in New South Wales, Australia. We addressed four main questions: (1) Have above- and belowground plant biomass recovered to natural levels? (2) Have organic carbon stocks of soils recovered? (3) Are microbial communities similar between natural and regenerated saltmarshes? and (4) Are microbial communities at both habitats associated to ecosys-

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tem characteristics? For both soil organic carbon stocks and belowground biomass, we found no significant differences between natural and regenerated habitats ($F_{(1,14)} = 0.47$, $p = 0.5$; $F_{(1,42)} = 0.08$, $p = 0.76$). Aboveground biomass was higher in the natural habitat compared to the regenerated habitat ($F_{(1,20)} = 27.3$, $p < 0.0001$), which may result from a site-specific effect: protection from erosion offered by a fringing mangrove forest in the natural habitat but not the regenerated habitat. Our microbial community assessment indicated that restored and natural saltmarsh habitats were similar at a phylum level, with the exception of a higher proportion of Proteobacteria in the rhizosphere of saltmarshes from the regenerated habitat ($p < 0.01$). Abundance of both *Desulfuromonas* and *Geobacter* was associated with high carbon and nitrogen densities in soils indicating that these genera may be key for the recovery of ecosystem characteristics in saltmarshes. Our restored and natural saltmarsh soils store at 30 cm depth similar levels of organic carbon: 47.9 Mg OC ha^{-1} to 64.6 Mg OC ha^{-1}. Conservation of urban saltmarshes could be important for 'blue carbon' programmes aimed at mitigating atmospheric carbon dioxide.

Key words: *Sarcocornia quinqueflora*; blue carbon; carbon sequestration; rehabilitation; Sydney Olympic Park; ^{210}Pb dating; microbial communities.

Highlights

- We used a range of metrics to assess the conditions of saltmarshes following ~ 20 years of restoration.
- We found soil and belowground organic carbon had recovered to natural levels.
- *Desulfuromonas* and *Geobacter* are associated with high levels of organic carbon and nitrogen within soils.

Introduction

Saltmarshes can be highly efficient at sequestering 'blue carbon' and provide other valuable ecosystem services such as sediment trapping, nutrient cycling and erosion control. Saltmarshes can also provide habitat for species that are commercially important such as banana prawns (*Fenneropenaeus merguiensis*) and for native species (Mazumder and others 2006; Connolly 2009). Globally, saltmarsh vegetation cover has been significantly reduced since the mid-19th century as a result of many different human-caused changes, including land reclamation, drainage works to facilitate agricultural, industrial and urban use, as well as sea-level rise (Duarte and others 2008; Gedan and others 2009; Crooks and others 2011). Up to 50% of the world's saltmarshes have been lost to date, and as a consequence many saltmarsh species are listed as endangered or vulnerable. Thus, saltmarsh communities are considered as threatened in many countries and are often protected by law (Adam 2002; Dhanjal-Adams and others 2016; Rogers and others 2016).

In Australia, 85% of the population lives within a 50-km perimeter of the coast, which places considerable pressure on saltmarshes due to changes associated with coastal development. In New South Wales (NSW), over 60% of coastal wetlands have been lost or degraded and saltmarshes are classified as an endangered ecological community under the *Environment Protection and Biodiversity Conservation Act 1999*. This has led to substantial saltmarsh regeneration efforts in the past decades, for example via restoring natural tidal hydrology (Winning and Saintilan 2010).

One of the goals of restoring wetlands is to recover the habitat and thus the original assemblages of flora and fauna species, but there is also increasing global interest in restoring the ecosystem functions provided by saltmarshes, such as nutrient cycling (Burden and others 2013). Saltmarshes are particularly efficient at trapping sediments and capturing organic carbon (OC) that is produced in situ or transported from the nearshore during tidal exchange (McLeod and others 2011; Adame and others 2012). Microbial composition of the soils, which influences the biogeochemical services of saltmarshes, is also increasingly considered as a metric to assess restoration success of an ecosystem (Gellie and others 2017). Soils harbour thousands of microorganisms including bacteria, archaea and eukaryotes that are crucial in controlling diverse ecosystem functions such as respiration, OC and nitrogen assimilation and plant biomass (Fierer and others 2012). Progress in saltmarsh restoration is increasingly measured according to sediment trapping, OC sequestration and OC stocks (Burden and others 2013). However, the ideal metrics by which to measure wetland restoration progress depend on the 'set of goals' of each particular restored site (Zedler 2007).

OC stocks and rates of OC sequestration in saltmarshes and other blue carbon ecosystems vary

with species composition of the wetland community and geomorphology (Saintilan and others 2013; Lovelock and others 2014). Soil OC stocks of saltmarshes with highly developed roots such as the rush *Juncus kraussii* contain significantly higher levels of soil OC than saltmarshes dominated by the grass *Sporobolus virginicus* and the chenopod *Sarcocornia quinqueflora* (Kelleway and others 2016). Roots in saltmarshes represent more than 50% of the total biomass of a plant, and root biomass and production are considered significant contributors to soil formation and carbon stocks of marsh communities (Chmura and others 2003). Additionally, saltmarshes in fluvial geomorphological settings have been found to trap and retain almost twice the amount of OC to that of marine influenced saltmarshes (Macreadie and others 2017).

Sediment trapping is important for OC burial as it contributes to OC sequestration and is important for maintaining the position of wetlands in the intertidal zone, especially within the context of increasing global sea levels (Church and White 2006). Rates of sediment accretion and OC sequestration can be measured using sediment dating techniques such as lead-210 dating (^{210}Pb; $T_{1/2}$ = 22.26 years) to date sediment accumulated in the last 150 years, or radiocarbon (^{14}C) to examine ages up to about 50,000 years. Studying responses of saltmarshes to sea-level rise is particularly important for determining their future resilience (Lovelock and Ellison 2007; Day and others 2008; Duarte and others 2013).

Previous studies have shown that once natural hydrology is restored, saltmarsh biomass rapidly achieves 100% cover. However, recovery of soil OC stocks in saltmarshes may require considerable time as soil OC accumulation from root biomass, plant litter and transported organic particles can be slow (Zedler 2000). Some studies show that levels of soil OC in restored wetlands can be similar to pre-disturbance levels after 7 to 20 years following restoration (Card and others 2010; Osland and others 2013), but other studies suggest that once landscape modification occurs, restored wetlands may never achieve the original levels of soil OC (Craft and others 1999; Zedler 2000; Moreno-Mateos and others 2012). Accordingly, the relative merits and potential of saltmarsh restoration efforts need to be further explored.

Here, we quantified aboveground and belowground biomass and their associated OC stocks, root production, soil OC stocks, soil nitrogen, soil respiration and microbial communities to assess restored compared to natural saltmarshes within a setting that has undergone major anthropogenic influence, Haslams Creek within the Sydney Olympic Park in NSW, eastern Australia. Haslams Creek was chosen as a study site because it presents a unique opportunity to evaluate ~ 20 years of regeneration efforts in areas with similar geomorphological (landscape) features, that is, located within the same riverine channel, at similar tidal elevations at ~ 0.9 m AHD, and with similar pore-water salinities of ~ 35–40 ppt. We examine the following questions: (1) After ~ 20 years of habitat regeneration has plant biomass, above and belowground recovered to natural levels? (2) Have OC stocks of soils recovered? (3) Are microbial communities similar between natural and regenerated saltmarshes? (4) Are microbial communities at both habitats associated to ecosystem characteristics?

Methods

Study Sites

This study was conducted at natural and regenerated saltmarsh habitats along Haslams Creek (33°50′24.04″S, 151°03′48.57″E and 33°50′27.66″S, 151°03′31.16″E, respectively), a riverine channel that joins the Parramatta River at Homebush Bay (Figure 1; Williams and others 2011). Our natural and regenerated areas were chosen at similar tidal elevations at ~ 0.9 m AHD, and exhibit similar pore-water salinities of ~ 35–40 ppt. Our natural habitat is fringed by a mangrove forest dominated by *Avicennia marina*. The climate of the region is temperate with mean minimum/maximum temperatures of 13.9°C and 23.6°C, respectively. Average annual rainfall from the nearest meteorological station is 1135 mm (Station 066013, Australian Bureau of Meteorology 2018).

The Sydney Olympic Park is located 14 km west of the Sydney central business district, and its wetlands have been subjected to a range of developments since the mid-nineteenth century. From 1911 to 1988, two brick pits were operational at the head of Homebush Bay. In the 1950s, the Parramatta River was dredged to create industrial land. The area was eventually converted to a dumping site for 9 million cubic metres of domestic, commercial and industrial waste produced in the Sydney region.

Active natural ecosystem regeneration efforts at the Sydney Olympic Park commenced in 1993, when the area was designated to host the Sydney Olympic Games in 2000 (Burchett and others 1998). Restoration of saltmarshes at Haslams Creek started by widening the creek that in the past had

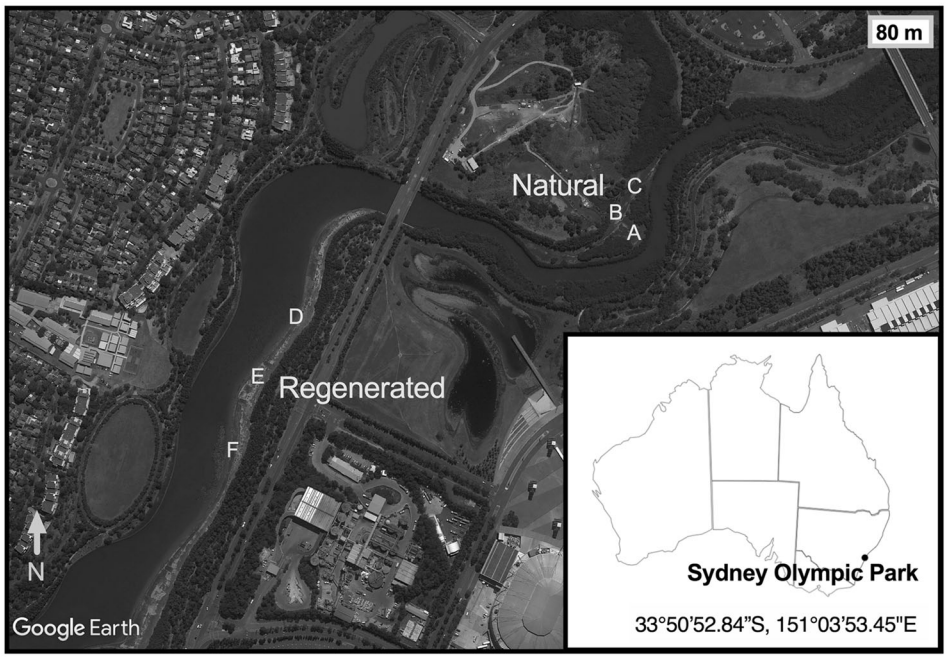


Figure 1. Study area at Haslams Creek, Sydney Olympic Park, New South Wales (33°50′52.84″S, 151°03′53.45″E). This study was conducted at a natural and a regenerated saltmarsh habitat (33°50′24.04″S, 151°03′48.57″E and 33°50′27.66″S, 151°03′31.16″E, respectively). Letters indicate the location of each of our plots within our natural (**A**, **B** and **C**) and regenerated (**D**, **E** and **F**) habitats.

been converted to a narrow, heavily polluted concrete channel. Following this hydrological rehabilitation, gabion walls and sandy soil with rubble were used to construct saltmarsh beds in 1998, and saltmarsh plants were transplanted within the area. Despite these restoration efforts, many areas remained unvegetated. From 2006 to 2007, rubble was removed and organic mangrove soil was incorporated into the top soil to improve soil properties and revegetation of the restored area. To date, with permission from the *Fisheries Management Act 1994*, ongoing active regeneration efforts include the annual cutting and removing of the invasive species *Juncus acutus* and of mangrove seedlings encroaching into the saltmarsh community.

Experimental Design

We chose three plots of 5 m × 30 m within the high intertidal zone of Haslams Creek at our natural and regenerated habitats. At each plot, we established six subplots of 0.25 m × 0.25 m. Within these subplots, we measured aboveground and belowground biomass and their associated OC stocks, root production, soil OC, soil nitrogen and soil respiration. Additionally, at three cores from the natural habitat, we assessed sediment age, sediment accretion and OC sequestration. We also examined microbial communities of bulk soil, rhizosphere and roots at both habitats. Our study areas were dominated by the chenopod *Sarcocornia quinqueflora*.

OC from Aboveground and Belowground Biomass

To examine aboveground OC, aboveground biomass of *Sarcocornia quinqueflora* was harvested within four of the six subplots established at each plot. Four plots were chosen in order to assess aboveground biomass variability without causing further damage to the protected saltmarsh vegetation. Harvested biomass was oven-dried at 60°C for 72 h and weighted. The OC stocks of total aboveground biomass were determined by using a conversion factor of 0.45 (the proportion of carbon in aboveground biomass) multiplied by the dry biomass (kg m^{-2}) and converted to Mg OC ha^{-1} (Howard and others 2014).

To determine OC from belowground biomass, we collected 3 to 4 cores of 30 cm depth from random locations within each of the three of the subplots established at each plot (a total of 10 cores). Our plots were dominated by *Sarcocornia quinqueflora* and no other species were present. The selected subplots were 1, 3 and 6 or 1, 2 and 6, as in some plots, bedrock was present at ~ 30 cm depth, which im-

peded collection of intact 30-cm-length cores. Cores were collected with a stainless-steel gauge auger of 2.5 cm diameter (Dormer Soil Samplers; Australia) and subdivided by depth (0–5, 5–10, 10–15, 15–20 and 20–30 cm). The collected material was sealed in plastic bags and stored at 4°C until laboratory analyses were performed. Soils were washed through a 1-mm sieving mesh to separate roots from soil. Belowground biomass was oven-dried at 60°C for 48 h and weigh was obtained. Belowground biomass for each core was assessed by adding up biomass per core segment (that is, 5 cm, 10 cm) and divided by the area of the corer as: $\pi * r^2$. The OC stock of total belowground biomass was determined by using a conversion factor of 0.34 (the proportion of carbon in belowground biomass) multiplied by the dry biomass (kg m^{-2}) and converted to Mg OC ha^{-1}. The use of conversion factors instead of other methods (that is, combustion of a subsample) to determine proportion of OC in aboveground and belowground biomass for OC accounting projects is a reliable method that reduces assessing times and costs (Howard and others 2014).

Root Production

Root production was assessed by installing 6 in-growth root bags along the six subplots per plot, but final sample size varied from 10 to 18 root bags per habitat as some of the bags were lost after burial. In-growth bags were 5 cm in diameter and 20 cm long made of nylon mesh (2 mm) and filled with natural root-free sediment. Root in-growth bags were collected after an 11-month period that encompassed seasonal variation in climate similar to other studies that have used this approach (Adame and others 2014; Lovelock and others 2015a, b). Roots were washed through a 1-mm-sieving mesh. Dry weight was assessed after oven drying material at 72°C for 48 h. Root biomass was determined as dry weight per horizontal projected surface area of the in-growth root bag per month.

Soil OC Stocks and Soil Nitrogen

Soil OC stocks and nitrogen content were assessed by collecting one core at three of the subplots per plot by using a stainless-steel gauge auger (2.5 cm diameter; Dormer Soil Samplers; Australia). We chose subplots 1, 3 and 6 or 1, 2 and 6 because in some subplots bedrock was present at ~ 30 cm depth and collection of intact 30 cm-length cores was not possible at all subplots. Each core was divided into depth intervals of 0–10 cm, 10–20 cm and 20–30 cm. To determine soil bulk density, we assessed the volume of the sediment by water displacement with a graduated cylinder and then sediment was dried at 60°C for 48 h to a constant mass. Soil bulk density was calculated as dry weight divided by volume. Samples were homogenized with a Retsch three-dimensional Vibrator Mill (Type MM 2 Haan; Germany). The samples to determine OC were processed by acidification with 1% hydrochloric acid and rinsed with Milli-Q water (Howard and others 2014). Both OC and nitrogen sediment were determined using an elemental analyser (Thermo Fisher Flash 2000 HT EA; Thermo Electron Corporation, USA). We determined soil OC stocks as (Eq. 1),

$$\begin{aligned}&\text{Soil OC stocks}\left(\text{g OC cm}^{-2}\right)\\&=\text{soil bulk density}\left(\text{g cm}^{-3}\right)\\&\quad\times\text{OC content}(1/100\,\%)\\&\quad\times\text{thickness of the assessed soil layer(cm)}\end{aligned}\tag{1}$$

Then we converted g OC cm^{-2} to Mg OC ha^{-1} as (Eq. 2),

$$\begin{aligned}&\text{Soil OC stocks}\left(\text{Mg OC ha}^{-1}\right)\\&=\text{Soil OC stocks}\left(\text{g OC cm}^{-2}\right)\\&\quad\times\left(1\,\text{Mg}/1\times10^{6}\,\text{g}\right)\times\left(1\times10^{8}\,\text{cm}^{2}/1\,\text{ha}\right)\end{aligned}\tag{2}$$

Finally, we added up the amount of OC from each core layer to the total sampling depth (that is, 30 cm).

We also determined soil OC density and soil nitrogen density as (see Eqs. 3 and 4),

$$\begin{aligned}\text{Soil OC density}\left(\text{g cm}^{-3}\right)&=\text{soil bulk density}\left(\text{g cm}^{-3}\right)\\&\quad\times\text{OC content}\,(1/100\%)\end{aligned}\tag{3}$$

$$\begin{aligned}&\text{Soil nitrogen density}\left(\text{g cm}^{-3}\right)\\&=\text{soil bulk density}\left(\text{g cm}^{-3}\right)\\&\quad\times\ N\ \text{content}\,(1/100\%)\end{aligned}$$

Soil Respiration

Soil respiration was measured by collecting one top-soil sample (0–10 cm) and one deep-soil sample (20–30 cm) at each of three subplots. Samples were collected with a stainless-steel gauge auger. A carbon dioxide (CO_2) burst Solvita Kit was utilized to quantify respiration (Haney and others 2008). In brief, free-root soil was coarse-sieved (2 mm) and dried at 40°C for 24 h. Immediately after drying, 40 g of soil were placed in a Solvita soil beaker to

determine (1) soil bulk density and (2) water amount to be added. A CO_2 probe was inserted into a Solvita glass jar containing the beaker with soil and was tightly screwed. Jars were maintained at constant temperature of 20°C, and values of released CO_2 were recorded after 24 h. Assessment of respiration using the Solvita Kit has proven to be a cost-effective method. Particularly, results have been used as an indicator to assess restoration of soils from different origins and depths (for example, Muñoz-Rojas and others 2016). Examination of respiration with the Solvita kit is performed after drying (that is, simulating a drying-rewetting event) and under controlled laboratory conditions thus, results are expected to be significantly higher than those obtained under natural conditions (Haney and others 2008).

Dating and OC Sequestration Rates

We dated three sediment cores of 99 cm depth, collected with a stainless-steel gauge auger from a subplot of our natural habitat, to determine OC sequestration rates. The sampled subplot was the only area where deep cores were available, as bedrock was present at ~ 30 cm depth in all of the other subplots. At the regenerated habitat, shallow sediment has been severely mixed and thus is not appropriate for ^{210}Pb dating techniques, which rely on non-manipulated natural layering of sediments.

Dating of the sediment cores was performed with two techniques: shallow samples (0–20 cm depth) and deeper samples (59–99 cm) were dated by ^{210}Pb (Appleby 2001) and radiocarbon (^{14}C) (Hua 2009), respectively. For ^{210}Pb dating we selected 7 shallow samples of 1 cm thickness every 2–4 cm for each core. Each sample was dried to estimate the soil bulk density and then processed for the determination of polonium-210 (^{210}Po) and radium-226 (^{226}Ra) activities by alpha spectrometry (Atahan and others 2014). Unsupported ^{210}Pb activity of each sample was then calculated from the activity of ^{210}Po (the proxy for total ^{210}Pb) minus the ^{226}Ra activity (the proxy for supported ^{210}Pb). To validate our ^{210}Pb chronologies, we analysed caesium-137 (^{137}Cs) activities by gamma spectrometry in five samples from each of our sediment cores. The presence of a peak in ^{137}Cs activity identifies the 1964 depth (Appleby 2001; Leslie and Hancock 2008).

Rates of OC sequestration from shallow samples were determined from mass accumulation rates as (Eq. 5),

$$\begin{aligned}&\text{OC sequestration}\left(\text{g OC cm}^{-2}\,\text{year}^{-1}\right)\\&\quad=\text{Mass accumulation rates}\left(\text{g cm}^{-2}\,\text{year}^{-1}\right)\\&\qquad\times\text{OC content}\,(1/100\,\%)\end{aligned}\tag{5}$$

Then we converted g OC cm^{-2} $year^{-1}$ to g OC m^{-2} $year^{-1}$ as (Eq. 6),

$$\begin{aligned}&\text{OC sequestration}\left(\text{g OC m}^{-2}\,\text{year}^{-1}\right)\\&\quad=\text{OC sequestration}\left(\text{g OC cm}^{-2}\,\text{year}^{-1}\right)\\&\qquad\times\left(1\times10^{4}\,\text{cm}^{2}/1\,\text{m}^{2}\right)\end{aligned}\tag{6}$$

Radiocarbon (^{14}C) dating on bulk organic sediment was performed for the three collected cores from two depth intervals 59–60 cm and 98–99 cm. Samples were chosen at these two intervals in order to determine OC sequestration from the whole core profile. In addition, a sediment sample in Core 3 at depth 15–16 cm (see Table S1), which was dated by ^{210}Pb, was analysed for ^{14}C for the determination of the radiocarbon reservoir age of the bulk organic sediments. Samples were cleaned using the standard acid–alkali acid pre-treatment to remove possible carbon contamination including carbonates and humic acids. The pre-treated samples were then converted to CO_2 and reduced to graphite (Hua and others 2001) for accelerator mass spectrometry (AMS) ^{14}C analysis using the STAR AMS Facility at the Australian Nuclear Science and Technology Organisation (ANSTO; Fink and others 2004). The results are reported as conventional radiocarbon ages (Stuiver and Polach 1977) and were corrected for the bulk-sediment reservoir effect before being converted to calendar ages using the SHCal13 calibration curve (Hogg and others 2013) and the OxCal calibration program (Bronk 2009).

Rates of OC sequestration from samples at 60 and 99 cm depth were determined by adding up the OC stocks from the assessed core layers (0–10, 10–20, 20–30, 30–60 and 60–99 cm) in g OC m^{-2} and dividing these values by sediment age to obtain g OC m^{-2} $year^{-1}$.

Microbial Community Analysis

We collected triplicate samples of bulk soil, rhizosphere and roots from the top 10 cm of soil at three plots from our natural and regenerated habitats ($n_{\text{bulk soil/rhizosphere/root material}} = 3 \times 3 \times 6$). All material was snap-frozen in liquid nitrogen and transported from the field to the laboratory within a dry shipper in cryogenic tubes and stored at − 86°C until further analyses were performed. Bulk soil samples represent the root-free sediment. Rhizosphere samples were obtained by washing up

roots with a standard phosphate-buffered saline solution (PBS) and once washed in PBS, root material was also collected in a different vial. Deoxyribonucleic acid (DNA) extractions were performed with a MO BIO Power Soil DNA Isolation Kit (Qiagen, Venlo, Netherlands) according to the instructions of the manufacturer. Extracted DNA was quantified using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA, USA).

Preparation of DNA Libraries and Sequencing

One of the extracted DNA samples among triplicates was selected from bulk soil, rhizosphere and roots for all three plots from both habitats for DNA library preparation and sequencing (for a total of 18 samples). Metagenome sequins (Synthetic DNA standards emulating a natural microbial community; Hardwick and others 2018) were spiked into the saltmarsh DNA samples at 5% fractional abundance. The Nextera XT sample prep kit (Illumina®) was used to prepare DNA libraries according to the instructions of the manufacturer. Prepared libraries were quantified on a Qubit Fluorometer and verified on an Agilent 2100 Bio Analyser (Agilent technologies, CA, USA). Libraries were sequenced on a HiSeq 2500 instrument (Illumina®, CA, USA) with 125 bp paired-end reads at the Kinghorn Centre for Clinical Genomics, Sydney, Australia.

Bioinformatic Analyses

FastQC (v0.11.5) (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to confirm sequence quality of reads. Reads were trimmed using Cutadapt (v1.8.1) with the Trim Galore wrapper (v0.4.1) in-paired mode. To quantify sequins and construct standard curves, reads were mapped with Bowtie2 (v2.3.2; Langmead and Salzberg 2012) to a genome index comprising sequin DNA sequences. Average fold coverage of each sequin was calculated from mapped BAM files using the BEDTools genomecov feature (v2.25.0).

The MG-RAST pipeline (v4.0.2; Meyer and others 2008) was used to examine the taxonomic composition of each DNA sample, by inputting quality-filtered raw sequencing reads directly. Default options for quality control, including removal of artificial duplicate reads, trimming and screening for human DNA, were used. Reads were compared against the RefSeq, SEED, KEGG, Subsystems and Greengenes databases using BLAT parameters of 60% similarity, 15 bp and E-value of 10^{-5}. Samples were normalized using the RUVg procedure, which uses spike-ins that remain at equimolar concentrations between mixtures as negative controls. The MG-RAST pipeline also estimates Shannon α-diversity also named Shannon species richness for each sample (Meyer and others 2008).

Statistical Analyses

A one-way analysis of variance (ANOVA) was used to test for differences in aboveground and belowground biomass and in OC content from aboveground, belowground and soil between natural and regenerated habitats, using habitat (Natural/Regenerated) as a fixed factor and plot as a random factor nested within habitat. A two-way ANOVA was used to test for differences between habitat and depth (0–10, 10–20 and 20–30 cm) for belowground biomass, bulk density, soil OC density, soil nitrogen density and soil respiration. In this two-way ANOVA, habitat and depth were fixed factors and plot was a random factor nested within habitat. A two-way ANOVA design was also used to analyse the metagenomic samples, with habitat and sample type (bulk soil, rhizosphere and roots) as fixed factors and plot as a random factor. We tested for differences in Shannon α-diversity, proportion of metagenome sequences by domain (that is, Bacteria, Eukaryota, Archaea) and proportion of metagenome sequences by phyla. We considered the eleven most abundant phyla, comprising 66 to 92% of relative abundance in metagenome sequences: Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Chloroflexi, Cyanobacteria, Planctomycetes, Verrucomicrobia, Deinococcus-Thermus, Acidobacteria and Chlorobi. We used post hoc tests to examine differences between levels of treatments where significant effects involving fixed factors were detected in the ANOVA analyses. We also used a multidimensional scaling ordination (MDS) with a Bray–Curtis dissimilarity metric to visualize the microbial communities of our samples of bulk soil, rhizosphere and roots. MDS ordination was performed using the vegan package in R (Oksanen and others 2018).

We used linear regression analyses to test the relationship between belowground biomass and aboveground biomass, bulk density and belowground biomass and nitrogen density and belowground biomass. We also used linear regression analyses to test the resulting ^{210}Pb ages versus depth, respiration and aboveground biomass and respiration and belowground biomass.

We used Pearson correlations to test the association between soil OC stock and root production,

soil OC stock and log (belowground biomass). We also examined the correlation between aboveground biomass and abundance of phyla and class, organic carbon density versus abundance of genus and nitrogen density versus abundance of genus. Parametric test assumptions of normality and homogeneity of variances were tested using a Bartlett's test. Our analyses were performed using Prism version 7.0c for Mac (GraphPad Software, La Jolla, CA, USA) and the software R (R Core Team 2018).

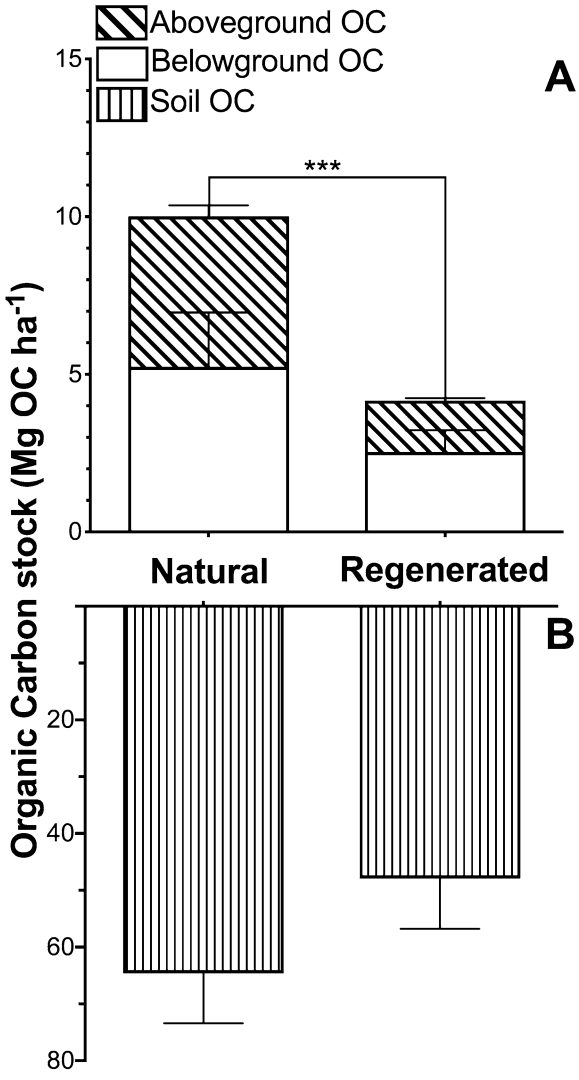


Figure 2. Organic carbon (OC) stocks at our studied natural and regenerated habitats at Haslams Creek, Sydney Olympic Park, New South Wales. **A** Aboveground OC stock (Mg OC ha^{-1}) and belowground OC stock (Mg OC ha^{-1}) and **B** soil OC stock (Mg OC ha^{-1}). Values are means and standard errors. We found significant differences in aboveground OC between the two habitats with a significant level of $^{***}p < 0.0001$. Note different scales between panel **A** and panel **B**.

Figure 3. A Belowground biomass (mg cm^{-3}) at ▶ different depths (0–5, 5–10, 10–15, 15–20, 20–30 cm). Belowground biomass was significantly higher at the top 0–5 cm with a significant level of $^{*}p < 0.01$. **B** Root production (mg cm^{-2} $month^{-1}$) of saltmarshes at our studied natural and regenerated habitats, Sydney Olympic Park, New South Wales. Values are means and standard errors. **C** Relationship between soil organic carbon stock (Mg OC ha^{-1}) and root production (mg cm^{-2} $month^{-1}$); $r^2 = -0.32$; non-significant ($p = 0.53$). **D** Correlation between soil carbon stock and Log (belowground biomass [mg cm^{-2}]); $r^2 = 0.82$; $p = 0.04$. **E** Linear regression between bulk density (mg cm^{-3}) and belowground biomass (mg cm^{-2}), the regression was $y = -0.01x + 1.6$, $r^2 = 0.47$, $p = 0.001$. **F** Linear regression between nitrogen density (mg cm^{-3}) and belowground biomass (mg cm^{-2}), the regression was $y = 0.001x + 0.1$, $r^2 = 0.28$, $p = 0.02$.

Results

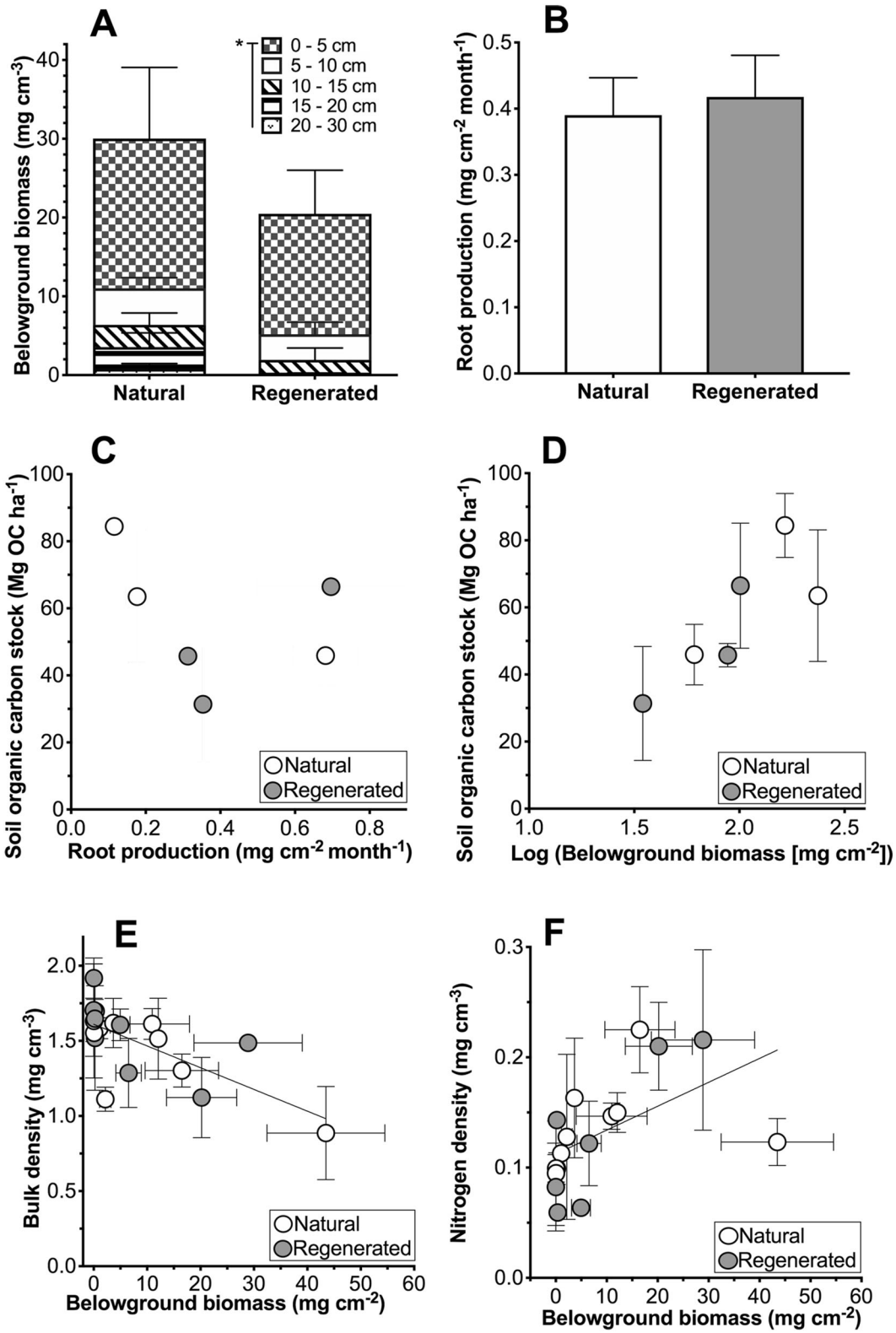
Saltmarsh Biomass, Aboveground and Belowground OC Stocks

Aboveground biomass was higher in the natural compared to the regenerated habitat ($F_{(1,20)} = 27.3$, $p < 0.0001$). Values of aboveground biomass varied from 0.36 ± 0.01 kg m^{-2} in the regenerated habitat to 1.06 ± 0.07 kg m^{-2} in the natural habitat. In contrast, belowground biomass varied from 0.78 ± 0.16 kg m^{-2} to 1.55 ± 0.35 kg m^{-2} and there were no significant differences between habitats ($F_{(1,42)} = 0.08$, $p = 0.76$). There was a significant linear relationship between aboveground and belowground biomass for *Sarcocornia quinqueflora* (Figure S1, $r^2 = 0.42$, $p = 0.0006$).

OC from aboveground biomass was higher in the natural habitat (4.77 ± 0.34 Mg OC ha^{-1}) than in the regenerated habitat (1.62 ± 0.08 Mg OC ha^{-1}) (Figure 2A, $F_{(1,20)} = 27.3$, $p < 0.0001$), whereas carbon from belowground biomass was not significantly different between natural and regenerated habitats (Figure 2A, $F_{(1,42)} = 0.08$, $p = 0.76$).

Belowground Biomass by Depth and Root Production

Our analyses show that belowground biomass was similar between habitats but varied among depths (habitat: $F_{(1,219)} = 0.62$, $p = 0.43$, depth: $F_{(1,219)} = 47.5$, $p < 0.0001$, habitat × depth: $F_{(1,219)} = 0.41$, $p = 0.52$; Table S2). The highest


A
*
0 - 5 cm
5 - 10 cm
10 - 15 cm
15 - 20 cm
20 - 30 cm
Belowground biomass (mg cm-3)
Natural
Regenerated
B
Root production (mg cm-2 month-1)
Natural
Regenerated
C
Soil organic carbon stock (Mg OC ha-1)
Root production (mg cm-2 month-1)
Natural
Regenerated
D
Soil organic carbon stock (Mg OC ha-1)
Log (Belowground biomass [mg cm-2])
Natural
Regenerated
E
Bulk density (mg cm-3)
Belowground biomass (mg cm-2)
Natural
Regenerated
F
Nitrogen density (mg cm-3)
Belowground biomass (mg cm-2)
Natural
Regenerated

biomass was found in the top 0 to 5 cm of soil (Figure 3A).

After 11 months of field deployment, we found no consistent differences in root production between natural and regenerated habitats (Figure 3B, $F_{(1,79)} = 0.01$, $p = 0.92$).

Soil Bulk Density, OC and N

Soil bulk density was similar between habitats but was significantly lower in the top 0 to 10 cm of the soil profile (Table 1 and S2; habitat: $F_{(1,48)} = 0.003$, $p = 0.95$, depth: $F_{(1,48)} = 6.52$, $p = 0.01$, habitat x depth: $F_{(1,48)} = 1.43$, $p = 0.23$).

Soil OC density from 0 to 30 cm depth varied from 15.5 ± 12 mg cm^{-3} in the regenerated habitat to 28 ± 5.4 mg cm^{-3} in the natural habitat (Table 1). OC density was similar between natural and regenerated habitats but was significantly higher in the top 0–10 cm compared to the rest of the soil profile (Table 1 and S2; habitat: $F_{(1,48)} = 1.18$, $p = 0.28$, depth: $F_{(1,48)} = 4.63$, $p = 0.03$, habitat × depth: $F_{(1,48)} = 1.71$, $p = 0.19$). We were only able to examine differences in soil nitrogen density between the two habitats for the top 0–20 cm, as nitrogen % values for the bottom 20–30 cm for the regenerated habitat were too low. We found that nitrogen density was similar between natural and regenerated habitats (1.61 ± 0.02 mg cm^{-3} to 1.47 ± 0.22 mg cm^{-3}, respectively). Soil N was higher in the top 0–10 cm of soil compared to deeper in the soil profile (Table 1 and S2; habitat: $F_{(1,33)} = 0.004$, $p = 0.95$, depth: $F_{(1,33)} = 4.25$, $p = 0.04$, habitat × depth: $F_{(1,33)} = 0.24$, $p = 0.62$).

Soil OC stock at 30 cm depth of the regenerated habitat (47.9 ± 8.9 Mg OC ha^{-1}) did not differ from that of the natural habitat (64.6 ± 8.8 Mg OC ha^{-1}; $F_{(1,14)} = 0.47$, $p = 0.5$; Figure 2B). Our analyses show that the correlation between soil OC stock and root production was not significant (Figure 3C; $r^2 = -0.32$; $p = 0.53$), but the correlation between soil OC stock and log (belowground biomass) was significant (Figure 3D, $r^2 = 0.82$, $p = 0.04$). Additionally, we found a significant negative relationship between bulk density and belowground biomass (Figure 3E, $r^2 = 0.47$, $p = 0.001$) and a positive relationship between nitrogen density and belowground biomass (Figure 3F, $r^2 = 0.28$, $p = 0.02$).

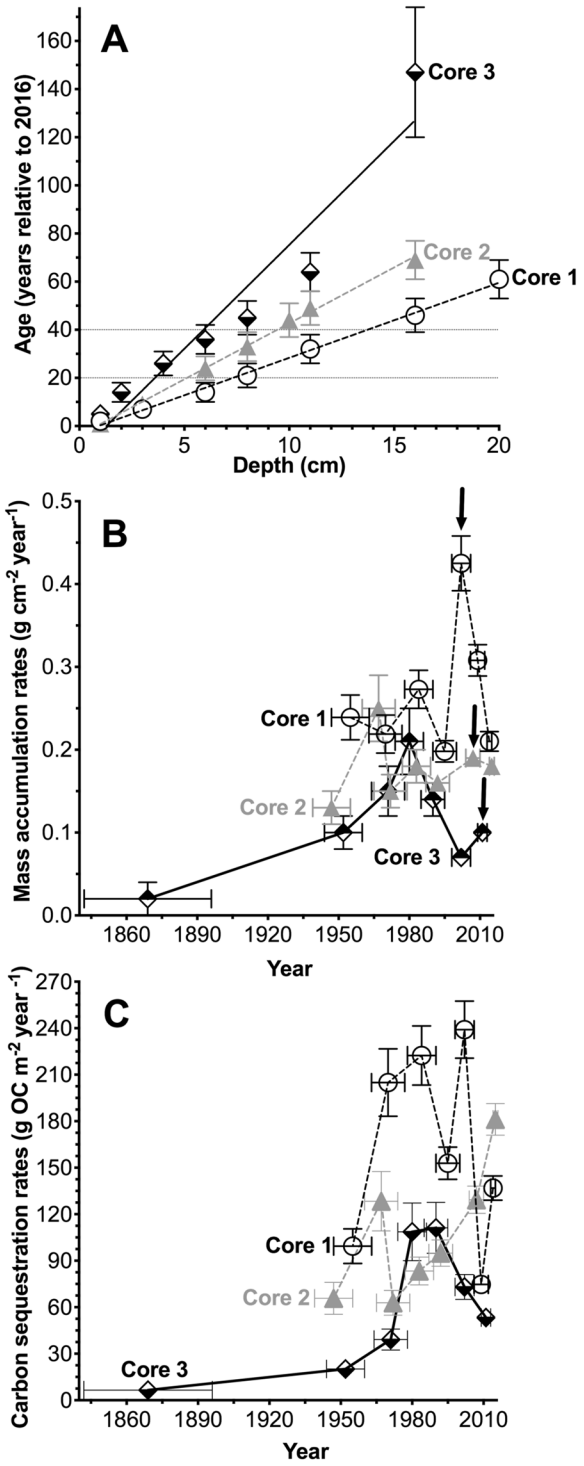
OC Sequestration Rates

The results of ^{210}Pb dating of sediment in the natural habitat are presented in Table S1, with the oldest ages of 61 ± 8, 69 ± 8 and 147 ± 27 years old (relative to 2016, year in which we sampled our sediments). Sediment accretion and carbon sequestration varied over time (Figure 4A–C). Our results of ^{137}Cs validate our ^{210}Pb chronologies of core 1 and core 2. In brief, peaks of ^{137}Cs activity (observed as higher values, Table S3) mark the year 1964 (or ∼ 52 years of age in 2016) (Leslie and Hancock 2008). We found peaks in ^{137}Cs activities were in agreement with ages of core 1 and core 2 determined by ^{210}Pb chronologies. However, activities from core 3 do not validate the ^{210}Pb chronology, and this could be due to Cs being mobile in the sediment materials within this core. Radiocarbon dating of the three sediment cores showed median values of 761–969 calendar years before present (cal. years BP, with 0 BP being 1950) for samples at 59–60 cm and 1215–1638 cal. years BP for samples at 98–99 cm depth (Table 2). Mean carbon sequestration rate in the last ∼ 860 years in our natural habitat is 20 ± 2 g OC m^{-2} $year^{-1}$ and in the last ∼ 1398 years 16 ± 2 g OC m^{-2} $year^{-1}$ (Table 3).

Table 1. Soil Physicochemical Characteristics of the Studied Natural and Regenerated Habitats at the Sydney Olympic Park, New South Wales, Australia

Habitat	Depth (cm)	Bulk density (g cm^{-3})	OC content (%)	OC density (mg cm^{-3})	*N* content (%)	*N* density (mg cm^{-3})
Natural	0–10	1.27 ± 0.14	3.76 ± 1.78	30 ± 4.6	0.21 ± 0.08	1.8 ± 0.14
	10–20	1.60 ± 0.09	2.74 ± 1.20	28 ± 3.8	0.17 ± 0.09	1.6 ± 0.17
	20–30	1.43 ± 0.14	2.55 ± 1.84	26 ± 8.9	0.12 ± 0.06	1.4 ± 0.26
	Mean	1.22 ± 0.15	3.02 ± 0.37	28 ± 5.4	0.17 ± 0.05	1.61 ± 0.2
Regenerated	0–10	1.30 ± 0.20	2.22 ± 0.27	29 ± 2.7	0.13 ± 0.02	1.75 ± 0.17
	10–20	1.61 ± 0.10	0.44 ± 0.15	7.3 ± 1.4	0.06 ± 0.02	0.97 ± 0.19
	20–30	1.76 ± 0.11	0.60 ± 0.38	10 ± 3.5	N/A	N/A
	Mean	1.57 ± 0.07	1.09 ± 0.56	15.5 ± 12	0.10 ± 0.04	1.47 ± 0.22

OC organic carbon and N nitrogen. Values are means ± standard errors.



◀**Figure 4. A** Linear regression between age (years relative to 2016) and depth (cm), the regressions were as follows: $Y_{CORE1} = 3.2X - 2.8$, $r^2 = 0.99$, $p < 0.0001$, $Y_{CORE2} = 4.64X - 3.80$, $r^2 = 0.99$, $p < 0.0001$, $Y_{CORE3} = 8.6X - 11$, $r^2 = 0.91$, $p = 0.0006$. **B** Mass accumulation rates (g cm^{-2} $year^{-1}$). The arrows indicate peaks in accretion rates after El Niño (2002) intense storms. **C** Carbon sequestration rates (g OC m^{-2} $year^{-1}$) from three cores at our natural habitat, Haslams Creek, Sydney Olympic Park, New South Wales.

Soil Respiration

Soil respiration was significantly higher in the top soil (0–10 cm) compared to the deeper soil (20–30 cm) regardless of habitat (Figure 5A; Table S2; habitat: $F_{(1,30)} = 1.43$, $p = 0.70$, depth: $F_{(1,30)} = 12.2$, $p = 0.001$, habitat × depth: $F_{(1,30)} = 0.01$, $p = 0.91$). We found a positive trend between respiration and aboveground biomass, but the regression was not significant (Figure 5B, $r^2 = 0.3$, $p = 0.22$). We also found a significant linear relationship between soil respiration and belowground biomass (Figure 5C, $r^2 = 0.71$, $p = 0.03$).

Microbial Communities

Sequence abundance of microbial communities from our bulk soils, rhizosphere and roots was mostly comprised by Bacteria 67–96%, followed by Eukaryota 1.8–30% and Archaea 0.5–1.8% (Figure S2A–C). Bulk soil and rhizosphere samples were comprised of a similar sequence abundance of Bacteria, Eukaryota and Archaea in both habitats, but roots exhibited a higher sequence proportion of bacteria and a lower sequence proportion of Eukaryota in the natural habitat compared to the regenerated habitat (Figure S2C).

Alpha diversity for bulk soil and rhizosphere samples was similar between habitats, but alpha diversity of roots was highest in the natural habitat (Figure S3; Table S5, habitat: $F_{(1,12)} = 1.83$, $p = 0.20$, soil sample type: $F_{(2,12)} = 41.08$, $p < 0.0001$, habitat × soil sample type: $F_{(2,12)} = 8.3$, $p = 0.005$).

At a phylum level bulk soil and roots were similar between habitats but for rhizosphere samples, Proteobacteria were significantly more abundant in the regenerated habitat (Figure 6A–C). Among soil types, the abundance of phylum significantly varied but the two most abundant phyla were Proteobacteria followed by Bacteroidetes (Table S5; Figure 6A–D).

Table 2. Radiocarbon Results of Our Three Cores Sampled from the Natural Habitat at Haslams Creek, New South Wales, Australia

Core	Depth (cm)	*R*-corr. ^{14}C Age (BP)[a]		Calibrated age (cal. BP)[b]		
		Mean	1σ	1σ range	2σ range	Median
1	59–60	976	32	798–906	773–922	851
	98–99	1322	37	1181–1270	1090–1284	1215
2	59–60	902	31	734–789	685–900	761
	98–99	1498	33	1310–1361	1300–1406	1341
3	59–60	1115	31	932–1045	926–1058	969
	98–99	1761	34	1585–1700	1545–1708	1638

[a]*All the reservoir (R)-corrected ^{14}C ages were calculated using an R value of 310 ± 24 ^{14}C years derived from paired measurements of ^{210}Pb and ^{14}C on a sediment sample in core 3 at 15–16 cm (^{210}Pb age = 1869 ± 27 AD and conventional ^{14}C age = 456 ± 19 BP).*
[b]*Age calibration was performed using the SHCal13 data (Hogg and others 2013) and OxCal program V.4.2 (Bronk 2009).*

Table 3. Carbon Sequestration Rates Obtained from Radiocarbon Values at Our Natural Habitat at Haslams Creek, New South Wales, Australia

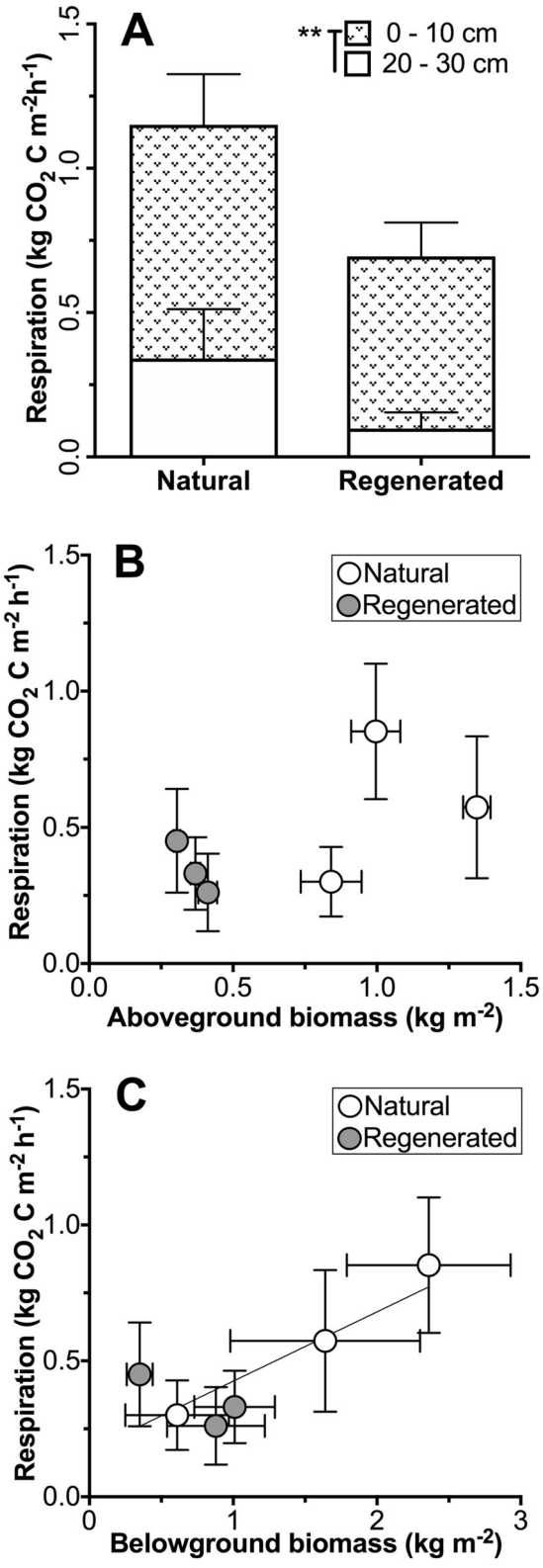
Core	Depth (cm)	Median age (cal BP)[a]	g OC m^{-2} year^{-1}
1	60	851	21
	99	1215	20
2	60	761	22
	99	1341	16
3	60	969	17
	99	1638	12
1, 2, 3	60		
	Mean	860	20
	SE	60	2
1, 2, 3	99		
	Mean	1398	16
	SE	125	2

[a]*Age calibration was performed using the SHCal13 data (Hogg and others 2013) and OxCal program V.4.2 (Bronk 2009). See Table 2 for details.*

We found a strong correlation between decreased aboveground biomass and increments in proportion of Proteobacteria in rhizosphere samples (r^2 = 0.87, p = 0.006, Figure 7A) and decreased aboveground biomass and increments in *Deltaproteobacteria* in rhizosphere samples (r^2 = 0.71, p = 0.03, Figure 7B). We also found significant correlations between increments in organic carbon density and higher proportion of *Desulfuromonas* in bulk soil (r^2 = 0.93, p = 0.006, Figure 7C) and between nitrogen density and proportion of *Desulfuromonas* in bulk soil (r^2 = 0.85, p = 0.03, Figure 7D). Additionally, we found significant correlations between organic carbon density and proportion of *Geobacter* in bulk soil (r^2 = 0.86, p = 0.006, Figure 7E) and nitrogen density and proportion of *Geobacter* in bulk soil (r^2 = 0.83, p = 0.01, Figure 7F).

Discussion

We assessed saltmarsh restoration according to a range of metrics at a natural and a regenerated habitat within Haslams Creek at the Sydney Olympic Park in New South Wales. We found that after ~ 20 years of regeneration efforts, belowground OC, soil OC and root production had recovered to natural levels. However, aboveground biomass and thus OC stock from aboveground biomass was still lower in the regenerated compared to the natural habitat. We also found that microbial communities at a phylum level were similar in bulk soil and roots between the two habitats, but that Proteobacteria were significantly more abundant in the rhizosphere of the regenerated compared to the natural saltmarshes. Two genera: *Desulfuromonas* and *Geobacter*, were corre-



◀**Figure 5.** **A** Soil respiration (kg CO_2 C m^{-2} h^{-1}) at 0–10 and 20–30 cm depth at our studied natural and regenerated habitats, Sydney Olympic Park, New South Wales. Values are means and standard errors. Respiration of soils was significantly higher at the top 0–10 cm, $^{**}p = 0.001$. **B** Relationship between soil respiration (kg CO_2 C m^{-2} h^{-1}) and aboveground biomass (kg m^{-2}); the regression was not significant, $r^2 = 0.3$, $p = 0.22$; and **C** soil respiration (kg CO_2 C m^{-2} h^{-1}) and belowground biomass (kg m^{-2}); the regression was $Y = 0.25X + 0.17$, $r^2 = 0.71$, $p = 0.03$.

lated with carbon and nitrogen density in soils. Typical metrics used to evaluate wetland restoration success include plant biomass and species diversity as well as soil OC and nitrogen accumulation (Moreno-Mateos and others 2012). However, the metrics selected to measure wetland restoration success largely depend on the goals of restoring a particular site (Zedler 2007). Within our study, we focused on measuring ecosystem functions associated with the carbon cycle within our restored urban saltmarshes.

We suggest that lower values of aboveground biomass and its concomitant OC stock in the regenerated compared to the natural habitat are associated with the presence of a fringing mangrove forest in the natural area. Despite occurring within close proximity, with similar tidal elevations at ~ 0.9 m AHD, and pore-water salinities of ~ 35–40 ppt, the fringing mangrove forest in the natural site can protect saltmarshes from daily wind and wave action. Previous studies have shown that wetland fringing vegetation reduces the velocity of water flow thereby reducing erosion of saltmarshes even if they are not fully submerged (Gedan and others 2009). Protection of saltmarshes from erosion by a fringing mangrove forest may be particularly important during large storm events. Thus, aboveground biomass of our regenerated habitat may never recover to levels similar to those of the natural habitat. Additionally, our regenerated area is located ~ 2.1 km away from the creek's mouth while the natural area is located 1.5 km away from the creek's mouth. Because water flows away from the creek's mouth in this urban environment, the regenerated area accumulates anthropogenic rubbish that may structurally damage the aboveground vegetation during tidal movement, although this idea remains to be tested.

Differences in aboveground biomass between habitats can impact inputs of OC within soils, but aboveground biomass also offers habitat for a range of organisms and it has shown to influence the

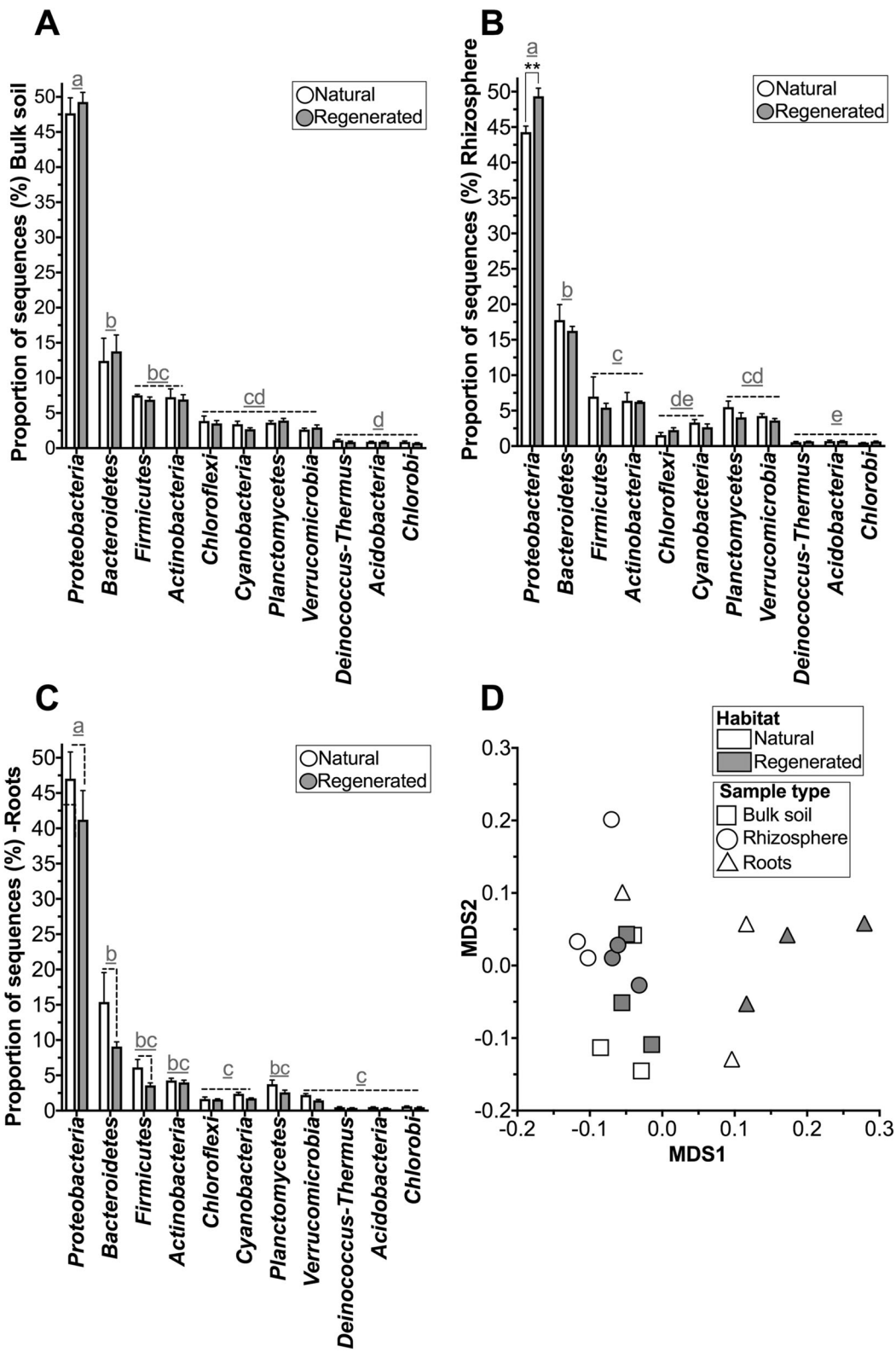


Figure 6. Proportion of sequences at the phylum level from **A** bulk soil, **B** rhizosphere and **C** roots. Different letters represent significant differences with $p < 0.05$ and ** indicates a significant level of $p < 0.01$). **D** Multidimensional scaling analysis performed with a Bray Curtis dissimilarity metric and a stress value 0.12.

abundance, density and diversity of invertebrates inhabiting saltmarshes (Levin and Talley 2002). At our study area we frequently observed crabs, for example, *Helograpsus haswellianus*, and snails, for example, *Salinator fragilis*. Further investigations would be necessary to understand the impacts of higher aboveground biomass in the natural compared to the regenerated habitat on fauna inhabiting our studied saltmarshes as well as impact on future trajectories of soil OC stocks.

Despite differences in aboveground biomass between the two habitats, we found a significant relationship between aboveground and belowground biomass for *Sarcocornia quinqueflora* ($r^2 = 0.42$, $p = 0.0006$). This relationship indicates that aboveground biomass and belowground biomass are not decoupled, and that environmental factors in addition to allometry govern partitioning of biomass in these saltmarshes. As such, belowground biomass in our study site is controlled by densities of bulk soil and nitrogen density (see below). In saltmarsh species, the belowground biomass can be much higher than that of the aboveground biomass, for example belowground biomass of *Spartina patens* and *Phragmites australis* is three to four times that of their aboveground biomass (Tripathee and Schafer 2015). Our values of aboveground biomass are similar to those reported for *Sarcocornia quinqueflora* in south-eastern Australia (Clarke and Jacoby 1994) and belowground biomass represented 52% to 60% of the total biomass.

Our study shows that soil OC stock of the regenerated habitat was similar to that of the natural habitat. Soil OC stocks from 0 to 30 cm depth were 64.6 Mg OC ha^{-1} in the natural habitat and 47.9 Mg OC ha^{-1} in the regenerated habitat. Values of soil OC at our study area in Haslams Creek are within the ranges reported for soil OC stocks in Australian saltmarshes. For example, Macreadie and others (2017) reported that saltmarshes in Australia store from 8.89 to 603.67 Mg OC ha^{-1} and that NSW saltmarshes store 69.5 ± 3.33 Mg OC ha^{-1}. We expect OC stocks in other saltmarsh areas of the Sydney Olympic Park to be at least as high as those in Haslams Creek. Other areas within the Sydney Olympic Park are dominated by species such as *Juncus kraussi* and *Sporobolous virginicus* that can store high levels of soil OC (Kelleway and others 2016). Given soil OC comprises the largest carbon pool within saltmarsh ecosystems, conservation and monitoring of these urban rehabilitated saltmarshes could be a priority for 'blue carbon' programmes aimed at mitigating atmospheric carbon dioxide.

The age of sediments at 10 to 20 cm depth from three cores in the natural habitat determined by ^{210}Pb, were 61 to 147 years. There was evidence that sediment accretion varied over time (that is, 0.07 g cm^{-2} $year^{-1}$ to 0.42 g cm^{-2} $year^{-1}$). Rates of sediment accretion are linked to inundation frequencies and sediment supply (Rogers and others 2005; Lovelock and others 2014). Rogers and others (2013) also found that accretion accelerated after storms followed by *El Niño* drought (2001–2006) in mangroves from Kooragang Island, New South Wales. Thus, variation in sediment accretion over time might be associated with tidal frequency, variation in sediment availability from the catchment and also strong storm events.

Sediment accretion is important for the maintenance of saltmarsh under rising sea levels as it enhances wetland soil surface elevation, maintaining its position in the intertidal zone (Kirwan and Megonigal 2013). But root production and soil subsidence also influence soil surface elevation (Rogers and others 2005). Further studies to determine rates of change of surface elevation at Haslams Creek would be valuable to understand the future of saltmarshes with expected sea-level rise in Sydney, Australia, which was 0.8 mm $year^{-1}$, for the period 1966 to 2010 (White and others 2014).

OC sequestration at 10 to 20 cm depth was 109 ± 13 g OC m^{-2} $year^{-1}$ (mean ± standard error). These values are higher than those reported by Macreadie and others (2017) (54.54 g OC m^{-2} $year^{-1}$) for Australian saltmarshes, but similar to those reported for marshes in the USA and mangroves (Chmura and others 2003; Lovelock and others 2014). Rates of carbon sequestration reported by Macreadie and others (2017) are based on 323 samples from a large range of geomorphological settings such as saline coastal lagoons, marine inlets and tidal creeks, thus reflecting the variability of OC sequestration across geomorphological settings. Higher values of OC sequestration were observed in our study because fluvial saltmarshes typically sequester higher amounts of sediment and OC than other saltmarsh settings. Respective sediment ages of 761–969 and 1215–1638 cal. year BP at 59–60 and 98–99 cm depth indicate that sediment carbon within the natural saltmarsh habitat at the Sydney Olympic Park has been stored for centuries. Mean OC sequestration rates in the last ~ 860 years in our natural habitat are similar, that is, 20 ± 2 g OC m^{-2} $year^{-1}$ than those estimated for the last ~ 1300 years, that is, 16 ± 2 g OC m^{-2} $year^{-1}$. Differences in OC sequestration along the soil profile may reflect

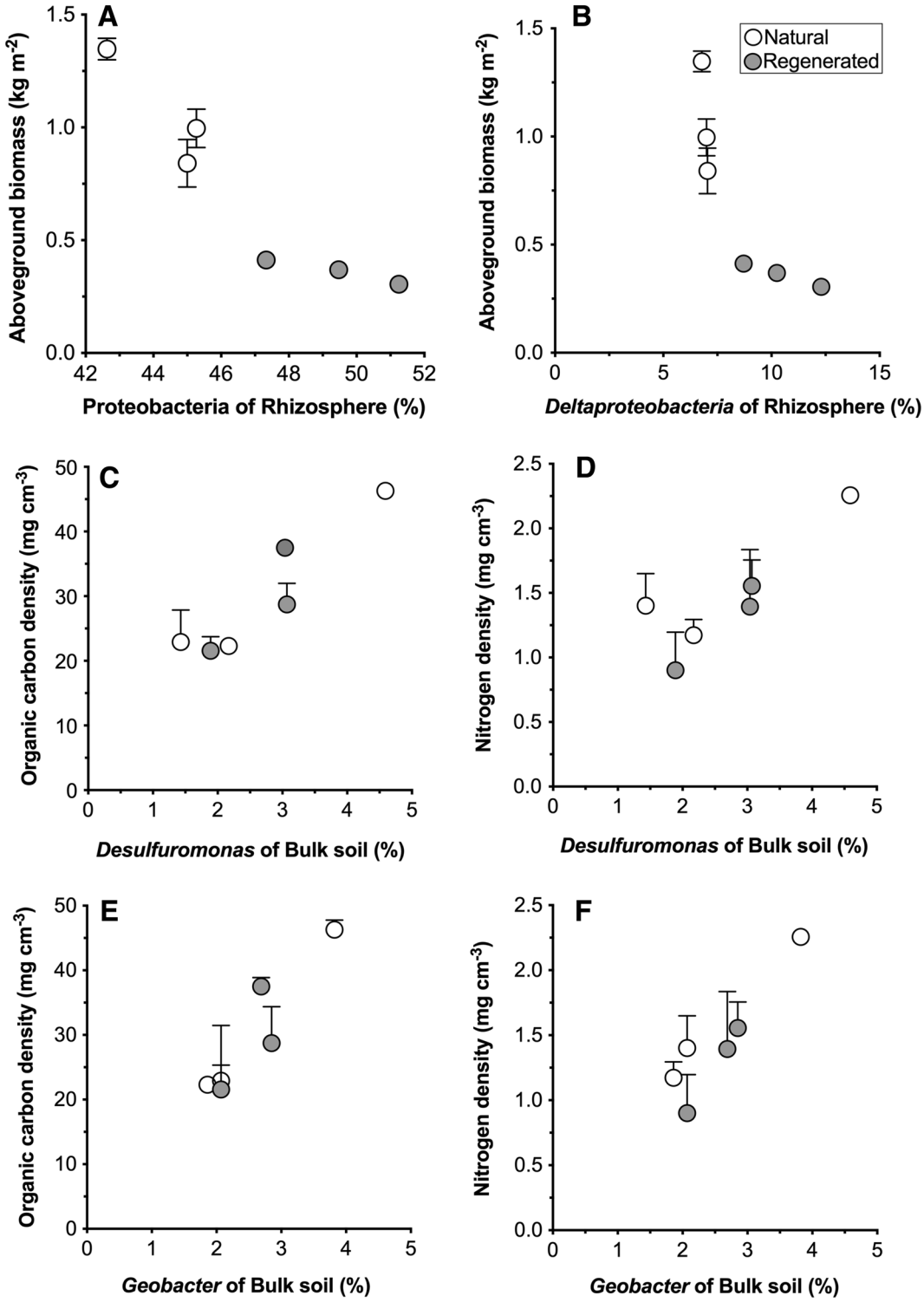


Figure 7. **A** Correlation between aboveground biomass (kg m^{-2}) and proportion of Proteobacteria in rhizosphere soil ($r^2 = 0.87$, $p = 0.006$). **B** Correlation between aboveground biomass (kg m^{-2}) and proportion of *Deltaproteobacteria* in rhizosphere soil ($r^2 = 0.71$, $p = 0.03$). **C** Correlation between organic carbon density (mg cm^{-3}) and proportion of *Desulfuromonas* in bulk soil ($r^2 = 0.93$, $p = 0.006$). **D** Correlation between nitrogen density (mg cm^{-3}) and proportion of *Desulfuromonas* in bulk soil ($r^2 = 0.85$, $p = 0.03$). **E** Correlation between organic carbon density (mg cm^{-3}) and proportion of *Geobacter* in bulk soil ($r^2 = 0.86$, $p = 0.006$ and **F** correlation between nitrogen density (mg cm^{-3}) and proportion of *Geobacter* in bulk soil ($r^2 = 0.83$, $p = 0.01$).

changes in tidal levels, and sediment and nutrient increments over time (Lovelock and others 2014).

Sarcocornia quinqueflora exhibits a relatively shallow root distribution compared to other saltmarsh species. We found that belowground biomass was highest in the top 0 to 5 cm of soil, but we encountered roots of *Sarcocornia quinqueflora* up to 30 cm depth. In contrast, Tripathee and Schafer (2015) found roots of *Spartina alterniflora*, *Spartina patens* and *Phragmites australis* distributed down to 55 cm depth. The high biomass and abundance of *Sarcocornia quinqueflora* roots in the top 5 cm of soil were associated with higher nitrogen density and lower bulk density (Figure 3E, F). Both bulk density and nitrogen concentrations of soils have been shown to influence root growth and distribution (Waisel and others 1996; McKee 2001). Nitrogen is likely to limit growth in wetland environments, and higher increments in root biomass have been observed in nutrient-rich patches versus sandy substrates (McKee 2001; Reef and others 2010). Additionally, high bulk densities, as in the bottom 20–30 cm of our saltmarsh soils, are likely to impede root growth (Waisel and others 1996).

Belowground biomass and root production are both important for accumulation of OC in saltmarshes and other wetland environments (Neubauer 2008). Our results support this idea as we found that root biomass significantly contributes to soil OC stocks. However, we found non-significant relationships between root production and soil OC stocks. Soil OC accumulation is a complex process and thus other factors such as inputs from aboveground litter, decomposition rates and carbon products from microorganisms also influence total soil OC and soil OC stock (Schmidt and others 2011). Further studies are needed to understand inputs, processing and accumulation of OC in this saltmarsh environment.

Belowground biomass also influences other ecosystem functions such as respiration. Both plant biomass and microbial community contribute to respiration from soils. Although soils are largely anoxic which suppresses respiration (Alongi 2009), bioturbation of the sediment by root growth allows for additional gas exchange and thus colonization of aerobic microorganisms where roots are distributed (Segers and Leffelaar 2001; Pi and others 2009; McLeod and others 2011). We found a strong significant linear relationship between belowground biomass and soil respiration. In addition, soil respiration was significantly higher in the top 10 centimetres of soil, where roots are more abundant, compared to the deeper soil (20–30 cm), where oxygen exchange is limited.

Investigation of variation in the microbial community shows that, at a phylum level, microbial communities were similar in bulk soil and roots between the two habitats, but that Proteobacteria were significantly more abundant in the rhizosphere of the regenerated saltmarshes compared to the natural saltmarshes. Similarities in microbial communities of roots are associated with plant exudates which determine the composition of the microbial community associated with plants (Xie and others 2015). The similarity in bulk soil microbial communities may also reflect both the vegetation and environmental similarities between habitats. However, we also found a higher proportion of Proteobacteria, particularly *Deltaproteobacteria*, in the rhizosphere of saltmarshes from the regenerated habitat. Higher proportions of Proteobacteria and *Deltaproteobacteria* in the regenerated compared to the natural habitat were associated with decreases in the aboveground biomass. Proteobacteria are typically more abundant in carbon rich soils, and Fierer and others (2007) have suggested that Proteobacteria preferentially consume labile soil organic carbon and exhibit high growth rates when resources are abundant. The higher percentage of Proteobacteria and *Deltaproteobacteria* in the regenerated habitat where aboveground biomass and its associated OC stock is lower is thus unexpected. This is particularly true given two genera of the *Deltaproteobacteria*: *Desulfuromonas* and *Geobacter* were more abundant in soils with higher OC density and N density. Both *Desulfuromonas* and *Geobacter* are anaerobic genera that participate in the mineralization of organic compounds by utilizing sulphate and iron respectively (Muyzer and Stams 2008; Calderoni and others 2017). *Desulfuromonas* and *Geobacter* have been found in abundance in anoxic environments (Shrestha and others 2009; Yamamoto and Takai 2011). Thus, *Desulfuromonas* and *Geobacter* may be key genera for assessments in the recovery of ecosystem characteristics such as carbon and nitrogen mineralization. Further characterization of soils and variation in the availability of different pools of carbon and nitrogen over the restoration sequence may provide productive avenues of further research to understand trends in the microbial communities.

Conclusions

Restoring ecosystem functions is a key goal of contemporary restoration. Using a range of metrics such as OC stocks, OC accumulation, root production, soil respiration and assessment of microbial

communities, we found that habitat restoration of saltmarshes within the Sydney Olympic Park in New South Wales has achieved similar levels of soil and belowground OC stocks within ~ 20 years. Soils in both restored and natural habitats are heterogeneous and, in both habitats, we found that two key genera, *Desulfuromonas* and *Geobacter*, are associated with high carbon and nitrogen density, suggesting that these microorganisms may play a key role in ecosystem function, especially within high resource patches. The functioning of these soils has implications for long-term carbon storage: sediment at 99-cm depth in our natural habitat has been stored in this saltmarsh environment for millennia, with a median of 1215 to 1638 cal. year BP. Soils of Haslams Creek store similar levels of OC compared to those reported in other sites in the region, and as such, monitoring and conserving these urban rehabilitated saltmarshes can be valuable for 'blue carbon' programmes aiming at mitigation of atmospheric carbon dioxide. Selecting metrics to assess the progress of wetland restoration depends on the goals of restoring a particular project; however, setting up the conditions necessary for long-term 'blue carbon' storage is often a key target. Given that the restoration trajectories of OC stocks can be difficult to predict, long-term monitoring of restored saltmarshes, as well as assessing multiple reference sites, may be required to observe restorative trends.

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