A study over 33 years shows that carbon and nitrogen stocks in a subtropical soil are increasing under native vegetation in a changing climate

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HIGHLIGHTS

- We measured SOC stocks in a subtropical soil at 0–0.3 m depths for 33 years.
- SOC stocks increased under native vegetation by 5.85 Mg C ha^{-1} over 33 years.
- Climate change actually increased SOC stocks in the clay Vertisol.
- It is likely that increases in CO_2 concentrations increased biomass productivity.
- Soil total nitrogen stocks increased by 0.57 Mg N ha^{-1} over 33 years.

GRAPHICAL ABSTRACT
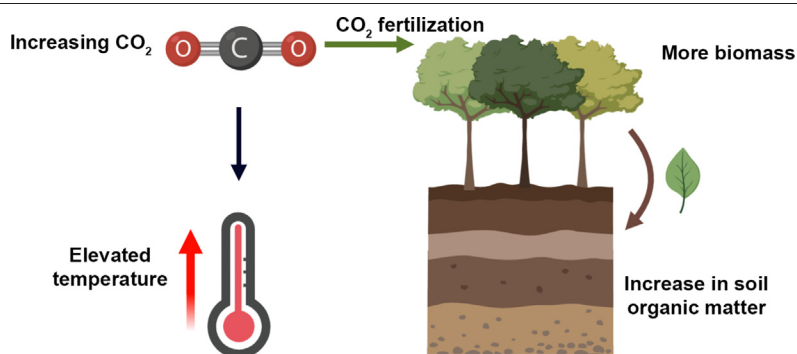
ABSTRACT

Soil plays a critical role in the global carbon (C) cycle. However, climate change and associated factors, such as warming, precipitation change, elevated carbon dioxide (CO_2), and atmospheric nitrogen (N) deposition, will affect soil organic carbon (SOC) stocks markedly – a decrease in SOC stocks is predicted to drive further planetary warming, although whether changes in climate and associated factors (including atmospheric N deposition) will cause a net increase in SOC or a net decrease is less certain. Using a subtropical soil, we have directly examined how changes over the last three decades are already impacting upon SOC stocks and soil total nitrogen (STN) in a Vertisol supporting native brigalow (*Acacia harpophylla* L.) vegetation. It was observed that SOC stocks increased under native vegetation by 5.85 Mg C ha^{-1} (0.177 ± 0.059 Mg C ha^{-1} y^{-1}) at a depth of 0–0.3 m over 33 years. This net increase in SOC stocks was not correlated with change in precipitation, which did not change during the study period. Net SOC stocks, however, were correlated with an increasing trend in mean annual temperatures, with an average increase of 0.89 °C. This occurred despite a likely co-occurrence of increased decomposition due to higher temperatures, presumably because the increase in the SOC was largely in the stable, mineral-associated fraction. The increases in CO_2 from 338 ppm$_v$ to 395 ppm$_v$ likely contributed to an increase in biomass, especially root biomass, resulting in the net increase in SOC stocks. Furthermore, STN stocks increased by 0.57 Mg N ha^{-1} (0.0174 ± 0.0041 Mg N ha^{-1} y^{-1}) at 0–0.3 m depth, due to increased atmospheric N deposition and potential N$_2$ fixation. Since SOC losses are often predicted in many regions due to global warming, these observations are relevant for sustainability of SOC stocks for productivity and climate models in semi-arid subtropical regions.

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1. Introduction

Organic carbon (C) and nitrogen (N) are the integral components of soil organic matter (SOM), which is essential for agricultural sustainability and terrestrial environmental stability. Of particular interest, soil is also an important sink for C, with soils storing ca. 2344 Gt of organic C in the top 3 m (ca. 1500 Gt in the top 1 m) (Jobbágy and Jackson, 2001; Scharlemann et al., 2014) and exceeding that in the atmosphere (870 Gt C in 2018) and vegetation (600 Gt C) combined (Crowther et al., 2016; Scharlemann et al., 2014).

The stock of soil organic C (SOC) at any given time represents the net change in organic C inputs and outputs, with changes in environmental conditions or land management practices potentially altering the SOC stocks markedly (Kopittke et al., 2017). For example, an increase in temperature increases the rate of SOC decomposition (Davidson and Janssens, 2006), but a concomitant increase in CO_2 concentrations may increase biomass productivity (Fensham and Fairfax, 2005; Pan et al., 2011; Peng et al., 2017), and hence increase inputs of organic C to soils (Oren et al., 2001). Also of interest, it is known that atmospheric N deposition has increased by an order of magnitude over the last 100 years (Galloway et al., 2008; Ren et al., 2017). Importantly, this increased deposition of N into soils can potentially decrease the loss of SOC and may even result in an increase in SOC (de Vries et al., 2014; Peterson and Melillo, 1985), especially as microbial necromass (Liang et al., 2019) although potentially N_2O emissions may increase in N-rich, high precipitation, ecosystems such as humid tropical rainforest (Dalal and Allen, 2008). Thus, both the inputs and outputs of organic C to soil are influenced by climate change and associated environmental factors, including increasing temperatures, variable precipitation, elevated CO_2 concentrations (CO_2 fertilization), and increasing atmospheric N deposition (de Vries et al., 2014; Hungate et al., 2007).

Despite its critical importance, there remains considerable uncertainty as to whether changes in climate and associated factors (including atmospheric N deposition) will cause a net increase in SOC or a net decrease. Certainly, it would appear likely that the magnitude of change in SOC with climate change will vary considerably around the world, largely depending upon latitude and other factors (Crowther et al., 2016). We are unaware of any studies for tropical and subtropical soils that provide a decades-long time series of field SOC compared to changes in climate and environmental conditions, specifically under native vegetation, showing how changes in climate and environmental conditions over the last few decades have already impacted upon SOM.

In the present study, for the first time, we examine how climate change and associated factors are already affecting SOC stocks under native vegetation in a subtropical soil. We examined the SOC stocks and soil total nitrogen (STN), and their natural abundances down to 0.3 m depth, over a 33-year period (1981–2014) in a Vertisol, supporting undisturbed, native, mature brigalow (*Acacia harpophylla* L.) C_3 vegetation. We examined the stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) as this provides insights into C and N cycling, including information on their input sources. For example, a decrease in $\delta^{13}C$ of the C_3 vegetation with decreasing mean annual precipitation over time indicates water stress (Robinson, 2001). Therefore, the objectives of this study were to: 1) ascertain the trends in SOM stocks (SOC and STN) and SOC fractions over a period of 33 years; 2) quantify the accompanying changes in the natural abundance $\delta^{13}C$ and $\delta^{15}N$ of SOM and infer their sources; and 3) characterize any possible relationships between SOM and climate change factors during this period. These factors include mean annual values for air temperature and precipitation, as well as atmospheric CO_2 concentration and N deposition. It was predicted that SOC stocks in all fractions would decrease over the 33 year experimental period given that rates of SOC decomposition increase at elevated temperatures, and that any increased productivity from CO_2 fertilization would not be sufficient to counter the increased rate of SOC decomposition.

2. Materials and methods

2.1. Sampling site

The study site is located at 24.81°S and 149.80°E (Queensland, Australia) at an altitude of 151 m above sea level in a subtropical climate (Fig. 1). At this site, the native vegetation has never been harvested or removed and remains undisturbed. Mean annual precipitation is 720 mm and annual potential evaporation is 2100 mm. The mean maximum temperature is 33.1 °C in January and mean minimum temperature is 6.5 °C in July (Cowie et al., 2007). The main soil types at the site are Grey Vertosols/Dermosols and Sodosols (Isbell, 2002), being Vertisols/Mollisols and Alfisols in Soil Taxonomy. Vertisols and Mollisols occupy 70% of the catchment area and Alfisols occupy the remaining 30%. Clay contents of the dominant soil (Vertisol) vary from 36% (0–0.1 m) to 44% (0.2–0.3 m), and respective pH of 6.7 and 8.1, SOC from 2.36% to 0.84%, and soil total N (STN) from 0.177% to 0.067% [see Table 1, also see Thornton and Shrestha, 2020]. The dominant native vegetation at the site is brigalow (*Acacia harpophylla* L.) which is a C_3 vegetation. Other species include belah (*Casuarina cristata* L.) and blackbutt (*Eucalyptus cambageana* L.) which are also C_3 vegetation. A detailed description of the site is available from Cowie et al. (2007).

2.2. Soil sampling

Soil sampling was typically conducted during the dry months of winter and spring (minimum biological activity) over a period of 33 years, in 1981, 1983, 1985, 1987, 1990, 1994, 1997, 2000, 2008, and 2014 at three monitoring sites supporting undisturbed, mature, native vegetation. The three permanent 20 m × 20 m monitoring sites (forming three replicates) are contained within the 16.8 ha 'Catchment 1' of the Brigalow Catchment Study. Each of these three replicates had similar aspect, slope, and vegetation (Cowie et al., 2007). Establishment of the three 20 m × 20 m replicates was done using double stratification.

From each of the three replicates at each sampling year, five samples were taken down to 0.3 m depth by a hydraulic-driven corer (0.05 m diameter) and divided into 0–0.1 m, 0.1–0.2 m, and 0.2–0.3 m depths. These five samples were bulked for respective depths, thus yielding one composite sample for each of the three depths for all three of the replicate sites. Hereafter, data are presented for depths of 0–0.1, 0.1–0.3, and 0–0.3 m. We include data for 0–0.3 m given that this is recommended by IPCC (2006). Soil cores from each depth were used for bulk density measurements. Bulk density was calculated as the mass of 105 °C oven-dry soil per volume of core sampled. Since the soils did not contain gravel, no corrections in bulk density were required.

Soil samples were dried at 40 °C, ground to pass <2 mm sieve, and stored at room temperature until analysis. Soil samples were analysed for soil pH, particle size distribution, and SOC fractionation (<0.25 mm) using mid-reflectance infrared spectrometer (MIR). For total C and N (see below), soil samples were ground <0.1 mm in a ring mill. All the soil samples were analysed simultaneously at the same point in time (i.e. after 33 years), with each parameter measured using the same instrument and the same operator. This was to ensure that the overall results were directly comparable.

2.3. Soil analysis for organic carbon, N and $\delta^{13}C$ and $\delta^{15}N$

Total SOC, STN, and natural abundance ^{13}C and ^{15}N values of the soil samples were determined using an Isoprime isotope ratio mass spectrometer (IRMS) coupled to a Eurovector elemental analyser (Isoprime-EuroEA 3000, Milan, Italy). The analytical precision for the measurement of ^{13}C and ^{15}N were ±0.02‰ and ±0.01‰, respectively. Samples containing inorganic carbonates were pre-treated with HCl before analysis. Total SOC and STN were also determined using TruMac CN (LECO Corporation, St Joseph, MI, USA), with the values presented for SOC and STN hereafter being these values. Samples containing
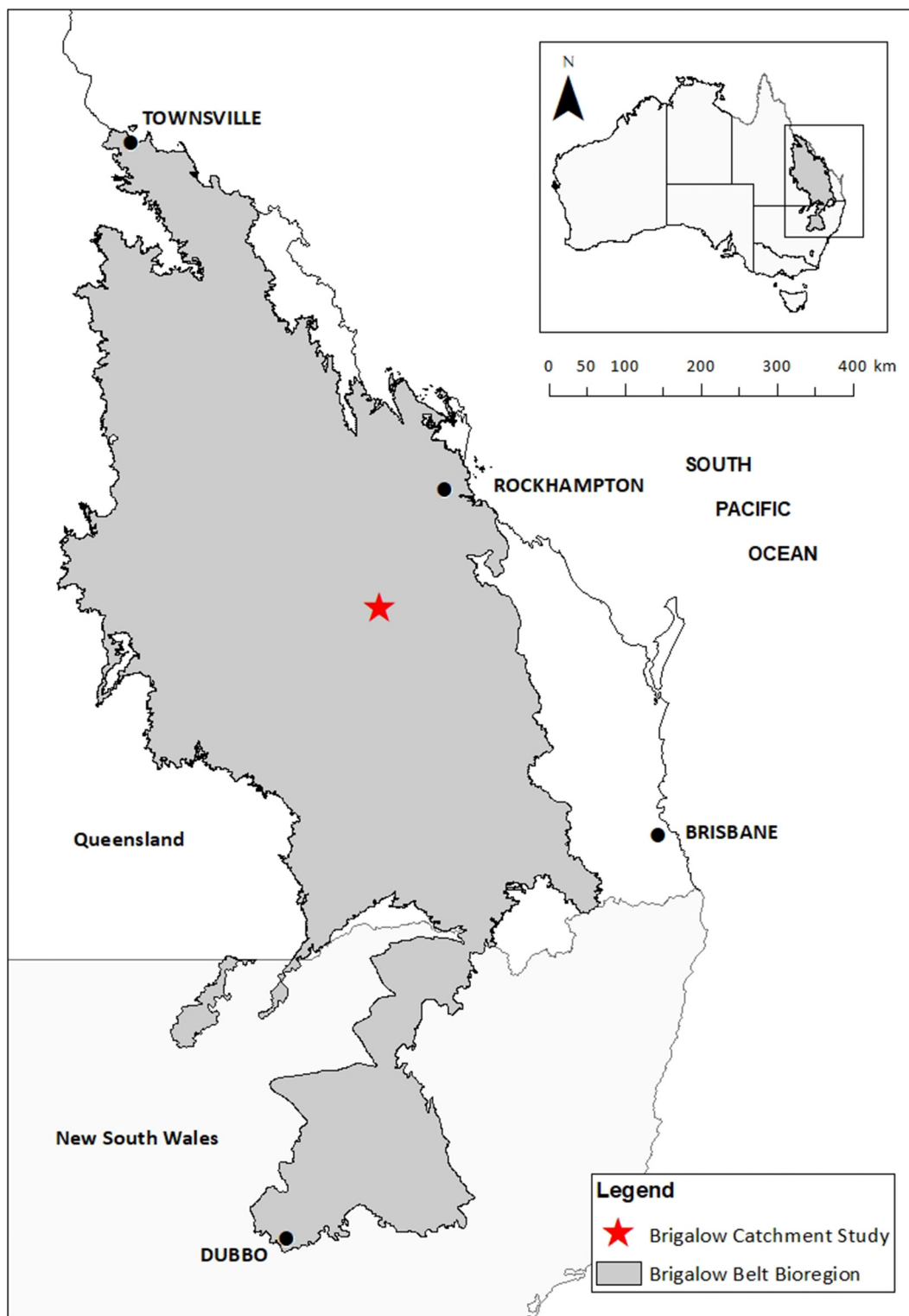
Fig. 1. Map showing the study site in Queensland, Australia.

carbonates were pre-treated with HSO_3 rather than HCl to avoid damage to the instrument. The isotope ratios were expressed using the 'delta' notation (δ), with units of 'per mil' or 'parts per thousand' (‰), relative to the marine limestone fossil Pee Dee Belemnite standard (Craig, 1953) for $\delta^{13}C$, using the following relationship:

$$\delta^{13}C\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000 \qquad (1)$$

where R is the molar ratio of $^{13}C/^{12}C$ of the sample or standard.

Samples containing approximately 50 µg N were weighed into 8×5 mm tin (Sn) capsules and analysed against a known set of standards. The isotope ratios were expressed using the 'delta' notation (δ), with parts per thousand (‰), relative to N_{air} standards (Peterson and Fry, 1987) for $\delta^{15}N$ using the following relationship:

$$\delta^{15}N\ (‰) = \left(R_{sample}/R_{standard} - 1\right) \times 1000 \qquad (2)$$

Table 1
Bulk density, clay content, pH, organic C, total N of the Vertisol at the study site (1981). See Thornton and Shrestha (2020).

Depth (m)	Bulk density (Mg m^{-3})	Clay content (%)	pH	Organic C (%)	Total N (%)
0.0–0.1	1.19	36	6.6	2.0	0.16
0.1–0.2	1.24	40	7.5	1.4	0.11
0.2–0.3	1.43	44	8.1	0.69	0.06

where R is the molar ratio of the heavy to light isotope (i.e. $^{15}N/^{14}N$) of the sample or standard.

2.4. Soil organic matter fractionation

The SOC fractions of soil samples were estimated using MIR on Thermo Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., MA, USA), which was calibrated before and during measurements using 312 standard soil samples. The detailed procedure of spectra analysis, including partial least-squares regression (PLSR), for estimating different fractions are given by Baldock et al. (2013). Briefly, 10 g soil sample (<2 mm) was dispersed in 40 mL solution of 5 g L^{-1} sodium hexametaphosphate and sieved to separate the >50 μm coarse fraction and the ≤50 μm fine fraction using an automated fractionation process. Using ^{13}C nuclear magnetic resonance (NMR) spectroscopy, the resistant organic C (ROC) content was determined. The particulate organic C (POC) content was determined as the non-ROC in the coarse fraction whilst the mineral-associated organic C (MAOC) was determined as the non-ROC in the fine fraction.

2.5. Soil bulk density and calculation of stocks

Average soil bulk density values, interpolated across the entire duration of the study, were 1.21 ± 0.01 Mg m^{-3} at 0–0.1 m, 1.31 ± 0.01 Mg m^{-3} at 0.1–0.2 m, and 1.46 ± 0.02 Mg m^{-3} at 0.2–0.3 m. For the 0–0.1 m layer, the bulk density values ranged from 1.16 to 1.27 Mg m^{-3}, with corresponding values being 1.28 to 1.35 Mg m^{-3} for 0.1–0.2 m, and 1.37 to 1.58 Mg m^{-3} for 0.2–0.3 m. The cumulative amounts of SOC and total N for 0–0.1 m, 0–0.2 m, and 0–0.3 m depths were, therefore, corrected for equivalent soil mass using the polynomial relationship ($r^2 > 0.99$) between the amount of soil carbon and soil mass (soil depth × bulk density) (Dalal et al., 2005a) at the minimum soil mass for each depth (Pringle et al., 2011). The minimum soil mass values were 1101 Mg ha^{-1} for 0–0.1 m, 2287 Mg ha^{-1} for 0–0.2 m, and 3682 Mg ha^{-1} for 0–0.3 m. Using these same values for equivalent soil mass, similar corrections were made for the stocks of the SOC fractions and STN.

2.6. Aeolian dust deposition

An aeolian dust sample was collected from the site in sufficient amount to analyse for total C, N, and natural abundance ^{13}C and ^{15}N values. Two replicate samples were collected in March 2020 and immediately analysed as outlined above. The amount of aeolian dust deposition was estimated for the site from McTainsh and Lynch (1996) and the amounts of C and N were then calculated from the annual amount of aeolian dust (31 Mg km^{-2} y^{-1}) and the concentrations of C and N in the aeolian dust.

2.7. Statistical analysis

The data were transformed where necessary to obtain normal distribution before statistical analysis. The results were analysed using one-way analysis of variance (ANOVA) and are presented as mean values with standard error of the means. The relationships between the period of monitoring and SOC and SOC fractions, and STN values were

evaluated using Pearson correlations and regression analysis (Snedecor and Cochran, 1967). All linear regression models were assessed at alpha = 0.05. The data were plotted using SigmaPlot v. 13.0.

3. Results

3.1. Changes in climate during the monitoring period

Mean annual precipitation varied from a minimum of 312 mm in 1993 to a maximum of 1350 mm in 2010, although it showed no trend over time during the entire period (Fig. 2a). It was also noted
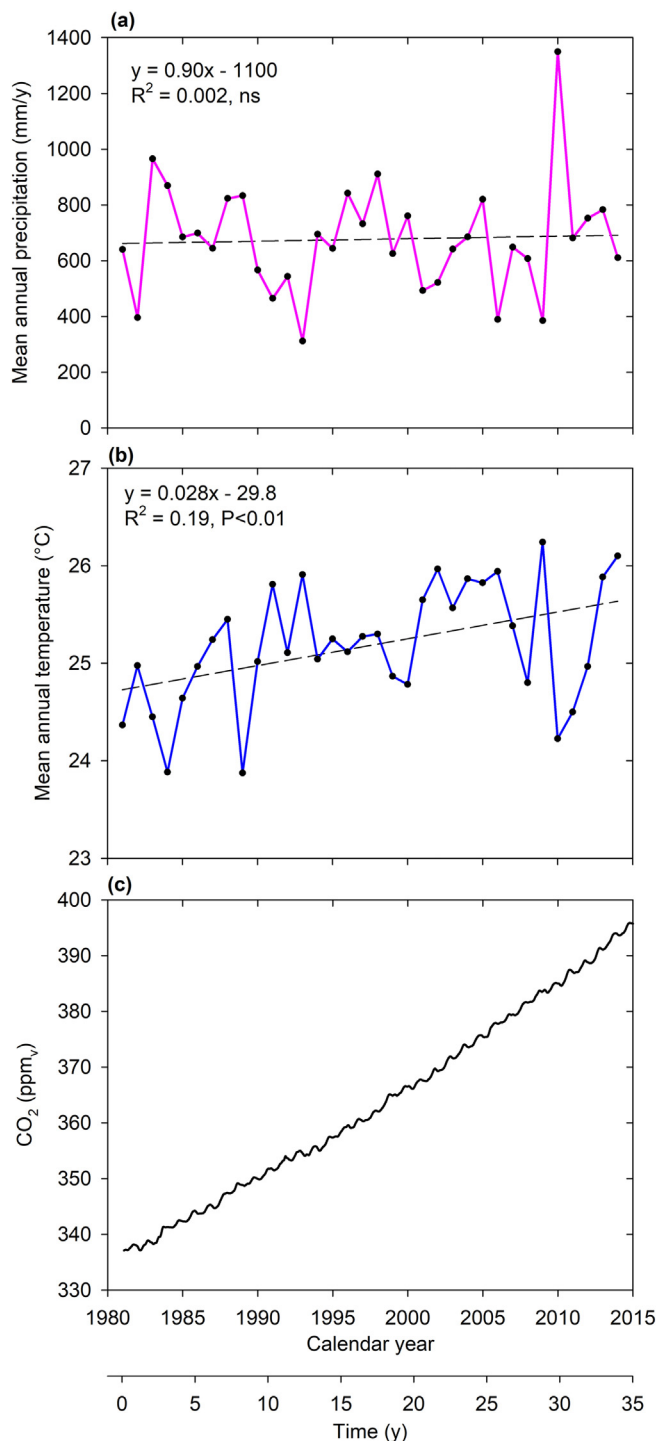

Fig. 2. (a) Mean annual precipitation, (b) mean annual temperature (MAT), and (c) carbon dioxide (CO_2) concentration, from 1981 to 2014 (CSIRO, 2020).

that mean annual precipitation during the monitoring period from 1981 to 2014 (680 mm) was similar to the longer-term mean annual precipitation from 1962 to 2016 (720 mm). Mean annual temperature, calculated using SILO PPD (scientific information for land owners patched point data, a database of Australian climate data), increased by 0.028 °C y^{-1} [mean annual temperature (°C) = 0.028 year + 24.7 °C, R^2 = 0.19, $P < 0.01$, $n = 33$], or an increase of 0.89 °C (Fig. 2b). The atmospheric CO_2 concentration at the international atmosphere monitoring site in the southern hemisphere, Cape Grim (40.68°S, 144.68°E), Tasmania (Australia), the closest atmosphere monitoring site to the study site, increased from 338 ppm$_v$ (720 Gt C) in 1981 to 395 ppm$_v$ (841 Gt C) in 2014 (CSIRO, 2020) (Fig. 2c), an increase of 57 ppm$_v$ CO_2 or additional 121 Gt C in the atmosphere during the monitoring period.

3.2. Soil organic carbon stocks and natural abundance $\delta^{13}C$ values

The soil organic C stocks at 0–0.1 m depth predicted from the linear regression were 28.2 Mg ha^{-1} in 1981 and 29.9 Mg ha^{-1} in 2014 (Fig. 3a), representing an increase of 0.055 Mg C ha^{-1} y^{-1} at 0–0.1 m. The corresponding values from the linear regression for the SOC stocks at 0.1–0.3 m depths were 26.7 Mg ha^{-1} in 1981 and 30.6 Mg ha^{-1} in 2014 (Fig. 3a), representing an increase of 0.12 Mg C ha^{-1}y^{-1}. The cumulative SOC stocks at 0–0.3 m depths were 54.8 Mg ha^{-1} in 1981 and 60.6 Mg ha^{-1} in 2014 (Fig. 3a), representing an increase of 0.17 Mg C ha^{-1} y^{-1} ($P < 0.05$). However, the natural abundance $\delta^{13}C$ values of SOC showed no significant trends at any of the three depth intervals considered (Fig. 3b).

3.3. Soil organic carbon distribution in different soil fractions

The size of the POC fraction at a depth of 0–0.1 m remained essentially similar although the MAOC fraction tended to increase at a
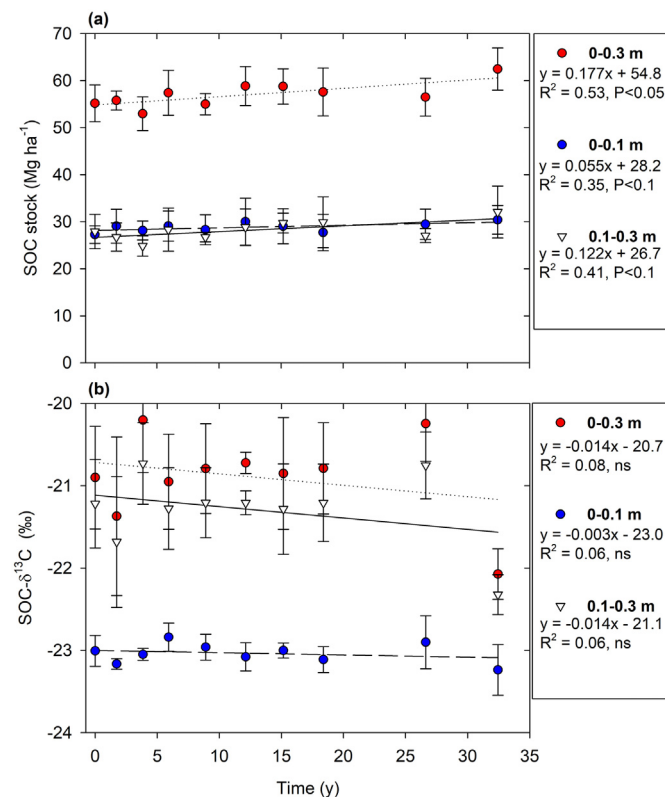
depth of 0–0.1 m during the monitoring period (Fig. 4a). The amount of the ROC fraction at 0–0.1 m remained similar during the monitoring period.

At a depth of 0.1–0.3 m, the MAOC fraction increased linearly at the rate of 0.139 ± 0.039 Mg ha^{-1} y^{-1} whereas the POC and ROC fractions showed no significant trend during the monitoring period (Fig. 4b). At a depth of 0–0.3 m, the MAOC fraction increased linearly at the rate of 0.183 ± 0.038 Mg C ha^{-1} y^{-1} (Fig. 4b). In contrast, the POC and the ROC fractions remained constant during the monitoring period (Fig. 4c).

3.4. Soil total nitrogen stocks and natural abundance $\delta^{15}N$ values

Soil total N stocks estimated from the linear regression at a depth of 0–0.1 m were 2.0 Mg ha^{-1} in 1981 and 2.3 Mg ha^{-1} in 2014, representing a significant increase of 0.0077 Mg N ha^{-1} y^{-1} (Fig. 5a). At 0.1–0.3 m depth, STN stocks were 2.15 Mg ha^{-1} in 1981 and 2.5 Mg ha^{-1} in 2014, representing a linear increase of 0.0097 Mg ha^{-1} y^{-1} (Fig. 5a). At a depth of 0–0.3 m depth, the STN stocks predicted by the linear regression were 4.18 Mg ha^{-1} in 1981 and 4.75 Mg ha^{-1} in 2014, representing an increase of 0.017 ± 0.0040 Mg N ha^{-1} y^{-1}

Fig. 3. Changes in (a) soil organic C (SOC) stocks at depths of 0–0.1 m, 0.1–0.3 m, and 0–0.3 m, and (b) natural abundance SOC-δ^{13}C values at a natural brigalow vegetation site over a 33-year period. Data are the arithmetic mean of three replicates ± standard error of the mean.
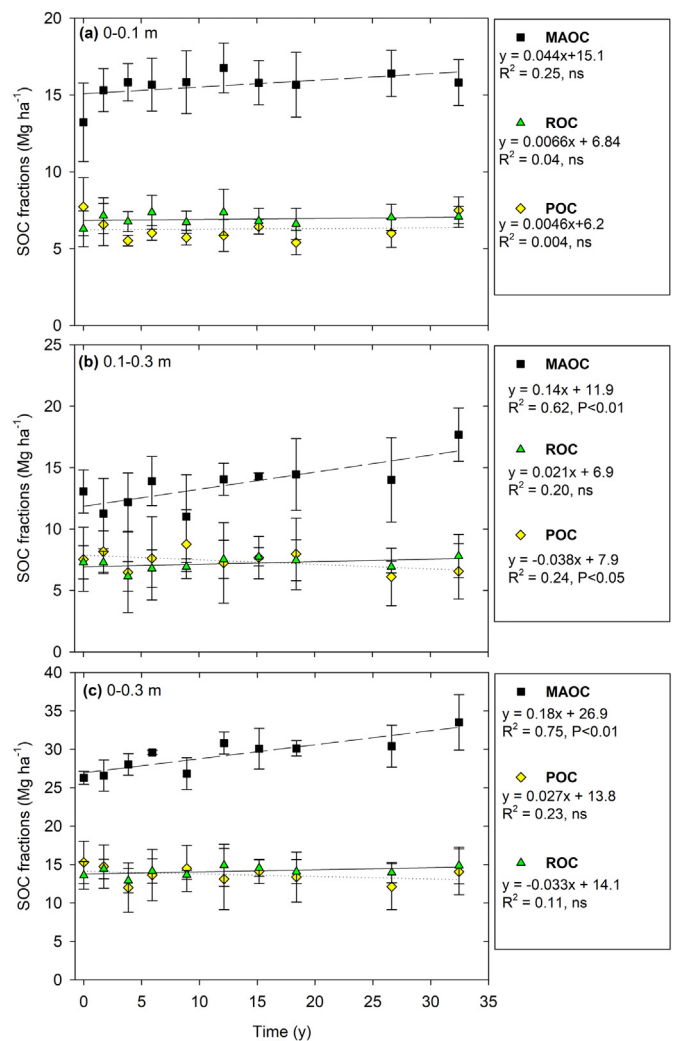
Fig. 4. Changes in soil organic C (SOC) fractions at (a) 0–0.1 m depth, (b) 0.1–0.3 m depth, and (c) 0–0.3 m depth for a natural brigalow vegetation site over a 33-y period. The C fractions are particulate organic C (POC), the mineral-associated C (MAOC), and resistant organic C (ROC). Data are the arithmetic mean of three replicates ± standard error of the mean.
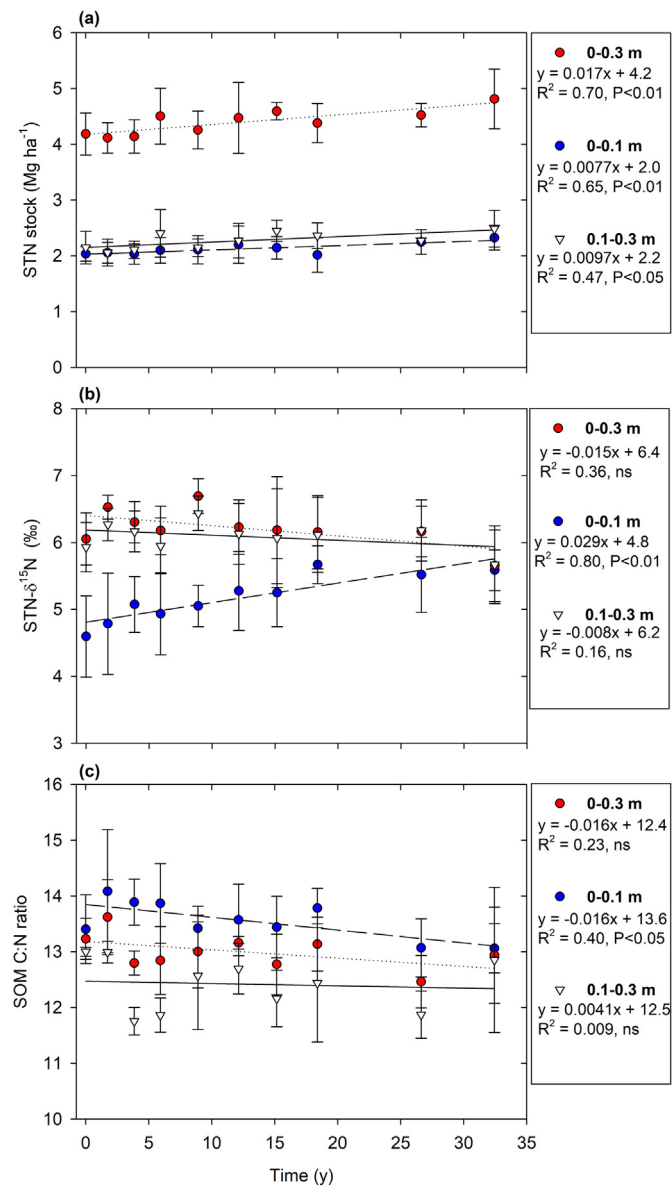
Fig. 5. Changes in (a) soil total N (STN) stocks at 0–0.1 m, 0.1–0.3 m, and 0–0.3 m depths, (b) natural abundance STN-δ^{15}N values, and (c) C:N ratio at a natural brigalow vegetation site over a 33-y period. Data are the arithmetic mean of three replicates ± standard error of the mean.

(Fig. 5a). The natural abundance δ^{15}N values of STN linearly increased from 4.6 ± 0.6‰ in 1981 to 5.6 ± 0.3‰ in 2014 at 0–0.1 m depth, corresponding to a significant increase of 0.03 ± 0.005‰ δ^{15}N y^{-1} (Fig. 5b). However, at 0.1–0.3 m and 0–0.3 m depths, the δ^{15}N value showed no significant trends across the monitoring period (Fig. 5b).

3.5. Soil C:N ratio

The soil C:N ratio decreased at 0–0.1 m depth across the monitoring period, from an average value of 13.4 ± 0.6 in 1981 to 13.1 ± 0.4 in 2014 ($P < 0.05$, Fig. 5c). A similar trend was observed for the C:N ratio at 0.1–0.3 m and 0–0.3 m depths, although these changes in the C:N ratio were not significant (Fig. 5c). From the linear rates of increase in the SOC and STN stocks (Figs. 3a and 5a), we calculated the ΔSOC:ΔSTN ratios, which were 7.1 at a depth of 0–0.1 m, 12.1 at a depth of 0.1–0.3 m and 10.2 at a depth of 0–0.3 m.

3.6. Relationship between SOC stocks and environmental factors

The SOC stocks at 0–0.3 m depths showed significant ($P < 0.05$) increase with increasing mean annual temperature ($P < 0.05$, Fig. 6a). However, the SOC stocks (0–0.3 m) were not affected by mean annual
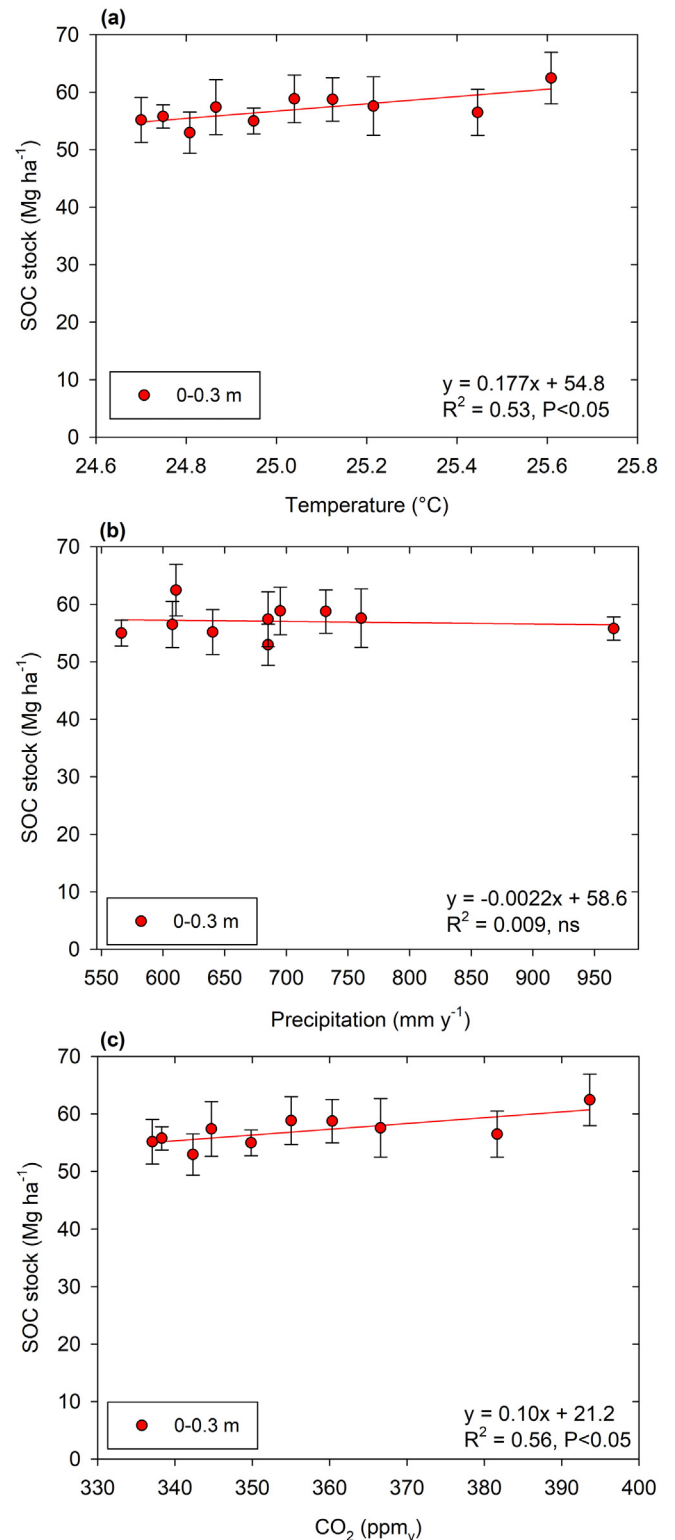

Fig. 6. Changes in soil organic C (SOC) stocks at 0–0.3 m depths at a natural brigalow vegetation site over a 33-year period compared to (a) mean annual temperature, (b) annual precipitation, and (c) carbon dioxide concentration. Data are the arithmetic mean of three replicates ± standard error of the mean.

precipitation (Fig. 6b). Soil organic C stocks (0–0.3 m) increased significantly ($P < 0.05$) with increasing atmospheric CO_2 concentrations, from a stock of 55.2 ± 3.9 Mg ha^{-1} at a CO_2 concentration of 338 ppm$_v$ in 1981 to a stock of 62.5 ± 8.5 Mg ha^{-1} at a CO_2 concentration of 395 ppm$_v$ in 2014 ($P < 0.05$, Fig. 6c).

3.7. Aeolian dust deposition

The aeolian dust sample was found to have an average total C concentration of 5.3%, a total N concentration of 0.57%, a $\delta^{13}C$ value of −20.5‰, and a $\delta^{15}N$ value of 6.0‰. Accordingly, the C:N ratio for the aeolian dust was 9.24.

4. Discussion

4.1. SOC stocks increased over time as climate and environmental conditions changed

In this study, we observed that the rates of SOC stock change across the monitoring period (1981–2014) were 0.055 ± 0.026 Mg C ha^{-1} y^{-1} at a depth of 0–0.1 m, 0.122 ± 0.052 Mg C ha^{-1} y^{-1} at a depth of 0.1–0.3 m, and 0.177 ± 0.059 Mg C ha^{-1} y^{-1} at a depth of 0–0.3 m in a native Brigalow forest of subtropical Queensland, Australia (Fig. 2a). Across the entire soil depth (0–0.3 m) using the guidelines from IPCC (2006), almost all of this increase in the SOC stock occurred due to a marked increase in the MAOC (Fig. 4b) in the 0.1–0.3 m depth where the clay content is higher (44%), with this being a stable SOC fraction. Presumably this increase occurred due to increased inputs (see below) rather than a redistribution of existing SOC. Our observation here that SOC stocks are increasing over time under native vegetation is of critical importance given the role of soil as an important sink for C. Furthermore, it is noted that for many soils, models generally predict that SOC stocks will decrease over time due to climate change [for example, see Crowther et al., 2016]. In our present study, in which we observed changes over three decades in a subtropical (low latitude) soil in a semi-arid region, we observed that SOC stocks increased. These direct measurements are in general agreement with predictions that losses in SOC are likely to be greatest in high latitude soils and lowest in low latitude soils (Crowther et al., 2016; Smith, 2012). Given that climate change (increasing temperatures, variable precipitation), and associated factors (elevated CO_2 concentrations, and increasing atmospheric N deposition) can impact upon both inputs and outputs of SOC, it is necessary to consider the likely mechanisms by which SOC increased over time.

4.2. Possible environmental control factors of SOC stocks

It is necessary to first understand what might cause the net increase in SOC stocks in this subtropical soil (Fig. 3a). This increase in SOC stocks could be due to increased biomass productivity, either from changes in precipitation or due to CO_2 fertilization (Lenka and Lal, 2012), as persistent increase in C sinks has been observed in the World's forests (Pan et al., 2011). However, we found no significant relationship between mean annual precipitation and SOC stocks (Fig. 6b) or SOC $\delta^{13}C$ at either 0–0.1 m or 0.1–0.3 m depths. Biomass C input to the soil was primarily from C$_3$ vegetation. The vegetation $\delta^{13}C$ signature is an indicator of long-term plant water availability (Ehleringer, 1993), and thus the lack of any significant trend in annual precipitation observed at this site may not have had a significant effect on changes in biomass $\delta^{13}C$, and hence, on SOC $\delta^{13}C$ values (Ehleringer et al., 2000).

Thus, changes in precipitation could not account for the observed changes in SOC stocks. Rather, we contend that the increase in SOC stocks was due, at least in part, to increasing atmospheric CO_2 concentrations (Fig. 6c), although further studies are required to verify this. Indeed, across the monitoring period, atmospheric CO_2 concentrations increased from 338 to 395 ppm$_v$ (Fig. 2). This elevated CO_2 can increase

SOC stocks by increasing plant biomass, especially in C$_3$ vegetation (Lenka and Lal, 2012). Furthermore, especially with increased N availability, SOC stocks can also increase at elevated CO_2 because of increases in the root:shoot ratio, primarily by increasing fine root production (Cusack et al., 2010; Schulte-Uebbing and de Vries, 2018). These previous observations that elevated CO_2 can increase SOC stocks are consistent with our findings in which we observed a consistent increase in the SOC stock over time, especially in the stabilised MAOC at 0.1–0.3 m depth (Figs. 3a and 4b). Thus, it remains possible that the increase in SOC stocks observed under native vegetation (Fig. 3) is due to the CO_2 fertilization effect. However, further work is required to verify this hypothesis, including studies with direct measurements of changes in biomass production considering the fact that a large and persistent carbon sink has been observed in the world's forests (Pan et al., 2011). It is also important to note that although increasing CO_2 concentrations would be expected to result in the values for SOC-$\delta^{13}C$ becoming more positive, since atmospheric-$\delta^{13}C$ are simultaneously becoming more negative over time [decreasing from −7.6 in 1981 to −8.4 in 2014, Graven et al., 2017], with these two effects potentially opposing each other and resulting in no net change in the SOC-$\delta^{13}C$ (Fig. 3b). However, further work would be required to confirm this hypothesis regarding the SOC- $\delta^{13}C$.

Although precipitation showed no trend in the present study (and could not account for changes in net SOC stocks), other previous studies have found that increases in precipitation are important in controlling SOC stocks. Indeed, some studies have found that increases in precipitation appear to be more important than increases in temperature (Garten et al., 2009; Hungate et al., 2007; Longbottom et al., 2014). For example, in a grassland soil in semiarid northern China, it was found that variations in soil respiration, microbial respiration, and microbial biomass were positively related to fluctuations in precipitation (Liu et al., 2009). In contrast, however, other studies have suggested that decreases in precipitation may have little effect on SOC stock since it lowers soil CO_2 efflux and slows down cycling in coarse organic matter (Chen et al., 2018).

It should also be considered whether the increase in SOC stocks could be attributed to the input of C from aeolian dust. The C inputs from aeolian dust correspond to an input of 0.017 Mg C ha^{-1} y^{-1}. In contrast, the increase in SOC stock across the monitoring period was found to be 0.177 ± 0.059 Mg C ha^{-1} y^{-1} at a depth of 0–0.3 m. Thus, C inputs from aeolian dust could only account for ca. 10% of the total increase in SOC stocks observed at a depth of 0–0.3 m. When considering the trend in SOC increase only at 0–0.1 m depth, which equated to 0.055 ± 0.025 Mg C ha^{-1} y^{-1}, aeolian dust accounts for almost 30% of the total increase in SOC stocks at 0–0.1 m depth.

4.3. Why did increasing temperature not cause a net decrease in SOC stocks?

Across the 33 year monitoring period, we found that temperature increased by 0.89 °C (Fig. 2) as did net SOC stocks at all depths (Fig. 3a). However, this increase in temperature was expected to cause a net decrease in SOC due to enhanced mineralization. Therefore, our hypothesis that increase in temperature results in SOC loss is rejected. Indeed, it is known that SOC decomposition follows Michaelis-Menten and Arrhenius models, with SOC decomposition increasing by a factor of two for every 10 °C increase in temperature (Davidson and Janssens, 2006). Thus, why did increasing temperature not cause a net decrease in the SOC stock due to increasing decomposition? In this regard, it is noteworthy that much of the increase in SOC stock was due to an increase in the MAOC in the 0.1–0.3 m depth (Fig. 4b). Given that the MAOC fraction is considered to be relatively stable, SOC decomposition may have been inhibited. In other words, SOC loss may be minimal as a consequence of increasing temperature (Conant et al., 2011; Giardina and Ryan, 2000), although this is not universally accepted. For example, Karhu et al. (2019) found that whilst the mechanisms by which SOC

are stabilised within the soil affect its temperature sensitivity, the temperature sensitivity was unrelated to the age of the MAOC. This uncertainty is evident in the 'carbon quality-temperature hypothesis', which states that more recalcitrant SOM should have higher temperature sensitivity, but the effect of temperature on the more recalcitrant SOC fractions remains unclear (Reynolds et al., 2017). In this regard, Conant et al. (2011) reviewed the effect of climate on SOC stocks and observed that the impacts of climate warming on SOC stocks have shown contradictory findings from field and controlled climate experiments. Specifically, given that SOC mineralization relies upon many factors (such as temperature, physical protection of the SOC, and so forth), studies need to ensure that they consider these multiple factors in tandem so that they are reflective of field conditions (Conant et al., 2011).

4.4. Does increasing atmospheric N deposition also play a role in increasing SOC?

It is also necessary to consider the potential role of increasing atmospheric N deposition, with atmospheric N deposition increasing substantially as global synthetic N use (agriculture and other industries) has increased in the last 70 years (Ackerman et al., 2019; Galloway et al., 2008; Liu et al., 2013; Ren et al., 2017). Atmospheric bulk N deposition, mostly wet deposition of NH_4^+-N and NO_3^--N, has been reported to vary from 4 kg N ha^{-1} to 60 kg N ha^{-1}. Although we did not measure total N deposition in the present study, Hunter and Cowie (1989) estimated a total N deposition rate at this site of 17 kg N ha^{-1} y^{-1} (0.017 Mg N ha^{-1} y^{-1}), accounting for both wet deposition and dry deposition as well as organic and inorganic forms of N (Kjeldahl digestion). Of this, we calculated that the aeolian dust contained 0.57% N, and assuming a deposition rate of 31 Mg km^{-2} y^{-1} (McTainsh and Lynch, 1996), this corresponds to 0.0018 Mg N ha^{-1} y^{-1} (i.e. approximately 10% of the estimated total N deposition at this site at 0–0.3 m and 22% at 0–0.1 m depth). However, this aeolian dust deposition rate was not measured at our study site and hence care needs to be taken in this regard.

The increase in N deposition observed globally over the last 70 years can potentially result in increases in STN stocks (Chang et al., 2019; Tipping et al., 2017). Indeed, in the present study, we found that both SOC and STN increased from 1981 to 2014 under native vegetation site. For example, at 0–0.1 m depth, SOC increased by 0.055 Mg C ha^{-1} y^{-1} and STN by 0.0077 kg N ha^{-1} y^{-1}; while at 0–0.3 m depth, SOC increased at the rate of 0.177 Mg C ha^{-1} y^{-1} and STN increased at the rate of 0.0174 Mg N ha^{-1} y^{-1} (Figs. 3 and 5), with a C:N ratio of 10.2. Accordingly, we found a significant increase in SOC stocks with increasing STN stocks for all three depths (Fig. 7). Addition of N to the soil allows for an increase in the retention of plant C within the MAOC
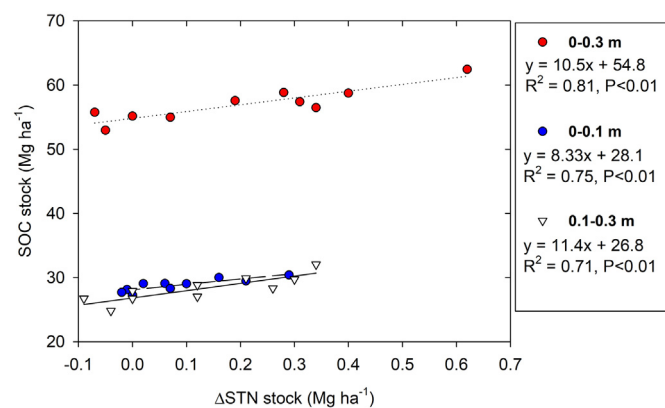

Fig. 7. Relationship between soil organic C (SOC) stocks at depths of 0–0.1 m, 0.1–0.3 m, and 0–0.3 m and changes in soil total N (ΔSTN) stocks for a natural brigalow vegetation site over a 33-y period.

fraction given that the MAOC pool has a relatively fixed stoichiometry (Finn et al., 2016). Using meta-analysis, de Vries et al. (2014) estimated an average soil C response of 9.1 kg C for each kg of N; our value of 10.2 kg C for each kg of N is close to their estimated value.

It is possible that this additional N deposition (and the concomitant increase in STN) made an important contribution to the observed net increase in SOC stocks (Tipping et al., 2017). This results in additional SOC storage or reduced SOC decomposition, especially in the stabilised SOM (Ramirez et al., 2010; Tan et al., 2017; Zak et al., 2017), thereby maintaining C and N stoichiometry of the MAOC fraction (Finn et al., 2016). We also noted that soil δ^{15}N values increased at 0–0.1 m depth as the monitoring period increased (Fig. 5b), which suggests proteins/amino acids as the N source (Ostle et al., 1999), likely as the N-transformed microbial biomass products (Robinson, 2001) or necromass (Liang et al., 2019). These observations are in agreement with Kopittke et al. (2018) who found that N-rich microbial biomass products provide new MAOC on microaggregates. However, it is also possible that this increase in δ^{15}N values was due in part to an increase in discrimination during nitrification and denitrification, or to greater abiotic losses of NO_x and NH_3. It is also noteworthy that although elevated CO_2 can increase SOC stocks through increased biomass production in natural ecosystems, N limitations can restrict this increased biomass production and the concomitant increase in SOC stocks (Liu et al., 2013; Luo et al., 2004; Oren et al., 2001). It is useful to consider the potential source of this additional N. The STN- δ^{15}N at 0–0.1 m depth increased significantly (0.03‰ y^{-1}, Fig. 5b), and an increase in the STN stock of about 0.250 Mg N ha^{-1} (Fig. 5a). The origin of this N was less likely from N_2 fixation source/s, which would have resulted in decrease in δ^{15}N values due to N_2 fixation (N_2 air, δ^{15}N = 0). However, N_2 fixation cannot be completely ruled out given that N transformations result in an increased δ^{15}N value and N_2 fixation results in a decreased value (Robinson, 2001), thus the net change in δ^{15}N value depends on the magnitude of both of these processes. In this context, Dalal et al. (2005b) estimated that a native, mature acacia (*Acacia aneura* L.) fixed as much as 25 kg N ha^{-1} y^{-1} in a semi-arid region of Australia. The likely source of at least a portion of this N is from the wet and dry deposition of atmospheric N. Indeed, the sample of deposited aeolian dust at the site had a δ^{15}N value of 6.05‰. Using a mixing model (Balesdent and Mariotti, 1996; Robinson, 2001), 22.4% of the increase in STN at 0–0.1 m is attributed to the aeolian dust N. Furthermore, soil δ^{15}N enrichment could also occur due to microbial N transformation processes during SOM turnover (Robinson, 2001) although this should have been accompanied by δ^{13}C enrichment, which remained essentially similar (−23.0 ± 0.2‰) during the monitoring period. However, given that δ^{15}N values of the deposited atmospheric N and total N deposition were measured only in one year, further work is required to understand the role of amount and nature of N deposition on SOC and STN stocks.

Thus, overall, our data show that over a period of 33 years, net SOC stock increased by 5.85 Mg C ha^{-1} (0.177 ± 0.059 Mg C ha^{-1} y^{-1}) at a depth of 0–0.3 m in a subtropical native brigalow forest. This net increase in SOC stocks may be due to the increase in CO_2 from 338 ppm$_v$ to 395 ppm$_v$ contributing to increased biomass productivity, as has been observed for world's forests (Pan et al., 2011), although further work is required in this regard. Total annual precipitation could not account for the net increase in SOC stocks. Interestingly, although temperature is known to increase the rate of SOC decomposition, the temperature increase of 0.89 °C in this experiment did not cause a net decrease in SOC. Instead, we observed that most of the increase in the SOC stock was due to an increase in MAOC, and given that this C fraction is relatively stable, it was likely that the loss of this additional SOC due to increasing temperature may be minimal. Furthermore, the comparatively low precipitation at this site would have reduced the magnitude of the increase in SOC decomposition caused by this increasing temperature. Finally, we also found that STN stocks increased by 0.57 Mg N ha^{-1} (0.0174 ± 0.004 Mg N ha^{-1} y^{-1}) at 0–0.3 m depth over the monitoring period, likely due to increasing atmospheric N deposition and N_2

fixation. It is possible that this additional N accretion to SOM may slow soil microbial respiration, resulting in reduced SOC decomposition, contributing to an increase in STN and SOC stocks. The results of this study for a subtropical, semi-arid region highlights how global predictions of ecosystem response to climate change, which are biased towards soil in the northern hemisphere, require further study and optimization for soils in subtropical Australia.

5. Conclusion

We examined the trend in SOC dynamics in a semi-arid subtropical region for a period of 33 years. There was no trend in mean annual precipitation but the mean annual temperature increased by 0.89 °C, and atmospheric CO_2 concentration increased by 57 ppm$_v$ during this period. We found that SOC stocks increased from 54.8 ± 3.9 Mg C ha^{-1} in 1981 to 60.6 ± 8.4 Mg C ha^{-1} in 2014; the corresponding increase in STN stocks was from 4.18 ± 0.38 Mg N ha^{-1} and 4.75 ± 0.54 Mg N ha^{-1} at 0–0.3 m depth. It is possible that CO_2 fertilization may have increased biomass productivity (Fensham and Fairfax, 2005; Pan et al., 2011; Peng et al., 2017), and C inputs, which were stabilised in mineral associated organic C, possibly due to N inputs from not only N$_2$ fixation, but also from both wet and dry atmospheric N deposition, although further studies are required. These factors and processes may have reduced the adverse effect of global warming on SOC decomposition and loss since N accretion slows down the turnover of C inputs and stabilises C in stable SOC fraction. These findings are relevant for productivity as well as global climate models, which should consider SOC dynamics and CO_2 feedback in a regional context. Further observations on long-term SOC dynamics in different regions, especially in semi-arid tropical and subtropical natural ecosystems are warranted.

CRediT authorship contribution statement

Ram C. Dalal: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Craig M. Thornton:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Diane E. Allen:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Peter M. Kopittke:** Data curation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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