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Carbon sequestration under subtropical perennial pastures I: Overall trends

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Abstract. The use of subtropical perennial grasses in temperate grazing systems is increasingly being promoted for production and environmental benefits. This study employed a combination of elemental and stable isotope analyses to explore whether pastures sown to either kikuyu (*Pennisetum clandestinum*) or a combination of panic (*Panicum maximum*) and Rhodes grass (*Chloris gayana*) could increase soil organic carbon (SOC) levels in five regions across southern Australia. Carbon was sequestered under kikuyu at a rate of 0.90 ± 0.25 Mg C ha^{-1} year^{-1} along the south coast of Western Australia. Lower but still significant sequestration rates were found for kikuyu in South Australia (0.26 ± 0.13 Mg C ha^{-1} year^{-1}). No changes in SOC were found for panic–Rhodes grass pasture systems in the northern district of Western Australia. Additionally, we found no changes in SOC when kikuyu-based pastures were established on formerly cropped paddocks in the Namoi Catchment of New South Wales. Stable isotope results corroborated these findings and suggested that, where SOC has accumulated, the gains have been dominated by SOC derived from the perennial vegetation and have been concentrated in the upper 10 cm of soil.

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Introduction

The introduction of perennial grasses, especially in lower and more seasonal rainfall zones, can provide production and environmental benefits (Sanford *et al*. 2003; Nichols *et al*. 2012). As such, many farmers have looked, or are looking, to convert some or all of their annual pastures to perennial based systems. In addition to these agronomic benefits, converting to a perennial-based pasture system may increase soil organic carbon (SOC) stocks. With the advent of the Australian government's Carbon Farming Initiative in July 2012, farmers now have a potential added incentive to convert some of their annual pastures over to perennials. However, there has been very little quantitative research exploring the SOC implications of converting to a perennial-based pasture system.

Perennial grasses, compared with annual grasses, generally allocate a greater fraction of productivity to the maintenance of a deeper and more extensive root system (Jackson and Roy 1986; Nie *et al*. 2008), including associated mycorrhizal fungi (Wilson and Hartnett 1998). Increased below-ground C allocation coupled with an increase in duration of C inputs and decreased surface erosion (Kort *et al*. 1998) due to greater grass cover through dry months, relative to annual grass pasture, should lead to increased soil C stocks. Respiratory losses of SOC may also be reduced under perennial pastures because off-season rains are effectively and rapidly utilised relative to typical summer fallow of annual grasses (Paydar *et al*. 2005). However, not all perennial grasses exhibit these growth forms and strategies that are favourable for building SOC stocks (Nie *et al*. 2008).

Analytical data to support these theoretical assertions remain limited. Young *et al*. (2009) found significant increases in SOC stocks (0.15–0.35 Mg C ha^{-1} year^{-1}) over 6 years of repeated-measurements when various perennial pastures were established on a formerly cultivated Vertosol in central New South Wales (NSW). In a replicated field trial, Chan *et al*. (2011) found no difference in sequestration rates to 30 cm between annual and perennial [mixture of phalaris (*Phalaris aquatica* L.), cocksfoot (*Dactylis glomerata* L.), and lucerne (*Medicago sativa* L.)] pastures when established on a formerly rotationally cropped, acidic soil in NSW. Those authors attributed the lack of difference to similar levels of biomass production for the two pasture types (Li *et al*. 2006). Chan *et al*. (2010) also found no difference in SOC stocks between pairs of commercial annual- and perennial-based pasture systems in southern NSW and northern Victoria. Low replication ($n = 4$) in this latter study may have prevented detection of differences given the natural variability in SOC across the landscape. In a warm temperate region of NSW with non-limiting irrigation, Neal *et al*. (2013) found that kikuyu (*Pennisetum clandestinum* Hochst. ex Chiov.) led to a significant increase in SOC after 3 years. Importantly, those authors found that temperate forages, despite matching the above-ground yield of kikuyu, did not change SOC levels.

The present study was designed to investigate whether sowing subtropical perennial grasses into temperate pasture systems leads to increased SOC levels. We have focussed on examining changes in SOC where the C_4 subtropical perennial grasses kikuyu and a mix of Gatton panic (*Panicum maximum* Jacq.) and Rhodes grass (*Chloris gayana* Kunth) have been sown into formerly C_3-dominated pastures or cropping systems. By limiting this study to locations where C_3 to C_4 transitions have occurred, we can utilise stable carbon isotope measurements to distinguish how much of the SOC found under the perennial grasses is attributable to the new perennial grasses, because C_3 and C_4 plants have unique isotopic fingerprints (Cerling *et al*. 1989). Thus, isotope measurements yield an independent means to assess changes in SOC levels between annual- and perennial-based pastures that is less reliant on the quality of the perennial–annual pairing.

Here, we report on changes in SOC stocks and the accumulation of C_4-derived SOC following the introduction of subtropical perennial grasses in several agronomically important regions across southern agricultural Australia. Known and potential sources of variability in the calculation of SOC change are then explored to build a case for the integrity of these findings. Finally, potential reasons for the variation in results between regions and species type are discussed. In the companion paper (Sanderman *et al*. 2013, this issue), we further explore reasons for regional differences in SOC sequestration rates by tracing the flow of new C into fractions of SOC.

Methodology

We have adopted a paired-site approach in this study. For each identified pasture where subtropical perennial grasses have replaced C_3 temperate vegetation, we also sampled a neighbouring C_3-based system that was representative of the state of the perennial pasture at the time of conversion from temperate to subtropical perennial vegetation. Within each region, field sites were selected to span a range of perennial pasture ages, thus enabling us to adopt a chronosequence approach to comment on the multi-decadal changes that may be occurring in SOC. As the extent of use of subtropical perennial grasses is still fairly limited, we had to be opportunistic in our site selection. In each region we worked with local agronomists and/or farming systems groups to identify appropriate sites. To ensure that the paired sites were as similar as possible, all sites were inspected before sampling to ascertain similarity in soil type and management history.

Field locations

Forty-two locations were included in this project from five agricultural regions (Table 1):

(1) Fleurieu Peninsula, South Australia (SA). In this high-rainfall region, four kikuyu-based pastures were sampled

Table 1. Selected regional attributes

Soil type presented to Suborder of the Australian Soil Classification (Isbell 2002), with dominant surface soil texture given in parentheses. MAT, MAP: Mean annual temperature and precipitation, respectively; standard deviation of MAT, MAP, and slope given in parentheses

Region	Perennial grass	*n*	Pasture age range (years)	Dominant land use	Dominant soil type	MAT (°C)	MAP (mm)	Summer/annual rainfall ratio	Focal mean slope (%)
Fleurieu Peninsula, SA	Kikuyu	4	3–45	Dairy and beef cattle	Red Kurosol (variable)	14.2 (0.5)	804 (104)	0.20	9.3 (1.1)
Kangaroo Island, SA	Kikuyu	5	3–14	Sheep (wool and meat)	Red Sodosol (sandy loam)	15.1 (0.3)	579 (50)	0.18	1.7 (0.4)
Northern district, WA	Panic–Rhodes grass	14	3–18	Cattle and sheep (wool/meat)	Bleached-Orthic Tenosol (sand)	19.4 (0.8)	515 (60)	0.11	4.3 (2.0)
Southern district, WA	Kikuyu	14	3–33	Sheep (wool/meat) and cattle	Yellow-Orthic Tenosol or Yellow Sodosol (sand)	16.5 (0.3)	547 (106)	0.25	1.2 (0.8)
Namoi catchment, NSW	Kikuyu	5	2–18	Beef cattle	Red Chromosols (clayey loam)	18.2 (0.1)	618 (16)	0.51	1.6 (0.7)

in undulating terrain, with ages ranging from 3 to 45 years. Paired sites typically consisted of a mixed grass pasture dominated by perennial ryegrass (*Lolium perenne*). Soils were primarily high-fertility loams; however, site FP6 was a degraded sandy duplex soil. Dairy and beef cattle were the dominant land uses in this region.

(2) Kangaroo Island, SA. Five gently sloping pastures sown to kikuyu and ranging in age from 3 to 14 years were sampled, all on high-fertility sandy soils. Sheep grazing for meat and wool production dominates in this region.

(3) Northern Agricultural District of Western Australia (WA). Fourteen panic–Rhodes grass based perennial pastures were sampled, ranging from 3 to 18 years since establishment, on low-fertility sandy soils. Terrain ranged from flat plains to rolling hills. As with the southern district, set-stocking of sheep and cattle and low but variable fertiliser histories dominate in this region.

(4) Southern Agricultural District of WA. Fourteen kikuyu-based pastures on flat to gently sloping terrain were sampled, ranging in age from 3 to 33 years, all on low-fertility sandy soils. Set-stocking of sheep and cattle and low but variable fertiliser histories dominate in this region. Nine of the locations within this zone were sampled by the University of Western Australia as part of a broader study on SOC levels in WA (D. V. Murphy, F. C. Hoyle, A. Wherrett, D. Hall, J. Sanderman, K. W. Holmes, unpubl.). At these locations, only a single composite sample at each depth was available for each pasture.

(5) Namoi Catchment, NSW. Here, formerly cropped paddocks on gently sloping terrain have been sown to kikuyu-based pastures instead of temperate pasture grass species. Composite soil samples from five sets of kikuyu pasture and paired cropped sites were contributed to this project by King and Wilson (2010) as part of the NSW Monitoring, Evaluation and Reporting (MER) program (for collection methodologies see Chapman *et al*. 2011). Pasture ages ranged from 2 to 18 years since establishment.

At each sampling location, mean climatic data were extracted from the SILO database (www.longpaddock.qld.gov.au/silo/) and various topographic attributes were extracted from the Smoothed Digital Elevation Model of Australia, of which the focal mean slope was used in further analyses in this study (details can be found in Sanderman *et al*. 2012*a*).

Sampling strategy

Due to the fact that this project has used a paired sampling approach, a different field sampling strategy than was adopted by other projects in the Soil Carbon Research Program was needed to capture whether or not significant differences existed between each pair of perennial and annual pastures (Sanderman *et al*. 2012*a*). Placing a fixed grid 25 m by 25 m at any one location within a large paddock may not give an accurate representation of the mean and variance of SOC content of that paddock. To test which sampling strategy would best capture these properties, we intensively sampled two perennial paddocks—one sown to kikuyu and the other to a combination of panic and Rhodes grass. At five randomly chosen locations within a 4-ha region of each paddock, a grid 3 m by 3 m was laid down, and every 1-m intersection of that grid was sampled in 10-cm increments to 30 cm using hand-driven volumetric cores. Using hand-driven volumetric cores ensured that accurate bulk density values would be collected with each sample. This sampling design resulted in 80 cores being retrieved from the panic–Rhodes grass pasture and 64 cores from the kikuyu pasture (one grid had to be rejected in the kikuyu pasture due to a large contrast in soil type).

From these intensively sampled paddocks, Sanderman *et al*. (2012*b*) found that: (*1*) a random sampling approach was superior to a small, fixed grid; and (*2*) eight randomly placed cores were more than adequate to capture representations of the mean and variance of SOC stocks for the region of interest.

For the remainder of the sampling in this project except for the samples contributed from different studies, eight randomly located cores over an area of ~4 ha within the pasture of interest were collected using hand-driven volumetric cores (5 cm internal diameter) (AMS Soil Core Samplers, AMS Inc., American Falls, ID, USA) in 10-cm increments. In each perennial pasture, vegetation samples (shoot and root samples) were collected from three random locations to determine the isotope ratio of the soil carbon input material. All samples were collected between June and August to minimise variability due to seasonality of grass productivity.

Analytical methods

All soil samples were air-dried in a 40°C forced-air oven within a few days of field collection. Samples were then weighed and sieved to 2 mm. Material >2 mm was weighed to determine gravel content, and for samples where it was amenable (primarily in the sandy soils of WA), coarse root material was further separated from this fraction. The fine-earth fraction (<2 mm) was then subsampled using a riffle-box splitter for subsequent analyses. Residual moisture content was determined by drying a 20-g subsample at 105°C for 24 h. A second subsample was finely ground (MM400 Mixer Mill; RETSCH, Haan, Germany) before further analyses. Details of sample preparatory procedures are given in Baldock *et al*. (2013*a*, this issue). Plant samples were dried in a 60°C oven and then finely ground before isotope analysis.

Total C and nitrogen (N) content was determined by high-temperature oxidative combustion (CNS2000; LECO Corp., St. Joseph, MI, USA). Almost all samples were found to be non-calcareous; thus, the total carbon (TC) measurement equates to total organic C (TOC). The few samples that were found to be calcareous based on a fizz test were pre-treated with sulfurous acid to remove inorganic C (IC) before analysis for TC. Analytical precision determined by repeat TOC analysis of 10% of the samples was better than 0.05 mg C g^{-1} for samples containing >4 mg C g^{-1} and better than 0.2 mg C g^{-1} for samples containing less C.

The isotopic composition of soil and plant samples was determined by coupled continuous isotope ratio mass spectrometry on a 20-20 Isotope Ratio Mass Spectrometer (Sercon Ltd, Crewe, UK) coupled with a Europa Solid/Liquid Elemental Analyser (Sercon Ltd) for sample introduction. Stable carbon isotope data are reported relative to the Pee Dee Belemnite (PDB) standard (Craig 1957) as:

$$\delta^{13}C = \frac{(R_{sample} - R_{std})}{R_{std}} \times 1000$$

where r is $^{13}C/^{12}C$ of the sample and standard, respectively. Precision of the isotope analysis determined by duplicate analysis of 10% of the samples was typically better than 0.05‰.

At all sampled paddocks, a composite sample was created for each depth by combining aliquots of each individual core in proportion to the bulk density of that core. A subsample from each of these composite samples was finely ground and analysed by mid-infrared (MIR) spectroscopy. Clay content (mg clay g^{-1} <2 mm soil) and pH were then predicted from the MIR spectra using a partial least-squares regression approach (Janik and Skjemstad 1995; Janik 2006; Baldock *et al*. 2013*b*, this issue).

Data analyses

Carbon stock data have been reported as the C content (Mg C ha^{-1}) for each soil layer by multiplying the TOC concentration (mg C g $soil^{-1}$) by the bulk density (Mg m^{-3}) by depth and by a correction for gravel content.

Data have also been reported on an equivalent-mass basis. For each pair of sites, the SOC content was adjusted to the lesser soil mass of the perennial and control treatment. For the paired site with the greater soil mass, SOC was removed from the 20–30 cm layer in proportion to the amount of soil mass that needed to be subtracted to reach the equivalent mass of the lighter pair.

Changes in SOC, whether on a fixed-depth or equivalent-mass basis, were reported as ΔSOC ($C_{perennial} - C_{control}$, Mg C ha^{-1}), and the annual rate of change was defined as ΔSOC divided by the perennial pasture age (Mg C ha^{-1} $year^{-1}$).

Isotope data were interpreted using a two-component linear mixing model between the old C_3 soil (C_{C3}), measured in the paired control site, and new soil carbon from the C_4 vegetation (C_{C4}):

$$C_S \times \delta^{13}C_S = (C_{C3} \times \delta^{13}C_{C3}) + (C_{C4} \times \delta^{13}C_{C4});$$

where C_S is the carbon stock of the entire soil ($C_S = C_{C3} + C_{C4}$). The fraction of total SOC attributable to the new C_4 carbon inputs ($f_{C4} = C_{C4}/C_S$) was calculated as:

$$f_{C4} = \frac{\delta^{13}C_S - \delta^{13}C_{C3}}{\delta^{13}C_{C4} - \delta^{13}C_{C3}}$$

Uncertainty in end-member values was accounted for by propagating the variance in each term following Phillips and Gregg (2001). The $\delta^{13}C$ and f_{C4} values for the 0–30 layer were calculated as SOC mass-weighted mean values of the individual horizons. Finally, the mass of new C_4-derived SOC was calculated by multiplying f_{C4} by the mass of SOC found in that perennial pasture.

Statistical analyses

At the regional level, two tests were performed, a two-way ANOVA (region × pasture type) and a pair-wise comparison such as a one-way, repeated-measures ANOVA (pasture type) for each region, to test for significant increases in SOC and $\delta^{13}C$. Due to the fairly low numbers of replications within each region, the variability between different farms within each region may swamp any potential differences between pasture types using the first of these approaches. The repeated-measures test eliminates some of the site-to-site variability within each region and only tests that the mean pasture type difference is significantly different from zero. These two statistical tests were performed on the 0–10 and 0–30 cm data. The SOC mass was log-transformed to normalise data. A critical assumption here was that the control paddock is representative of the state of the perennial paddock upon conversion to the perennial vegetation.

As we sampled to span a range of pasture ages, a chronosequence approach was also utilised in which the changes in SOC properties are plotted against pasture age. The chronosequence approach relies on the assumption that all other state factors (i.e. soil properties, land form, climate, and vegetation) were constant within a region and only pasture age varies (Jenny 1941). Soil type and land form were broadly similar within each region (Table 1). Vegetation was purposely selected to be similar across sites within each region. There was found to be a significant range of climatic properties within each region. Importantly, with the exception of a weak correlation ($R^2 = 0.33$, $P = 0.03$) in the Southern District of WA, climatic properties (rainfall or temperature) were unrelated to pasture age (Fig. 1). The oldest site in this region was found to be driving this correlation, and when removed, there was no residual correlation between rainfall and age ($R^2 = 0.08$). Given the similarity in soil type, landform, climate, and vegetation, the two regions in SA have been combined for the purposes of the regression analysis. Several different regression models were tested on the chronosequence data. All parametric analyses were performed in SigmaPlot, version 12 (Systat Software Inc., Chicago, IL, USA).

Results

Regional mean changes in SOC

Across the project ($n = 42$), subtropical perennial pastures stored 3.3 ± 8.6 Mg C ha^{-1} (mean ± s.d.) more SOC than their paired temperate counterparts ($P = 0.014$) to a depth of 30 cm (Table 2), translating to a mean annualised rate of change of 0.23 ± 0.85 Mg C ha^{-1} $year^{-1}$ ($P = 0.098$). At a regional level, significant increases in SOC stocks were only found for kikuyu in the Southern District, WA, and the Fleurieu Peninsula, SA. The annualised rate of change was only found to be significantly different from zero ($P = 0.001$) in the Southern District, with a mean rate of change of 0.61 ± 0.51 Mg C ha^{-1} $year^{-1}$. A much more detailed summary of bulk data from all sites is provided in Sanderman *et al*. (2012*b*).

In the Northern District of WA, SOC levels under pastures sown to a mixture of panic and Rhodes grass were, on average, no different from their annual counterparts (Table 2), with 24.8 ± 6.6 and 24.4 ± 7.4 Mg C ha^{-1} found in the perennial and annual paddocks, respectively. In fact, only one of 14 sites was found to have significantly more SOC under the panic–Rhodes pasture (data not shown).

On Kangaroo Island, the paired annual pasture for sites KI1–KI4 was on a slightly different soil type but it was the best representation of the perennial paddocks that we could find.

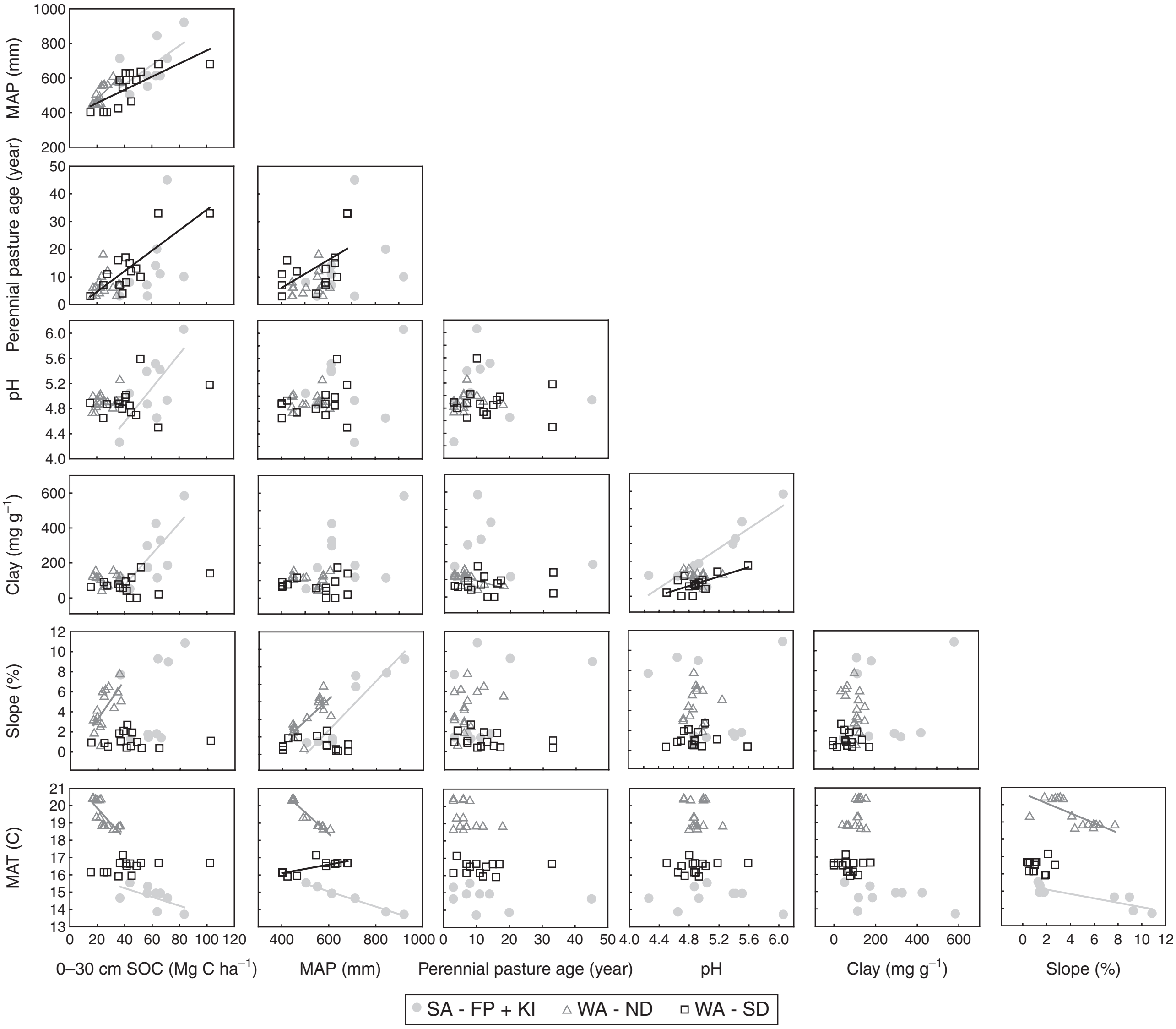

Fig. 1. Draftsman plot of perennial pasture SOC content to 30 cm, mean annual temperature (MAT), mean annual precipitation (MAP), slope (focal mean), weighted mean clay content, weighted mean pH, and perennial pasture age for the Northern and Southern Districts of Western Australia and for all of South Australia. Data for the Namoi catchment are not shown due to low sample size. Linear regression lines are shown where correlations are significant ($\alpha < 0.05$).

The annual soil had a higher watertable at the time of sampling due to a shallower depth to the clay pan. As a result, the rates of change calculated from the difference between the perennial and annual pairs (Table 2) should be interpreted with caution. For these sites on Kangaroo Island, the trend over time (i.e. Fig. 2) is likely the more accurate representation of the SOC dynamics.

On the Fleurieu Peninsula in South Australia, there was a large range in SOC content (36.5–83.5 Mg C ha^{-1} in the perennial pastures), but by focusing only on the ΔSOC values, we see that the subtropical perennial pastures had on average 5.8 Mg C ha^{-1} more than the temperate grass based pastures (Table 2).

In the Namoi Catchment of NSW, where kikuyu pastures have been sown into previously cropped paddocks, small but non-significant increases in SOC were found (Table 2), with the SOC stocks under the subtropical perennials averaging 26.2 ± 7.7 *v.* 22.9 ± 9.3 Mg C ha^{-1} in the cropped paddocks. Importantly, only one pasture in this region had been established >5 years ago.

Accumulation of C_4-derived SOC

Isotope measurements, as reported as the fraction of C_4-derived SOC (f_{C4}) present in the perennial soils, proved to be a more sensitive indicator of change in SOC stocks, with significant differences found in most regions (Table 2) and at greater depths than for SOC stocks alone (Sanderman *et al.* 2012*b*). Across all sites, the perennial paddocks contained an average of 9.2% new C_4-derived SOC, ranging from a mean of 16.9% under kikuyu in the Southern District of WA to a mean of only 4.8% under

Table 2. Overall soil organic carbon (SOC) trends: paired regional means reported on a fixed-depth and an equivalent-mass basis for the 0–30 cm total and for the 0–10 cm horizon only

The f_{C4} data are only reported on a fixed-depth basis because calculation on an equivalent-mass basis for the 0–10 cm horizon is not possible. *P*-statistic: one-way repeated-measures ANOVA

Region	ΔSOC ($Mg\,C\,ha^{-1}$)				Rate of change ($Mg\,C\,ha^{-1}\,year^{-1}$)				f_{C4} (%)	
	Fixed depth		Equivalent mass		Fixed depth		Equivalent mass		Fixed depth	
	Mean ± s.d.	*P*	Mean ± s.d.	*P*	Mean ± s.d.	*P*	Mean ± s.d.	*P*	Mean ± s.d.	*P*
					0–30 cm total					
Fleurieu Peninsula	5.77 ± 5.03	0.053	8.15 ± 5.35	0.071	0.58 ± 0.55	0.064	0.75 ± 0.50	0.091	12.0 ± 6.0	0.028
Kangaroo Island	–4.05 ± 4.34	0.099	–3.05 ± 4.89	0.213	–0.83 ± 1.01	0.138	–0.74 ± 1.04	0.189	3.5 ± 3.9	0.111
Northern District	0.46 ± 4.20	0.494	0.46 ± 4.27	0.488	0.17 ± 0.94	0.483	0.17 ± 0.96	0.482	4.8 ± 4.1	0.000
Southern District	9.06 ± 11.7	0.003	9.67 ± 12.43	0.002	0.61 ± 0.51	0.001	0.65 ± 0.61	0.001	16.9 ± 9.5	0.000
Namoi Catchment	3.33 ± 4.63	0.279	2.98 ± 4.33	0.262	1.42 ± 2.41	0.325	1.30 ± 2.26	0.334	7.4 ± 6.3	0.097
All	3.33 ± 8.55	0.014	3.81 ± 9.05	0.008	0.23 ± 0.85	0.098	0.26 ± 0.87	0.060	9.2 ± 8.6	0.000
					0–10 cm horizon					
Fleurieu Peninsula	3.28 ± 2.18	0.028	9.60 ± 5.84	0.046	0.33 ± 0.28	0.050	0.82 ± 0.64	0.084	17.5 ± 9.6	0.036
Kangaroo Island	–3.47 ± 2.02	0.018	–3.83 ± 2.50	0.027	–0.63 ± 0.69	0.111	–0.69 ± 0.70	0.092	4.8 ± 5.2	0.108
Northern District	–0.66 ± 2.78	0.357	–0.50 ± 2.88	0.495	–0.06 ± 0.54	0.668	–0.02 ± 0.58	0.89	4.8 ± 4.0	0.000
Southern District	6.31 ± 9.58	0.028	7.87 ± 10.84	0.018	0.40 ± 0.16	0.001	0.56 ± 0.08	0.000	20.5 ± 10	0.000
Namoi Catchment	1.34 ± 3.01	0.438	3.21 ± 5.55	0.266	0.80 ± 1.36	0.328	0.43 ± 0.82	0.387	11.5 ± 9.3	0.096
All	1.72 ± 6.69	0.099	3.12 ± 8.12	0.014	0.05 ± 0.56	0.574	0.18 ± 0.69	0.108	11.3 ± 10	0.000

panic–Rhodes in the Northern District of WA. In the Namoi Catchment, while SOC stocks had not changed, there was a significant shift in the isotopic composition of the SOC even after only a few years under kikuyu.

Chronosequence results

Several different regression models were fitted to the chronosequence data including linear, logarithmic, exponential rise to maximum, and a sigmodal function, but for all three regions included in the chronosequence analyses, the linear model gave the best fit (Fig. 2). The annual rate of change in ΔSOC was thus calculated as the slope of a linear regression between ΔSOC and perennial pasture age (Table 3). Results from this analysis indicated that the regression-based statistical approach yielded similar patterns between regions but with values ~25% greater than found in the paired ANOVA approach (Table 2). For the entire dataset, the regression-based analysis indicated that the annual rate of change in ΔSOC with pasture age (mean ± s.e.m.) in the 0–30 cm layer was $0.51 \pm 0.13\,Mg\,C\,ha^{-1}\,year^{-1}$, with an increase of $0.41 \pm 0.10\,Mg\,C\,ha^{-1}\,year^{-1}$ for the 0–10 cm layer only. The greatest rate of increase was found in the Southern District of WA. Importantly, the trend in the Southern District was independent of the oldest sites. If these two sites were removed, the slope of the linear regression only dropped from 0.90 ± 0.13 to $0.77 \pm 0.11\,Mg\,C\,ha^{-1}\,year^{-1}$, with $R^2 = 0.58$ ($P = 0.004$).

When the bulk SOC data were combined with the isotope data, we found that the annual rate of change in C_4-C across all sites was 0.41 ± 0.05 and $0.35 \pm 0.04\,Mg\,C\,ha^{-1}\,year^{-1}$ for the 0–30 and 0–10 cm layers, respectively (Table 3). The ratio of these regression slopes suggested that >80% of the total increase in SOC stocks was attributable to the new perennial vegetation. The Namoi Catchment data were not included in the regression analyses because of low replication and a narrow range of pasture ages.

Discussion

Sources of variability in SOC stock

There are a myriad of reasons why differences in SOC stocks may be found. Before even considering environmental and anthropogenic drivers of SOC change, the calculation of SOC stocks, itself, incorporates three separate measurements, all of which contribute to variation: SOC concentration, bulk density, and the rock fragment content. For the mean 0–10 cm data across all 42 sites, we found that variation in SOC concentration contributed the greatest amount of variance (66%), with variation in rock fragment content contributing 27%, and variability in bulk density contributed the remaining 7% (Fig. 3). On Kangaroo Island, where rock fragment content varied from 14 to 33% in the top 10 cm, nearly 40% of the variance in SOC stocks can be attributed to this single factor, which is independent of land use and management.

Beyond the error inherent in the calculation of SOC stocks, climate, soil properties, and resource quality are known to exert strong direct and indirect controls on SOC cycling (Sanderman and Amundson 2003), and this was the case for total SOC stocks within each region in this study (Fig. 1). However, much less is known about whether these environmental parameters affect the ability to change SOC stocks due to a shift in land management. The parameters ΔSOC and f_{C4} were found to be uncorrelated with the measured covariates in all regions except SA, where there were weakly significant correlations with mean annual precipitation (ΔSOC: $r = 0.69$, $P = 0.038$; f_{C4}: $r = 0.63$, $P = 0.069$), but this trend was primarily driven by the fact that greater SOC changes were found on the Fleurieu Peninsula where rainfall was 200–300 mm higher than on Kangaroo Island (Table 1).

Importantly, the ability to build SOC under subtropical perennial grasses appears to be independent of the amount of SOC already in the soil (Fig. 4). For all data and within each region, there were no correlations between ΔSOC or f_{C4} and the amount of SOC found in the paired annual pasture, despite

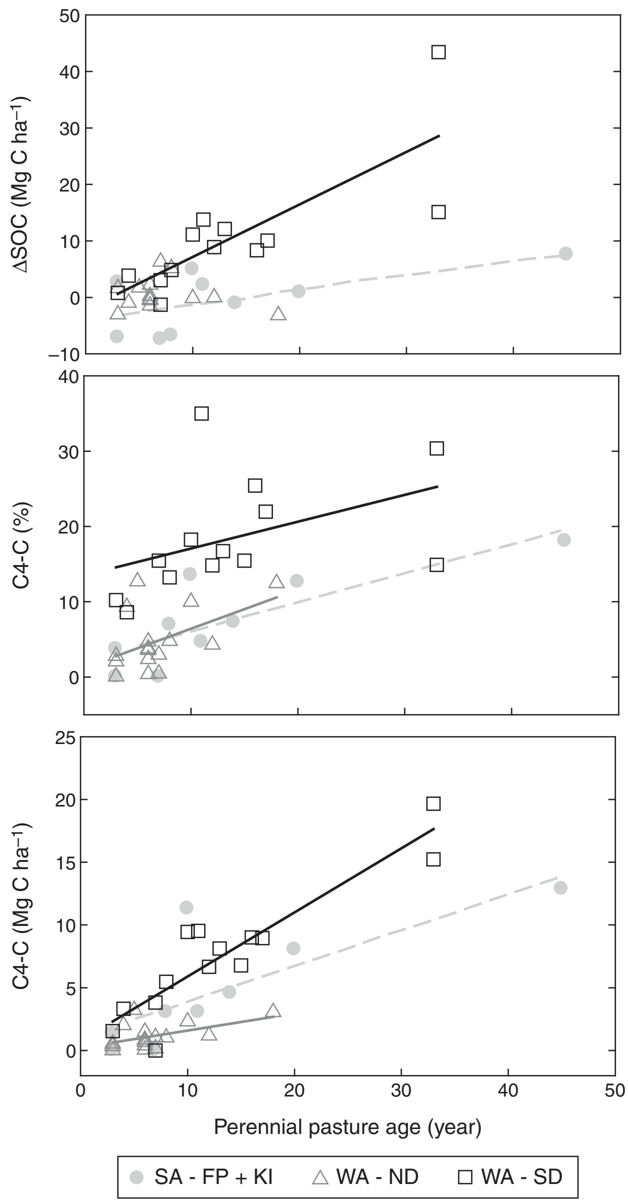

Fig. 2. Regressions against perennial age for the difference in SOC between the perennial and annual pair (ΔSOC, Mg C ha^{-1}) and the amount of C_4 perennial-derived SOC presented as a percentage of total perennial SOC and as mass of SOC. Linear regression lines are given where relationship is significant (regression statistics are summarised in Table 3).

C_{annual} varying from 13 to 73 Mg C ha^{-1}. This finding is in contrast to the often-stated conventional wisdom (e.g. Bruce *et al.* 2009) that it is harder to increase SOC levels in soils already high in SOC. It is possible that none of these sites were close to their 'potential maximum' SOC level. Additional modelling work would be needed to further explore this possibility. An alternative explanation is that the new SOC is simply accumulating in an unprotected fraction and that the more stable SOC pool has been saturated (cf. Stewart *et al.* 2007). This latter possibility appears to be supported by analysis of the isotopic composition of size-fractioned soil samples from the Southern District of WA (see companion paper, Sanderman *et al.* 2013).

A final source of variability in SOC stocks that we considered in this study was due to differences in soil mass found within our fixed sampling depth. Carbon accounting in Australia occurs to a fixed depth (Richards 2001). However, when trying to ascertain the true impact of a particular management practice on SOC stocks, calculating the stocks to an equivalent mass is preferred (Dalal and Mayer 1986) because differences due to compaction or inflation of the soil are removed. In this study, it was found that the extensive root system under kikuyu significantly reduced the bulk density in the upper 10 cm of soil, leading to a decrease in soil mass compared with the paired control sites (Fig. 5). The panic–Rhodes grass systems examined in the Northern District of WA did not change the soil bulk density. Given that there was less soil mass under kikuyu, the fixed-depth SOC calculation under-estimated the rate of change in SOC by 20% on the Fleurieu Peninsula, 13% on Kangaroo Island, and 7% across the Southern District of WA (Table 2). Due to these underestimations in SOC sequestration rates using a fixed-depth sampling strategy, it is recommended for systems such as kikuyu which build an extensive perennial root system that an equivalent-mass sampling strategy is undertaken.

Interpretation of isotope results

The fact that there were significant differences in the $\delta^{13}C$ value of SOC under the C_4 grasses does not necessarily equate to increases in SOC stocks, because the new C_4 carbon may simply be replacing older C_3 carbon that is being respired away by microorganisms. Modelling of the steady-state replacement of the existing C_3 SOC with new C_4 inputs would give an indication of whether the rate of change in isotope values was greater than would be expected if there were no changes in total SOC stocks occurring. This modelling exercise is beyond the scope of this study as it requires detailed knowledge of pasture composition and pasture production throughout the history of each of the pastures, and assumes that the model fully captures the dynamics of C in the soil (for elaboration see companion paper, Sanderman *et al.* 2013).

The isotope results can be combined with the ΔSOC results to build a case that the changes in $\delta^{13}C$ were likely greater than would have been expected based on steady-state replacement of the older C_3-SOC. The finding that the trends in ΔSOC and C_4-SOC were similar (Fig. 2) strongly suggests that it was the new perennial vegetation that contributed to the gains found in SOC in the perennial-based pastures. Additionally, the agreement of findings between the paired-site approach (i.e. ΔSOC) and the isotope approach validates the quality of the pairings and builds a strong case that real changes in SOC can occur with the introduction of kikuyu into pasture systems.

Does conversion to subtropical perennial pastures increase SOC levels?

Regardless of whether an ANOVA-based or regression-based statistical approach was taken, the data presented here suggest that subtropical perennial pastures can increase SOC levels, but it is dependent upon grass species and, to a lesser degree, soil characteristics. In WA and SA, pastures sown to kikuyu had higher SOC levels than paired annual-based systems. Pastures comprising a mix of panic and Rhodes grass had SOC levels similar to those found in the paired annual systems, and the isotope results suggested that the new perennial C is just slowly replacing the older C_3-SOC. Recently, Lawes and Robertson (2012) examined differences between 16 pairs of panic–Rhodes grass based perennial pastures and annual crop–pasture rotations and also found no significant differences between the land-use types. Because the panic–Rhodes grass systems were only in one region, it is a difficult to ascribe the lack of difference to one cause. However, the soils and climate in the Northern District are broadly similar to the Southern District of WA (Table 1), and the two pasture systems differed markedly in the amount of below-ground biomass. Nearly three times more coarse root (defined as the roots retained on 2-mm sieve) C was found in samples from soils under kikuyu compared with the panic–Rhodes grass pastures and the annual-based pastures in WA (Fig. 6). In a trial of several forage species, Neal *et al*. (2013) found that despite similar yields for several species, only kikuyu was able to increase SOC levels, suggesting that below-ground C allocation is indeed significantly greater than for many commonly sown temperate C_3 grasses. Thus, it appears that the spreading growth form of kikuyu combined with substantial below-ground C compared with both annual grasses and panic and Rhodes grass is the most likely explanation for the differences in SOC response between systems. Findings from a radiocarbon pulse-chase experiment conducted by Roper *et al*. (2013, this issue) support this notion. Those authors found that significantly more fixed $^{14}CO_2$ was recovered below ground under kikuyu than under panic grasses.

The effectiveness of kikuyu for increasing SOC levels varied between regions, with the largest increases found in the sandy soils of WA, smaller but still significant gains found in SA, and no changes found in the Naomi Catchment of NSW. Cooler winter temperatures in the SA regions relative to the Southern District of WA (11.9 *v.* 14.2°C) may confer a greater relative advantage to winter-active grasses, because kikuyu growth is greatly restricted over cold winter periods (Hill *et al*. 1985; Moore *et al*. 2006). In addition, the soils in SA are generally more fertile and, based upon farmers' responses, were more actively managed with regard to nutrient status. Thus, it was

Table 3. Regression summary for selected SOC measurements for the Southern District (SD) and Northern District (ND) of Western Australia, and for South Australian sites Fleurieu Peninsula (FP) and Kangaroo Island (KI) combined

Slope of linear regression ± s.e.m. only given when regression was significant ($\alpha \leq 0.10$). n.s., Not significant

Region	0–30 cm Slope	R^2	P	0–10 cm Slope	R^2	P
			Perennial SOC mass (Mg C ha^{-1})			
SD	1.84 ± 0.36	0.68	0.000	1.35 ± 0.24	0.73	0.000
ND		0.01	n.s.		0.09	n.s.
FP + KI		0.22	n.s.		0.12	n.s.
			Perennial $\delta^{13}C$ (‰)			
SD		0.15	n.s.		0.18	n.s.
ND	0.04 ± 0.03	0.19	0.090	0.05 ± 0.02	0.17	0.100
FP + KI	0.07 ± 0.02	0.59	0.019	0.11 ± 0.03	0.52	0.010
			ΔSOC (Mg C ha^{-1})			
SD	0.90 ± 0.25	0.61	0.002	0.77 ± 0.20	0.66	0.001
ND		0.06	n.s.		0.08	n.s.
FP + KI	0.26 ± 0.13	0.38	0.070	0.15 ± 0.08	0.35	0.090
All	0.51 ± 0.13	0.27	0.001	0.41 ± 0.10	0.28	0.000
			C_4-C (%)			
SD	0.47 ± 0.24	0.30[A]	0.047[A]	0.54 ± 0.27	0.33[A]	0.030[A]
ND	0.46 ± 0.25	0.19	0.099	0.44 ± 0.26	0.18	0.100
FP + KI	0.39 ± 0.11	0.64	0.007	0.61 ± 0.16	0.67	0.007
All	0.51 ± 0.13	0.27	0.001	0.71 ± 0.15	0.38	0.000
			C_4-C (Mg C ha^{-1})			
SD	0.51 ± 0.06	0.84	0.000	0.42 ± 0.05	0.86	0.000
ND	0.13 ± 0.06	0.27	0.041	0.08 ± 0.03	0.29	0.033
FP + KI	0.29 ± 0.09	0.60	0.013	0.24 ± 0.08	0.58	0.017
All	0.41 ± 0.05	0.61	0.000	0.35 ± 0.04	0.62	0.000

[A] R^2 and P given for regression with log(age) as the log-transformation significantly improved the fit.

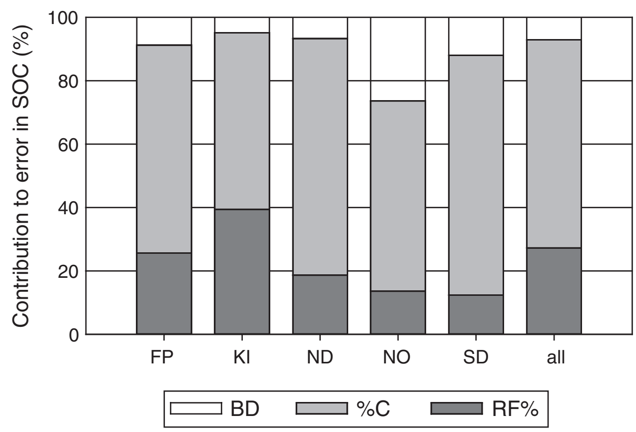

Fig. 3. Mean relative contribution of variance in bulk density (BD), carbon concentration (%C), and rock fragments (RF%) to variance in mean SOC mass in the 0–10 cm horizon for each region.

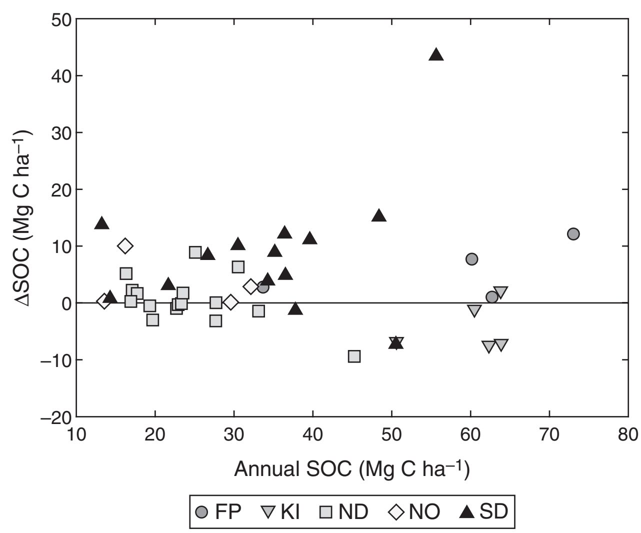

Fig. 4. Increase in SOC at 0–30 cm plotted against the amount of SOC found in the annual pasture for each pair of pastures. FP, Fleurieu Peninsula; KI, Kangaroo Island; ND, Northern District, NO, Namoi Catchment; SD, Southern District.

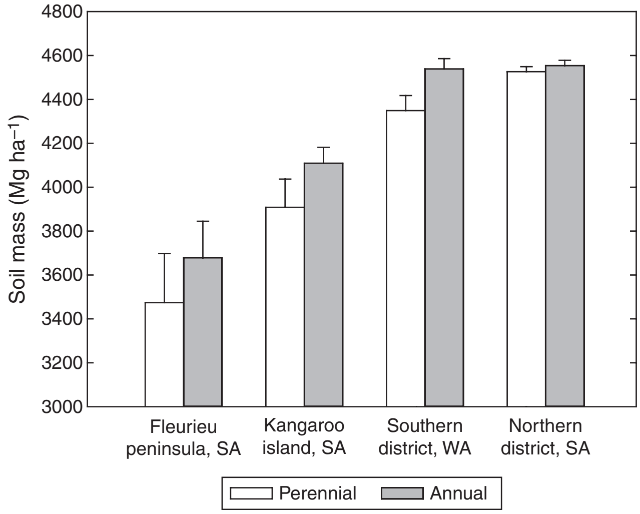

Fig. 5. Mean soil mass to 30 cm for each region. Error bars indicate s.e.m.

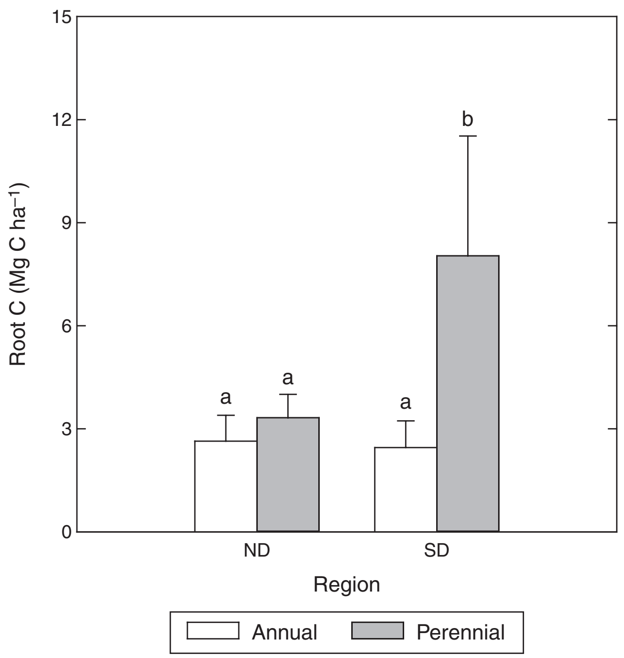

Fig. 6. Root (>2 mm) carbon stocks in upper 30 cm for the Northern (ND) and Southern (SD) Districts of Western Australia. Error bars indicate 95% confidence intervals. Significant differences in root carbon levels are indicated with different letters.

likely that the traditional, temperate grass based pastures in SA were already producing at high levels, and introduction of kikuyu was only leading to smaller net gains in productivity and C return to the soil. We did not sample along the southern coast of Victoria where kikuyu is also becoming an important part of the grazing landscape. Conditions are generally similar to the Fleurieu Peninsula but with slightly cooler temperatures and different soil types. Future research in this region would be valuable for confirming or refuting the above hypothesis regarding a smaller relative advantage for kikuyu in cooler, more fertile regions.

The results from the Naomi Catchment were particularly striking as that region receives the most summer rainfall and has the greatest annual temperature (Table 1); thus, it might be expected that a summer-active subtropical grass would have a greater advantage in this region over the cooler, Mediterranean regions along Australia's south coast. Additionally, in the Namoi Catchment region, the kikuyu was sown into formerly cropped paddocks. Conversion of cropped land to pasture typically increases SOC levels regardless of pasture type (Guo and Gifford 2002; Sanderman *et al.* 2010; Chan *et al.* 2011). Although the SOC stocks have not shifted significantly, there has been a rapid replacement of older SOC with new kikuyu-derived carbon (Table 2). The lack of significant differences in SOC stocks here may have been due to a combination of low replication and a low mean age of the perennial pastures.

The regression-based analysis is likely the more accurate of the two statistical approaches used in this study because the age of the perennial pastures varies substantially, thus potentially confounding results when only the mean values were computed for each region. We specifically sampled sites that spanned a range of pasture ages to be able to comment on the trend over time. At least for the first few decades after conversion to kikuyu, the increase in SOC appeared to be fairly linear (Fig. 2). A logarithmic model did no better job explaining the data for two main kikuyu regions than a simple linear model (data not shown). Our best estimates of the rate of change in SOC when a pasture was sown to kikuyu in the Southern District of WA was 0.90 ± 0.25 Mg C ha^{-1} year^{-1} and in South Australia was 0.26 ± 0.13 Mg C ha^{-1} year^{-1} over a period of at least 20 years.

Unfortunately, more specific management history (i.e. fertiliser history, grazing management, etc.) for these pastures was not variable enough within a region, or the data were not of a consistent enough quality to quantitatively test for more specific management drivers of changes in SOC or δ^{13}C values.

Conclusions

The combination of elemental and isotope measurements proved to be a powerful means for assessing changes in SOC stocks where subtropical perennials were sown into formerly C_3-based agricultural systems. We found that the ability of subtropical perennial grasses to sequester SOC varied with species and region.

Levels of soil C increased in kikuyu-based systems in the Southern Agricultural District of WA, and in Kangaroo Island and the Fleurieu Peninsula of SA. In these regions, the increase in C_4-SOC within the perennial pasture averaged 80% of the overall increase in SOC, indicating that new SOC attributed to the kikuyu was the dominant driver of SOC change. The majority (>70%) of the 0–30 cm change in SOC and C_4-SOC occurred in the upper 10 cm of soil. The SOC difference between the kikuyu and C_3-based pasture increased linearly with the age of the perennial pasture for 33 years in WA and for 45 years in SA. Mean SOC sequestration rates in the upper 30 cm were 0.90 ± 0.25 and 0.26 ± 0.13 Mg C ha^{-1} year^{-1} in WA and SA, respectively.

Given that ~120 000 ha has been sown to kikuyu in the Southern District of WA (Nichols *et al*. 2012), using results presented in Table 3, these pastures may be sequestering 0.29–0.51 Tg CO_2e annually. However, to assess the full greenhouse gas benefit of converting to a kikuyu-based pasture system, any additional methane or nitrous oxide emissions due to running higher livestock numbers would have to be discounted from this value (Thomas *et al*. 2012).

In the Namoi Catchment, NSW, we found no changes in overall SOC levels when cropped paddocks were converted to kikuyu-based pasture systems, despite rapid accumulation of C_4-SOC in these systems. Replication was low and there were only two pastures that had been in the ground for >5 years in this region; thus, confidence in the broader applicability of these findings is limited.

Perennial pastures based on a mixture of panic and Rhodes grass do not appear able to build SOC in the Northern Agricultural District of WA. Soil carbon levels were no different between the perennial and annual pastures and there was little indication of accumulation of new C_4-SOC in the perennial pastures (<5%), even after 18 years. The most likely reason for the greater response in the kikuyu-based pastures relative to the panic–Rhodes grass and annual grass based systems is a combination greater below-ground C allocation and more complete pasture coverage due to the spreading growth form of kikuyu.

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