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http://dx.doi.org/10.1071/SR12375

Allocation into soil organic matter fractions of ^{14}C captured via photosynthesis by two perennial grass pastures

M. M. Roper[A,C,E], *I. R. P. Fillery*[A,C], *R. Jongepier*[A,C], *P. Sanford*[D], *L. M. Macdonald*[B,C], *J. Sanderman*[B,C], *and J. A. Baldock*[B,C]

[A]CSIRO Plant Industry, Private Bag No. 5, Wembley, WA 6913, Australia.
[B]CSIRO Land and Water, Private Bag No. 2, Glen Osmond, SA 5064, Australia.
[C]CSIRO Sustainable Agriculture Flagship.
[D]Department of Agriculture and Food WA, Albany 6330, Australia.
[E]Corresponding author. Email: Margaret.Roper@csiro.au

Abstract. Perennial grass pastures are being increasingly adopted, but little is known about the flows of carbon (C) from photosynthesis into soil organic matter (SOM) that could be used for calculations in carbon accounting. Repeat-pulse labelling of perennial grass pastures (kikuyu and Rhodes grass) with ^{14}C in the field in Western Australia was used to trace the allocation of C to SOM fractions and to determine the stability of each fraction over an extended period. For kikuyu, >40% of the ^{14}C fed to the plants was allocated belowground within 10 days of labelling, and after 1 year half of this remained. Allocation of ^{14}C belowground under Rhodes grass ranged between 20 and 24% of ^{14}C applied and remained constant for up to 6 months. At least 90% of the ^{14}C belowground was found in the surface 300 mm of soil. The allocation of ^{14}C to the coarse (50 μm–2 mm) and fine (<50 μm) SOM fractions was similar in magnitude for the two grasses and remained stable through time. It was estimated that in 1 year ~1 t C ha^{-1} was assimilated into the coarse + fine SOM fractions under kikuyu. However, Rhodes grass was not uniformly distributed across the paddock, thereby reducing the estimates of assimilation of C belowground in these systems to one-tenth of that under kikuyu. Data obtained will help validate plant–soil models for assessing rates of C sequestration under perennial pastures.

Additional keywords: ^{14}C pulse-labelling, C sequestration, kikuyu, Rhodes grass, coarse SOM fraction, fine SOM fraction, soil carbon.

Received 21 December 2012, accepted 28 June 2013, published online 20 December 2013

Introduction

Replacement of annual grasses with perennial grasses has been promoted as an environment-friendly agricultural management practice for increasing rates of carbon (C) sequestration in soil. Perennial-based grass pastures have been shown to sequester more C belowground than annual pastures in Australian environments with summer rainfall (Young *et al*. 2009). However, studies undertaken in Australian agricultural zones with winter-dominant rainfall have typically measured little or no difference in C sequestration under perennial compared with annual systems (Chan *et al*. 2010, 2011; Lawes and Robertson 2012). Clarification of the potential of perennial grass systems to sequester additional soil C is urgently needed to provide supporting evidence for C farming initiatives based around perennial grass pastures.

Australian studies have typically determined whether C sequestration under perennial pastures is greater than under annual systems by comparing changes in total soil C (Young *et al*. 2009; Chan *et al*. 2010, 2011; Lawes and Robertson 2012). Ideally, the transition from cropping or annual systems to perennial grass pasture systems, should distinguish newly fixed C from pre-existing soil C stocks. This is difficult to achieve without the use of isotopes of C such as ^{14}C or ^{13}C. Also, isotopic labelling approaches provide a tool to understand plant C partitioning and the fate of root-derived C in soil systems, information needed to validate computer simulation models of C flow. Isotopic labelling has been used to determine the short-term fate of C under perennial ryegrass (*Lolium perenne*), but this species is restricted to irrigated agriculture (Domanski *et al*. 2001; Kuzyakov *et al*. 2001; Saggar and Hedley 2001; de Neergaard and Gorissen 2004; Sanaullah *et al*. 2012). Therefore, these findings have limited application to the Australian dryland agricultural industry, which is primarily interested in the C sequestration potential of subtropical species such as kikuyu (*Pennisetum clandestinum*) and mixtures of Rhodes grass (*Chloris gayana*) and panic (*Megathyrsus maximus*) grown under water-limiting conditions. Of particular interest is the effect that the introduction of

perennials has on the quantities of particulate soil organic matter (coarse SOM) and humus (fine SOM) (Skjemstad *et al*. 2004) in field soil conditions.

Continuous labelling with either ^{14}C or ^{13}C allows the determination of net C inputs to soil over the growing season (Danckwerts and Gordon 1987), while single-pulse labelling approaches follow the fate of recent photoassimilate over shorter time frames (Domanski *et al*. 2001; Kuzyakov *et al*. 2001). Continuous labelling approaches require careful control of labelling conditions and cannot be easily applied to field-scale experiments (Meharg 1994; Kuzyakov *et al*. 2001), while single-pulse labelling does not uniformly label all plant C pools and may underestimate soil C inputs (Hodge *et al*. 1996).

Repeat-pulse labelling has been suggested to overcome the technical constraints of continuous labelling and the short-term focus of single-pulse labelling approaches (Bromand *et al*. 2001). Because of its low natural abundance, ^{14}C labelling provides greater sensitivity than can be achieved through ^{13}C labelling, making it preferable to study the fate of C in SOM fractions over annual time-frames. The adoption here of a ^{14}C repeat-pulse labelling approach, under field conditions relevant to Australian agriculture, provides unique information on the fate of perennial grass-derived C in SOM fractions measured over long time-frames. Deriving this information on the flow of C through these SOM fractions is critical to validating models that aim to predict changes in soil C following the transition between production systems.

The objectives of this ^{14}C repeat-pulse labelling study were to: (*i*) quantify the belowground allocation of C from photosynthesis by perennial pastures, (*ii*) trace the fate of this C in time in SOM fractions including roots and coarse and fine fractions, and (*iii*) determine the stability of newly allocated C in each of the SOM pools over an extended period (6–12 months).

Materials and methods

Field locations

Field experiments were conducted in the wheatbelt region of Western Australia (WA) where the Mediterranean-type climate is suitable for temperate and subtropical, summer-active perennials. In the cooler Southern Region, kikuyu grass (*P. clandestinum* cv. Whittet) is commonly grown for pasture, and an 11-year-old kikuyu pasture in the Gnowellen district was selected. In the warmer Northern Region, a 13-year-old Rhodes grass site was chosen near Badgingarra. Details on the location of the field sites, soil characteristics, and climate are given in Table 1.

At each location, steel cores (700 mm long) were hydraulically driven into the ground to enclose the plant root system and contain assimilated ^{14}C. The diameter of the cores was determined by the habit of the grass pasture and was designed to include a whole plant as far as possible. Cores for kikuyu were 300 mm in diameter, and for Rhodes grass 711 mm in diameter. The cores protruded above ground level by 40 mm to allow attachment of the labelling chamber and cooling system.

Field labelling with ^{14}C

At each labelling period, replicates of 15 cores (kikuyu) and 12 cores (Rhodes grass) were labelled for 1.5 h on three consecutive weeks during a re-growth phase to enable a more even labelling of plant-derived C. Five kikuyu replicates and four Rhodes grass replicates were destructively sampled at three different times after labelling. The number of Rhodes grass replicates was reduced to four due to the increased core size and, hence, larger area sampled than for kikuyu. A flow diagram of the labelling and sampling procedure for kikuyu is shown in Fig. 1.

Approximately 1 week before labelling kikuyu pasture, plants were artificially grazed by cutting the shoots by hand to a few mm above ground level. The Rhodes grass plants were cut before installation of cores (86 days before first label), and because they recovered slowly, they were not cut again before labelling. Annual plant species were removed from the cores before labelling.

Pulse labelling was conducted 1 week after rainfall (~50 mm) or, when rainfall was unreliable, after watering with an equivalent total of 50 mm. Clear sunny days were chosen to ensure fast uptake of $^{14}CO_2$ by photosynthesis, and labelling was done when the soil surface was dry to reduce the potential for respiration and/or uptake of $^{14}CO_2$ by soil microbial communities (Yuan *et al*. 2012).

One set of 15 kikuyu cores was labelled on 16, 24, and 29 November 2010 (referred to as 'spring labelling'), and a second set of 15 cores was labelled on 23 February and 3 and 9 March 2011 (referred to as 'summer labelling'). One set of 12 Rhodes grass cores was labelled on 20 and 31 October and 10 November 2011. At the end of the 3-week labelling period, each core had a total exposure of 388.8 MBq ^{14}C. The amount of $^{14}CO_2$ applied was based on other short-term (7–21-day) ^{14}C pulse labelling studies (e.g. Bhupinderpal-Singh *et al*. 2005; Keith *et al*. 1986),

Table 1. Site locations and characteristics
Soil types from Isbell (2002). Weather data from Bureau of Meteorology (2012)

Location	Perennial grass	Mean age (years)	Land use	Dominant soil type	Mean. temp. (°C)		Annual rainfall (mm)
					Max.	Min.	
Badgingarra[A]	Rhodes grass	13	Sheep meat	Bleached-Orthic Tenosol (sand)	25.9	11.8	540
Gnowellen[B]	Kikuyu	11	Sheep fine wool	Yellow-Orthic Tenosol (sand)	20.7	9.5	478

[A]16 km SW of Badgingarra, Western Australia.
[B]17 km NNW of Wellstead, Western Australia.

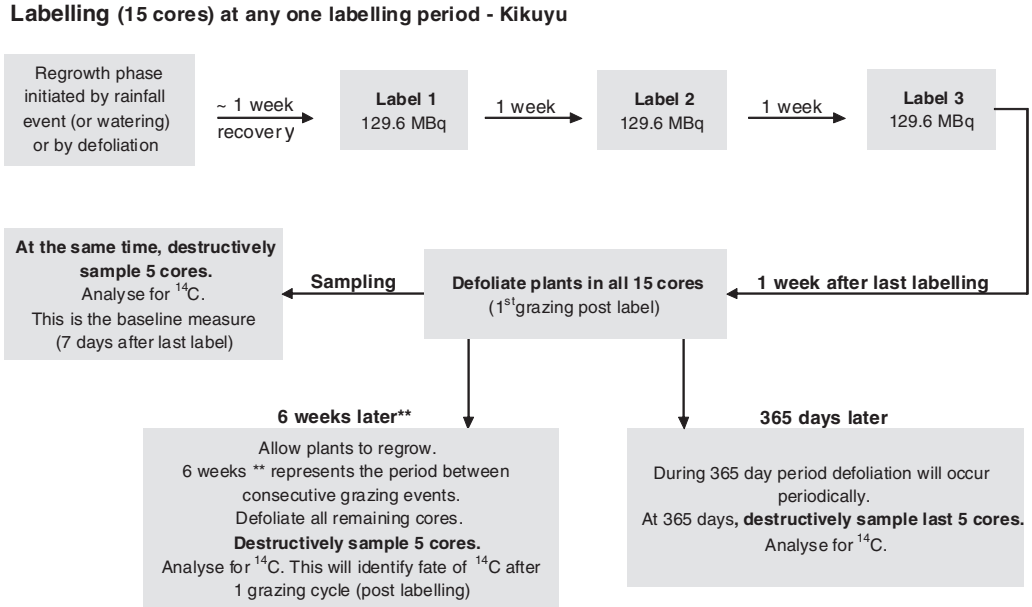

Fig. 1. Flow diagram of labelling and sampling procedure.

with additional quantities of ^{14}C factored in to account for potential losses over the 365 days planned for our experiments.

Labelling chambers

Clear plastic labelling chambers were custom-designed from commercially available plant-protection domes, which were cut to 110 mm high and closed with a clear acetate lid secured with silicone adhesive. A rubber strip on the chamber underside ensured a gas-tight seal with the steel core, which was additionally secured with electrical tape before labelling. Two Suba-Seals (Sigma-Aldrich Co., St. Louis, MO) in the chamber lid allowed injection of acid into two vials, one containing ^{14}C-labelled $NaHCO_3$ and the other non-labelled $NaHCO_3$ for generation of the pulse label and 'cold' chase. An aquarium pump was used to circulate ice-chilled water through a copper coil inside the chamber, which cooled the air and maintained the temperature at ~35°C. The air was circulated with a computer fan, and an anti-fog coating prevented condensation forming on the chamber interior. The chamber design used for kikuyu pasture is shown (Fig. 2).

The chamber system was adapted for the larger Rhodes grass cores by using custom-built two-piece Perspex chambers measuring 711 mm wide and 200 mm high (3 mm thick). The lid was kept separate from the enclosure, and was secured with strong fold-back (bulldog) clips during labelling. The size of the computer fan and cooling system was doubled to maintain the chamber temperature around 35°C.

Generation of $^{14}CO_2$

Prior to labelling, chambers were sealed onto the cores to allow for CO_2 depletion to below 150 ppm, to maximise assimilation of the $^{14}CO_2$ when introduced. The CO_2 depletion was monitored under identical conditions in one chamber supplied with unlabelled CO_2 using an infrared gas analyser. $^{14}CO_2$ was generated inside each chamber by injecting 10 mL of HCl (10%)

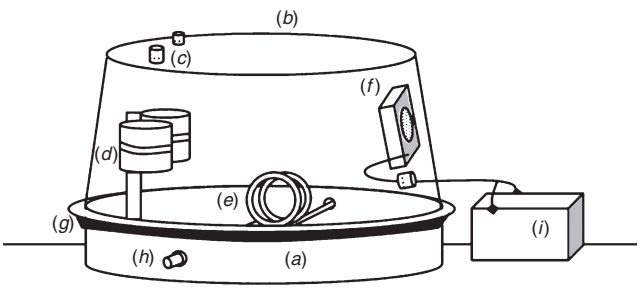

Fig. 2. Schematic diagram of field labelling chambers which enclosed perennial grasses within steel cores driven into the ground. (*a*) Steel core enclosing plants down to 700 mm; (*b*) clear plastic air-tight chamber; (*c*) Suba-Seal injection ports for acidifying $NaHCO_3$; (*d*) cups secured onto a stake to hold vials containing $NaHCO_3$ below the injection port; (*e*) coiled copper pipe for cooling system; (*f*) computer fan mounted to side wall to circulate air; (*g*) rubber seal to attach chamber to steel core; (*h*) copper pipe outlet (sealed with Blu-Tak) to be connected to aquarium pump circulating iced water; (*i*) 12 V battery to power fan.

through the Suba-Seal into the vial containing 12.96 mg of ^{14}C-labelled $NaHCO_3$ (129.6 MBq of ^{14}C). Following a labelling period of ~15 min, two cold chase periods (non-labelled CO_2) were created by (*i*) injecting 10 mL HCl into the second vial which contained 12.96 mg of non-labelled $NaHCO_3$; and (*ii*) dissolving 130 mg of non-labelled $NaHCO_3$ in 5 mL of water in a Bijou bottle and injecting 1 mL of this liquid into one of the reaction vials inside the chamber, followed by another 10 mL of HCl (10%). The cold chase periods raised the CO_2 concentration above the ambient level (>380 ppm) and ensured that all ^{14}C was assimilated. The chambers were left sealed for ~1 h after the last cold chase was applied, during which time the atmospheric CO_2 concentration reduced to the CO_2 compensation point due to photosynthetic uptake. Before chamber removal, two air samples were collected using 9-mL evacuated blood

collection tubes to check for remaining ^{14}C-CO_2. One mL of 0.5 M NaOH was added to the vacutainers and later mixed with 10 mL Ultima Gold scintillation cocktail (PerkinElmer, Waltham, MA) and analysed using a Tri-Carb Liquid Scintillation Counter (Packard, Meriden, CT).

Pulse labelling was repeated at ~7 days and 14 days following the first labelling event.

Plant sampling after labelling with ^{14}C

Shoots were cut from all labelled cores at ~1, 6, and 52 weeks (kikuyu), and 1, 6, and 28 weeks (Rhodes grass) after the last labelling event. Five kikuyu and four Rhodes grass soil cores were destructively sampled at each time. Previous work indicated that partitioning of the ^{14}C pulse is likely to have been complete after 5 days (Domanski *et al*. 2001). Thus, the first sampling provided a baseline measurement of ^{14}C allocation. The 6-week sampling represented a recommended grazing cycle (Kamstra *et al*. 1966). The shoot re-growth in cores that were not destructively sampled was trimmed to simulate grazing (defoliation), and shoot material was analysed for ^{14}C content. After the last label, kikuyu defoliation occurred at 10, 44, 82, 107, 140, 191, 277, and 365 days (spring labelling) and at 7, 40, 91, 265, and 365 days (summer labelling). Rhodes grass was cut from cores at 7, 42, 189, and 201 days after the last label and separated into shoots and runners (stolons). Because of the dependence of new growth on the stolons, only shoot material (not stolons) was removed from the cores to be destructively sampled at a later date. All plant material was oven-dried at 70°C for 24 h, weighed, and roughly ground in a spice grinder and then ball-milled to a fine powder.

Sampling and subsampling of cores belowground

Where cores were destructively sampled, all of the soil was collected from depth increments 0–100, 100–200, 200–300, 300–500, and 500–700 mm. Due to their large mass, soil samples were air-dried under cover on-site (0–200 mm layers were spread out on trays) for at least 4 weeks and then weighed. Samples were sieved to collect material >2 mm (roots, gravel, bark, debris) and soil <2 mm. The soil <2 mm was thoroughly mixed and subsampled down to 1.5 kg using a large riffle splitter. This subsample was transported to the laboratory. Soils were further subsampled using a small riffle splitter to obtain two soil samples for analysis: one for fractionation of SOM (~40 g), and the other to be finely ground in a Spex 8000 M Mixer/Mill (SPEX SamplePrep, Metuchen, NJ) for total C and ^{14}C (~100 g).

Roots were separated from debris >2 mm (e.g. bark, fine gravel, and other organic material) on the surface of a 2-mm sieve with tweezers. In the 500–700 mm layer, the roots had to be separated from gravel. This was done using a vacuum cleaner with an external cyclonic chamber (turbo dust filter) connected to a hose with a wide funnel opening that collected the roots using gentle suction, leaving the gravel behind for weighing and disposal. Roots were dried at 70°C for 24 h in a fan-forced oven and weighed. Dried root material was cut into small pieces and finely ground as per shoot material. Kikuyu roots from the surface 100-mm layer were dense and tough and had to be coarsely ground in a SM100 Cutting Mill (Retsch GmbH, Haan, Germany) before undergoing the two-step grinding process described above.

Fractionation of SOM (coarse and fine)

Automated vibratory wet sieving was used to fractionate soil samples into a coarse SOM fraction (50 µm–2 mm) and a fine SOM fraction (<50 µm). Soil (40 g) was dispersed by shaking overnight with 30 mL of Na hexametaphosphate (5 g L^{-1}), before fractionating as outlined by Sanderman *et al*. (2011).

The coarse SOM fraction, recovered on the 50-µm sieve, was separated from the coarse sand fraction by rinsing/floating the lighter organic material onto glass filter paper in a Buchner funnel, before drying (70°C) overnight in a Teflon-coated pie dish, weighing, and grinding in a small mortar and pestle or mixer-mill. The Teflon coating prevented residue from sticking to the surface of the container, allowing complete recovery of the coarse SOM fraction. The sand was dried and weighed to check the recovery rate of the fractionated samples.

The fine SOM fraction, recovered in the <50 µm filtrate, was flocculated with a saturated solution of $Al_2(SO_4)_3$ (1–2 mL) and centrifuged at 3500 rpm (1500*G*) for 10 min. The supernatant was decanted, the fine fraction pellet dried at 70°C overnight, and the dry sample weighed before pulverising the pellet by inserting a 10-mm ball bearing into the centrifuge tube and holding the tube against a vortex mixer. In contrast to the freeze-drying method of recovery described in Sanderman *et al*. (2011), a flocculation method was used to avoid a ^{14}C-related restriction on equipment use and any lengthy freeze-drying time lag. Mass recovery with flocculation was >99% C (data not shown).

Analysis of samples—combustion and ^{14}C counting

Samples of plant material (5 mg), coarse (5 mg), and fine (15 mg) SOM fractions, and soil (50 mg) were analysed for total C (data not shown) and ^{14}C through dry combustion at 1050°C (Roboprep CN analyser; Europa Scientific, Cambridge, UK). The Roboprep vent was modified to allow the oxidised carbon (CO_2) to be bubbled through a glass gas dispersion tube and trapped through 2 mL of 0.5 M NaOH contained in screw-top test tubes. Samples were analysed in duplicate, with one sample used to determine total C by titration and one to determine ^{14}C by liquid scintillation counting. The total C results were used to check the combustion and recovery efficiency against 5-mg wheat flour samples (39.6% C) as standard quality controls.

For total C analysis, the alkali trap was titrated against 0.04 M HCl using an automatic titrator (848 Titrino Plus; Metrohm AG, Herisau, Switzerland) in a two-endpoint titration (pH 8.3 to pH 3.8) (Skjemstad and Baldock 2008). For ^{14}C analysis, 10 mL of scintillation cocktail (Ultima Gold; PerkinElmer) was added to a 1.8-mL aliquot of the second alkali trap, shaken, and analysed on a Tri-Carb Liquid Scintillation Counter for up to 5 min per sample. Results were used to determine the total ^{14}C activity (Bq) for each SOM fraction, which could be further expressed as a percentage of the total ^{14}C applied.

Calculation of net allocation of C

Shoot biomass production and C content was measured for a 36-day period spanning the spring labelling event (3 November–9 December 2010) and for a 43-day period spanning the summer labelling event (1 February–16 March 2011). This allowed

calculation of the net assimilation of C to the shoots during this time. Using these values and knowing the relative proportions of ^{14}C in each SOM fraction compared with the shoots, the net allocation into the coarse and fine SOM fractions of C (kg C ha^{-1}) fixed in one day during spring or summer was calculated. Furthermore, from the ^{14}C content in SOM fractions at subsequent sampling dates, it was possible to calculate how much of this daily amount fixed in spring or summer remained at 6 weeks and 12 months after the end of the labelling events. For the single measurement period for Rhodes grass, a similar calculation was applied except that the shoot biomass production was measured over 114 days (the time from the last cut before labelling until the first cut post labelling).

Use of GrassGro model to estimate annual aboveground biomass C for kikuyu pasture

At the Gnowellen trial site, shoot biomass production was measured over just 1 year, giving a single snapshot of annual production. In order to gain a better estimate of production in the region, GrassGro version 3.2.4 (Moore *et al*. 1997) was used to estimate the monthly aboveground biomass production for kikuyu, averaged over the years 1990–2010. The climate file utilised for the simulation was constructed using SILO (www.longpaddock.qld.gov.au/silo/) and Department of Agriculture and Food Western Australia local weather stations. Soil parameters used in the simulation were based on the default values in the model for sand (depth 0–400 mm) over clayey sand (depth 400–4000 mm) modified to match measurements taken at a local experimental site with a similar soil profile; soil fertility scaler was set to 0.75. The standard kikuyu plant parameter set was utilised with a rooting depth of 3500 mm. The simulation was stocked at 6.5 Merino ewes ha^{-1}, which flexibly grazed the kikuyu pasture based on changes in liveweight, i.e. stock were moved to an adjacent annual pasture if liveweight gain was <0.010 kg head^{-1} day^{-1}. GrassGro simulations were calibrated against kikuyu, and Merino ewe measurements were taken from 2006 to 2008 at a grazing trial north of Wellstead and just 10 km south of our trial site at Gnowellen. The simulation was initiated by running the model for five growing seasons before the simulation to ensure appropriate start conditions. The values of dry matter (DM) production derived from the model were converted to kg C ha^{-1} using the average C content of shoot material measured at the trial site (39.7%).

Estimation of the annual belowground C assimilation into the roots and coarse and fine SOM fractions

The monthly accumulation of C derived from the GrassGro model was compared with that measured in the shoots for each labelling period. Knowing the proportion of roots and coarse and fine SOM C to shoot C, the amount of C in each fraction remaining after 1 year could be calculated.

For Rhodes grass, aboveground biomass C production was calculated using DM production measured in cores between July 2011 and May 2012. Biomass C was used to calculate the amount of C sequestered belowground in the roots and coarse and fine SOM fractions as for kikuyu.

Cores in the field for pulse labelling of Rhodes grass were positioned to include a whole plant as much as possible. Rhodes grass has a clumped habit and plants were not evenly distributed across the landscape. To extrapolate measures of C sequestered belowground from these cores to a per hectare basis would be an overestimation. To gain an estimate of C allocated belowground on an area basis, the frequency of Rhodes grass plants was determined by counting the number of plants in a 4-m^2 grid. The number of large clumps (>300 mm in diameter) within this total was also counted.

Statistical analyses

Standard errors of the mean were calculated within Excel (Microsoft). All other analyses were performed using the statistical package GENSTAT (Version 13.1, VSN International Ltd, Hemel Hempstead, UK). Prior to performing an ANOVA, the results were log-transformed to normalise the data. There were three fixed factors: three SOM fractions, two labelling periods, and three sampling dates.

Results

Dry matter production in cores

Table 2 shows the actual DM production (kg DM ha^{-1}) measured from the first cut before labelling until the last sampling at 365 days (kikuyu) and 201 days (Rhodes grass) after labelling. Most kikuyu production occurred during spring–early summer and least during late winter. Annual production of kikuyu was 5097 ± 326 kg DM ha^{-1} from the spring labelling (30 November 2010–29 November 2011) and 3769 ± 585 kg DM ha^{-1} from the summer labelling (9 March 2011–8 March 2012). Rhodes grass production, measured over 301 days from 26 July 2011 to 29 May 2012 was 3083 ± 586 kg DM ha^{-1}. The average daily rate calculated in May was used to estimate shoot production from

Table 2. Aboveground dry matter (DM) production (kg DM ha^{-1}) in kikuyu and Rhodes grass pastures during and after labelling with ^{14}C
Shoots were defoliated by cutting to a few mm above ground level. Values are means with standard error of the mean in parentheses

Date shoots cut	Days since last cut	DM production
	Kikuyu spring labelling	
9 Dec. 2010	36	1439.5 (73.8)
12 Jan. 2011	34	244.3 (14.5)
19 Feb. 2011	38	563.9 (24.6)
16 Mar. 2011	25	667.8 (58.4)
18 Apr. 2011	33	483.8 (57.1)
8 June 2011	51	250.3 (30.1)
23 Aug. 2011	76	141.5 (14.1)
29 Nov. 2011	98	2385.6 (69.9)
	Kikuyu summer labelling	
16 Mar. 2011	43	1241.4 (28.4)
18 Apr. 2011	37	379.1 (23.8)
8 June 2011	47	236.1 (36.8)
23 Aug. 2011	76	141.5 (14.2)
29 Nov. 2011	98	1499.8 (257.2)
8 Mar. 2012	100	1281.8 (131.0)
	Rhodes grass	
17 Nov. 2011	114	1856.2 (158.2)
22 Dec. 2011	35	480.4 (104.5)
17 May 2012	147	594.0 (97.6)
29 May 2012	12	153.1 (39.8)

29 May to 25 July 2012 to give an annual estimate of Rhodes grass production of 3810 kg DM ha^{-1}.

Allocation of ^{14}C assimilated by photosynthesis—kikuyu

Results of liquid scintillation analysis of air samples remaining at the end of the field labelling procedure indicated that the system assimilated 99.96% (spring labelling) and 99.90% (summer labelling) of the ^{14}C applied. It was assumed that this was all taken up by the plants in photosynthesis and that little or no ^{14}C was assimilated by other soil organisms. A total of 388.8 MBq of $^{14}CO_2$ was applied to each core over the 3-week period. These efficiencies represent 388.6 and 388.4 MBq of uptake in photosynthesis in the spring and summer labelling, respectively, a difference which is below detectable limits. Therefore, in the calculations, allocations of ^{14}C in the shoots and belowground were presented as proportions of the amount of ^{14}C applied, i.e. 388.8 MBq.

The allocation of ^{14}C into the shoots and belowground OM (as percentages of the total ^{14}C applied) for spring and summer labelling of kikuyu are shown in Fig. 3. In spring (Fig. 3*a*), the allocation of applied ^{14}C in the shoots at 10 days was 13.9%, but this declined rapidly with successive cuts; 41.1% of the ^{14}C was allocated belowground at 10 days, and after 1 year, more than half of this ^{14}C remained (25.4% of the total applied ^{14}C). The distribution of ^{14}C in each SOM fraction within 0–700 mm shows that, initially, most of the ^{14}C allocation was in the roots (28.2%), followed by coarse SOM fraction (11.3%) and fine SOM fraction (1.7%). The ^{14}C in the labile root fraction reduced over time to 7.7% at 365 days. On the other hand, allocation of ^{14}C into the coarse SOM fraction increased significantly ($P < 0.05$) from 11.3% at 10 days to 15.7% at 365 days but remained stable in the fine SOM fraction (1.7% at 10 days, and 1.9% at 365 days).

Seven days after the end of the summer labelling, 20.9% of applied ^{14}C was in the aboveground biomass (Fig. 3*b*). The allocation of ^{14}C belowground was 32.8%. After 365 days, 18.1% of the applied ^{14}C remained in the belowground fractions. The ^{14}C content of the shoot biomass reduced sharply after the first pasture cut (Fig. 3*b*) indicating little or no return of ^{14}C from belowground after successive simulated grazing. Patterns of change in ^{14}C allocations for the summer labelling were similar to spring, with the allocation of ^{14}C in the roots declining from 20.6% at 7 days to 7.3% at 365 days. There was no significant ($P > 0.05$) change in the ^{14}C allocation in the coarse SOM fraction (10.3% at 7 days to 9.2% at 365 days) or in the fine SOM fraction (2.0% at 7 days to 1.6% at 365 days) (Fig. 3*b*).

At both labelling times, the majority (76–84%) of the ^{14}C allocated belowground was found in the top 100 mm (Fig. 4), where most of the root mass occurred (data not shown). There was a sharp decline in allocation of ^{14}C at depths below 100 mm. More than 90% of ^{14}C recovered belowground was found in the top 300 mm. At 500–700 mm depth, there was a slight increase in ^{14}C activity, possibly associated with an accumulation of roots at a gravel layer that occurred at 600–700 mm depth. The fractionation method provided full recovery by mass (100.3%) of soil (<2 mm), consisting of <2 mm sand (93.8%), coarse SOM fraction (3.4%), and fine SOM fraction (3.1%) when checked using 0–100 mm soil samples collected at 365 days after spring and summer labelling, giving confidence about the values obtained from the fractionations.

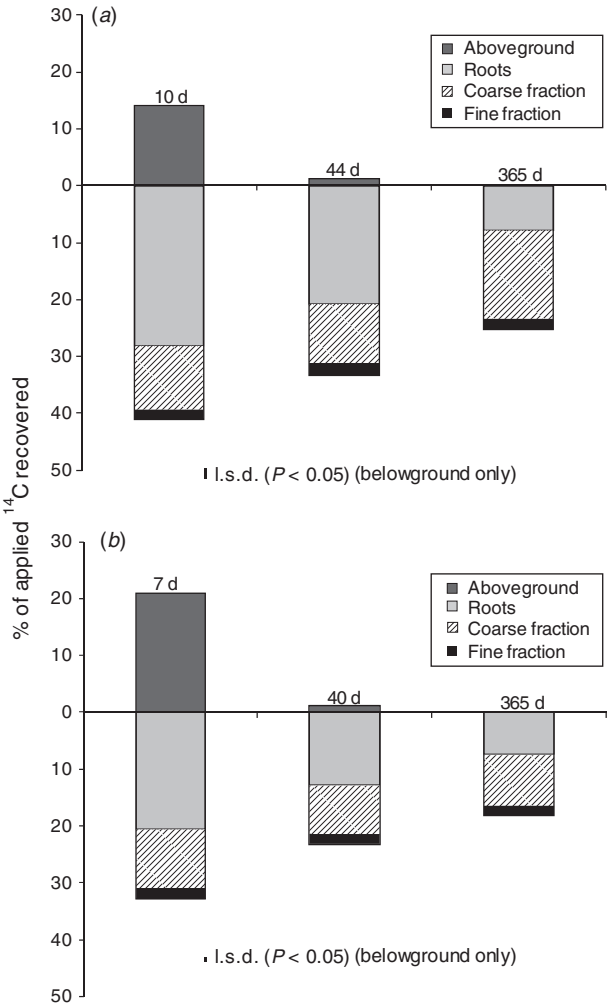

Fig. 3. Percentage of applied ^{14}C recovered aboveground and in roots, coarse SOM, and fine SOM (to 700 mm) under a kikuyu pasture at Gnowellen at: (*a*) 10, 44, and 365 days after the last $^{14}CO_2$ feed (spring labelling, November 2010); and (*b*) 7, 40, and 365 days after the last $^{14}CO_2$ feed (summer labelling, February–March 2011). Small vertical lines indicate l.s.d. ($P = 0.05$).

Calculation of net allocation of C under kikuyu

The net assimilation of C to the shoots over the 36-day spring pulse labelling event (3 November–9 December 2010) was 567.2 kg C ha^{-1}, equating to an assimilation rate of 15.8 kg C ha^{-1} day^{-1}. For the 43-day period spanning the summer labelling event (1 February–16 March 2011), net assimilation of C to the shoots totalled 488.6 kg C ha^{-1} or a daily rate of 11.36 kg C ha^{-1}. Comparison of the proportions of ^{14}C in each of the fractions belowground to the proportion of ^{14}C in the shoots allowed calculation of kg C ha^{-1} in the coarse and fine SOM fractions.

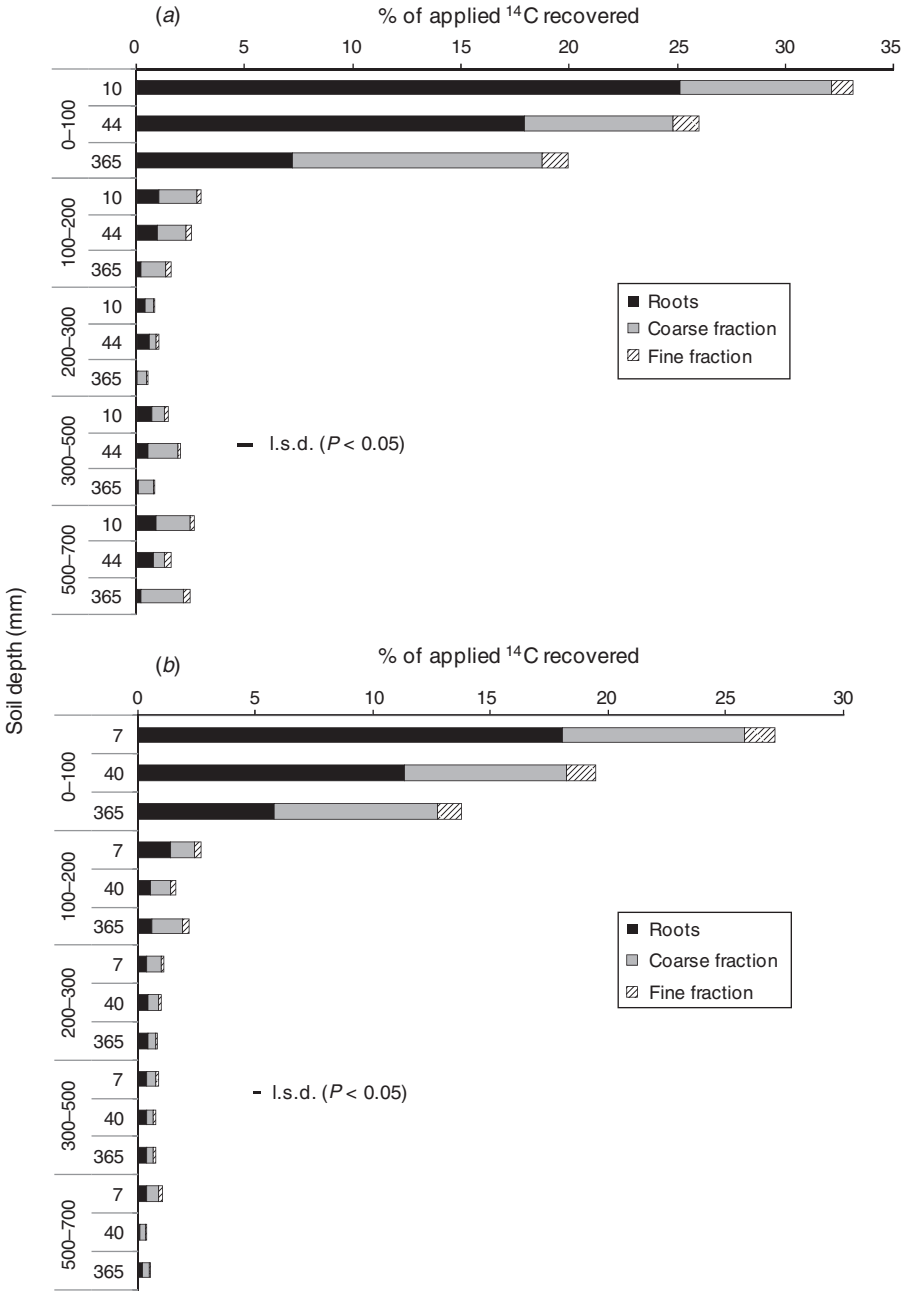

Fig. 4. Changes in the percentage of applied ^{14}C found at each depth with increasing duration since labelling of kikuyu pasture at Gnowellen in: (*a*) spring (November 2010), and (*b*) summer (February–March 2011). Small horizontal lines indicate l.s.d. ($P = 0.05$).

The net allocation of C (kg C ha^{-1}), calculated for 1 day of photosynthesis in spring or summer, into each of the belowground fractions was summed for the entire 700-mm profile and is shown in Table 3. There was a significant interaction among all three factors ($P < 0.05$; l.s.d. ($P = 0.05$) 0.13). Individual factors were significantly different ($P < 0.001$). At 10 days after the completion of the spring labelling, the sum of C allocated into the coarse and fine SOM fractions in the entire depth profile (0–700 mm) was 11.5 kg C ha^{-1}. This C pool increased significantly ($P < 0.001$) to 13.9 kg C ha^{-1} after 1 year. After the summer labelling, there was no significant ($P > 0.05$) change in the amounts of coarse + fine SOM fractions (from 6.7 kg C ha^{-1} at 7 days to 5.9 kg C ha^{-1} after 1 year).

Use of GrassGro simulation results to estimate assimilation of C into the roots and coarse and fine SOM fractions in 1 year under kikuyu

The long-term average C content of aboveground production per month determined from application of the GrassGro model

near Wellstead is shown in Fig. 5. Biomass production estimated by GrassGro peaked at 268 kg C ha^{-1} month^{-1} in November and was least (88 kg C ha^{-1} month^{-1}) in July. Between January and May, biomass production was steady and averaged 172 kg C ha^{-1} month^{-1}. Average annual production predicted by the model was 169 kg C ha^{-1} month^{-1}, which was similar to the average prediction for February–March (166 kg C ha^{-1} month^{-1}) when the summer labelling at the Gnowellen trial site was done. Therefore, the ratios of net C allocated measured during the summer labelling (February–March) were used to calculate an annual estimate of C assimilated into the coarse + fine SOM fractions. The amount of C calculated as remaining in the coarse + fine fraction after 12 months following a single day of photosynthesis in summer was 5.9 kg C ha^{-1} (Table 3). The allocation in the shoots during the summer labelling period was 11.4 kg C ha^{-1} in 1 day. Therefore, an estimate of the annual allocation of C into the coarse + fine SOM fractions could be determined: [(summer labelling coarse + fine SOM fraction)/summer labelling shoot C] × total C in shoots for the year (from the simulation) = (5.9/11.4) × 2033 = 1052 kg C ha^{-1} in 1 year. Using the same calculation, the annual allocation of C into individual fractions belowground to 700 mm was 713 kg C ha^{-1} (roots), 892 kg C ha^{-1} (coarse SOM fraction), and 161 kg C ha^{-1} (fine SOM fraction). At 0–300 mm depth, individual allocations were 659 kg C ha^{-1} (roots), 836 kg C ha^{-1} (coarse SOM fraction), and 141 kg C ha^{-1} (fine SOM fraction), giving a value for the coarse + fine SOM fraction at 0–300 mm of 978 kg C ha^{-1}.

Table 3. Net allocations into roots and soil organic matter (OM) fractions of C (kg C ha^{-1}) fixed in one day during spring or summer by a kikuyu pasture at Gnowellen

Net assimilation of C into shoots was used to calculate the amount of C allocated to belowground C pools by comparing proportions of ^{14}C in each of the fractions with that in the shoots. Amounts of C remaining at subsequent sampling dates were based on the proportional change in ^{14}C contents in each SOM fraction. Quantities are summed for the entire depth of 700 mm at three sampling dates after labelling (10, 44, and 365 days for spring labelling; 7, 40, and 365 days for summer labelling). A factorial ANOVA (3 SOM fractions × 3 sampling dates × 2 labelling dates) indicated a significant interaction among all factors ($P < 0.05$); l.s.d. ($P = 0.05$) 0.13. Individual factors were significantly different ($P < 0.001$)

Fraction	Sampling date 1	Sampling date 2	Sampling date 3
	Spring labelling		
Roots	28.8	21.1	7.9
Coarse SOM	10.1	10.7	11.9
Fine SOM	1.4	2.1	2.0
	Summer labelling		
Roots	11.1	6.9	4.0
Coarse SOM	5.6	4.7	5.0
Fine SOM	1.1	0.9	0.9

Allocation of ^{14}C assimilated by photosynthesis—Rhodes grass

An average of 99.5% of the $^{14}CO_2$ introduced to the chambers enclosing the Rhodes grass in spring 2011 was taken up by the soil–plant system during labelling. Therefore, allocations of ^{14}C in the shoots and belowground are presented as proportions of the amount of the total ^{14}C applied (388.8 MBq) and are shown in Fig. 6. The allocation of ^{14}C into aboveground biomass was higher than belowground. Some ^{14}C persisted aboveground into the 6-week sampling because only the shoots were previously removed and the stolons remained to allow re-growth after grazing. On day 7, for those cores where both stolons and shoots were cut, analysis indicated that the proportion of ^{14}C in each component was distributed across shoots (18.8%) and

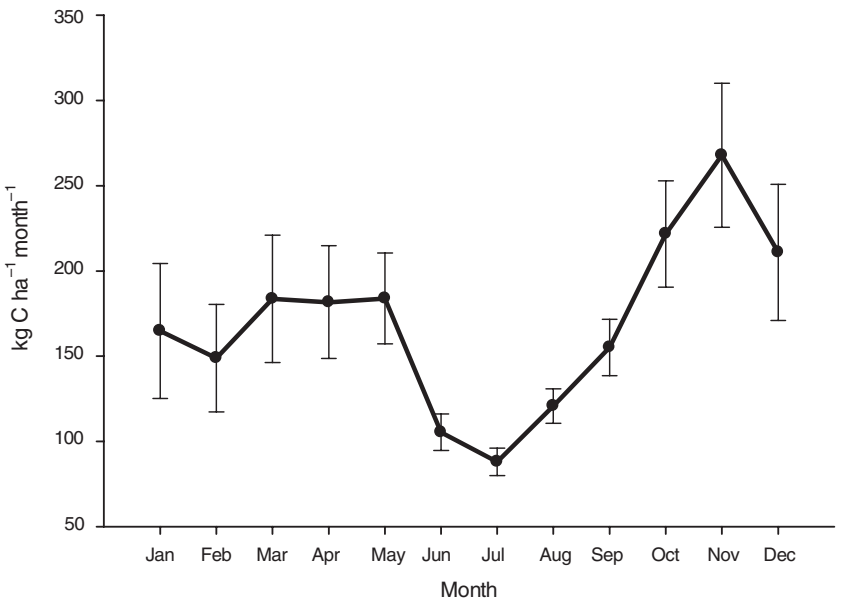

Fig. 5. Long-term (1990–2010) average monthly net C accumulation (kg C ha^{-1}) in aboveground biomass in kikuyu estimated using GrassGro. Error bars are ±standard errors of the mean ($P = 0.05$).

stolons (13.6%). At 44 days, ^{14}C aboveground (16.8% of applied ^{14}C recovered) consisted of 5.0% in the shoots and 11.8% in the stolons, and this was likely due to some translocation from retained stolons to the new shoots during the period from 7 to 44 days. At 201 days, 3.4% of applied ^{14}C was recovered from the stolons + shoots.

The ^{14}C allocated belowground reduced slightly after 6 months, with 21.5% of applied ^{14}C recovered at 7 days and 19.8% at 201 days. The total allocation of ^{14}C (% applied) into roots in the 700-mm depth profile under Rhodes grass was 9.2% at 7 days and increased to 10.8% at 201 days ($P < 0.05$) (Fig. 6).

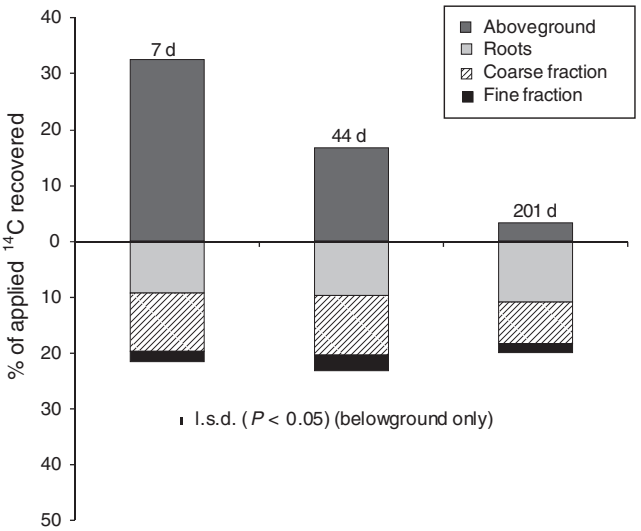

Fig. 6. Percentage of applied ^{14}C recovered aboveground and in roots, coarse SOM, and fine SOM (to 700 mm) under a Rhodes grass pasture near Badgingarra at 7, 44, and 201 days after the last $^{14}CO_2$ feed (October–November 2011). Small vertical line indicates l.s.d. ($P = 0.05$).

The allocation of ^{14}C in the coarse SOM fraction was stable for the first 6 weeks (10.5% at 7 days and 10.8% at 44 days) but reduced significantly ($P < 0.05$) to 7.5% at 201 days. The ^{14}C in the fine SOM fraction was 1.7% at 7 days and did not change significantly ($P > 0.05$) after 201 days (1.6%).

The distribution of ^{14}C belowground under Rhodes grass is shown in Fig. 7. At 7 days, the amount of ^{14}C recovered as a percentage of that applied was approximately one-third of that measured under kikuyu in the previous spring. As found with kikuyu, most of the assimilated ^{14}C (58%) occurred in the top 100 mm but it tapered off more gradually with depth under Rhodes grass. Ninety per cent of ^{14}C recovered belowground was found in the top 300 mm. The fractionation method for Rhodes grass provided full recovery by mass (99.6%) of soil (<2 mm), consisting of <2 mm sand (93.8%), coarse SOM fraction (2.0%), and fine SOM fraction (3.8%) when checked using 0–100 mm soil samples collected over all three sampling dates. Rhodes grass plants had a clumped distribution across the landscape. The average frequency (and standard deviation) was 10.8 (3.2) plants m^{-2}. On average there was only one large clump (>300 mm in diameter) per m^2.

Calculation of net assimilation of C into the coarse and fine SOM fractions under Rhodes grass

The net assimilation of C to the shoots in the 114-day period spanning the labelling event (26 July–17 November 2011) was 718 kg C ha^{-1}, or a daily rate of 6.3 kg C ha^{-1}. Using the proportions of ^{14}C in each of the fractions belowground compared with the proportion of ^{14}C in the shoots, the net allocation of C (kg C ha^{-1}), calculated for one day of photosynthesis into the coarse + fine SOM fractions at 0–700 mm depth, was 2.35 kg C ha^{-1} at 7 days after the last labelling event. At 201 days after labelling, 1.72 kg C ha^{-1} remained. In the absence of modelling data on the production of Rhodes grass in the region of the field trial, production

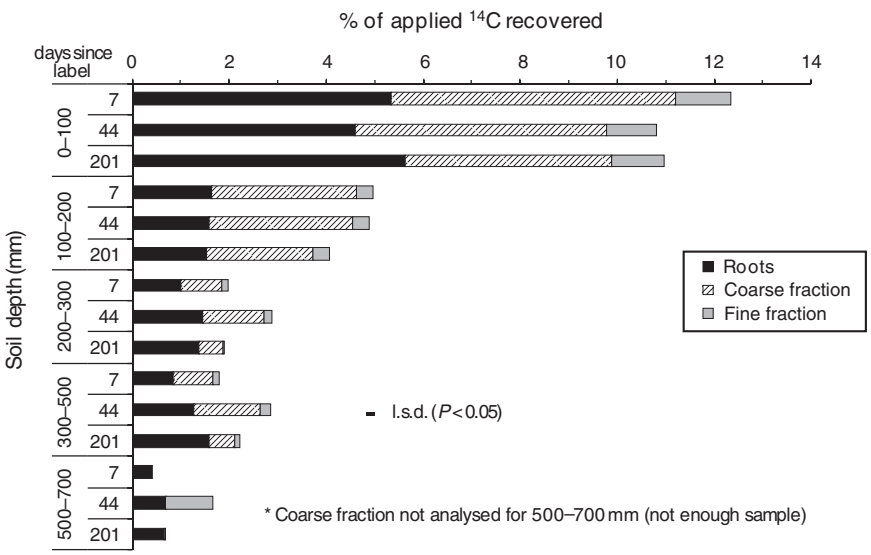

Fig. 7. Changes in the percentage of applied ^{14}C found at each depth with increasing duration since labelling of Rhodes grass pasture near Badgingarra in spring (October–November 2011). Small horizontal line indicates l.s.d. ($P = 0.05$).

estimates for the trial (3.8 t DM ha^{-1}) were used to calculate annual accumulation of C. Assuming a C content of 40%, DM production was converted to C production (1552 kg C ha^{-1}). Using the proportions of ^{14}C allocated to each fraction compared with that in the aboveground biomass at labelling, the amount remaining in the coarse + fine SOM fraction was calculated as 423 kg C ha^{-1}. This assumes an even coverage of Rhodes grass; however, on average one Rhodes grass clump occurred per m^2 and occupied an area of ~500 mm diameter. Adjusting the calculation for this, the annual assimilation of C into the coarse + fine SOM fraction was 83 kg C ha^{-1}.

Discussion

Dry matter production of perennial grass pastures

Measured annual DM production by kikuyu in the 12 months following the spring labelling was 5.1 t DM ha^{-1}, which agrees with the average production predicted by the GrassGro model (5.08 t DM ha^{-1}) for a site located near Wellstead, ~10 km south of our trial site. However, in the 12 months following the summer labelling, measured DM production (3.8 t DM ha^{-1}) was lower than predicted biomass. The annual estimate of Rhodes grass production of 3.8 t DM ha^{-1} is close to measurements of DM production by Ward *et al*. (2008) of 3–3.5 t DM ha^{-1} in two Rhodes grass pastures in the northern wheatbelt of WA.

Transfer of photosynthate belowground

The ^{14}C tracer pulse technique applied here enabled an assessment of the transfer of C captured by photosynthesis to belowground OM components for two perennial grass systems that have been identified as having potential to build soil C. The values obtained for kikuyu (30–40% of applied ^{14}C) are similar to those reported for mixed-species perennial pastures in New Zealand (Saggar *et al*. 1997) when plants were sampled about 1 week after ^{14}C pulse-labelling. The lower allocation of C belowground obtained for Rhodes grass (20–25% of applied ^{14}C) in the same period can in part be attributed to retention of ^{14}C in stolons (12–13% applied ^{14}C), which are entirely aboveground in this species but predominantly underground in kikuyu. The translocation of C in stolons to shoots during regrowth in part accounts for the prolonged recovery of ^{14}C in Rhodes grass shoots (Fig. 6), whereas negligible amounts of ^{14}C were recovered in shoots after the first defoliation of kikuyu pasture (Fig. 3). Greater retention of photosynthate in aboveground biomass, where it is accessible to grazing animals, should reduce the potential for sequestering soil C, and this is one of the arguments used to explain lower rates of C build-up under Rhodes grass than under kikuyu pasture (Sanderman *et al*. 2013*a*).

The smaller allocation of ^{14}C belowground for summer-labelled kikuyu compared with spring-labelled kikuyu (Fig. 3) has been observed in temperate perennial-based pasture in New Zealand, where 10–20% more ^{14}C was allocated belowground in spring compared with summer, autumn, and winter (Saggar and Hedley 2001). These authors attributed differences in allocation of C belowground to changes in pasture growth rate, with greater retention of ^{14}C in aboveground biomass during periods of higher rates of respiration when either low soil moisture or low temperature retarded pasture growth (Saggar and Hedley 2001). In this study the apparent ^{14}C respiration rate, or unaccounted-for ^{14}C, after either the first or second labelling dates in the kikuyu systems (Fig. 3) was not affected by season when labelling was done, but the rate of growth during and immediately after the pulse-labelling period was lower in summer than in spring (Table 2). Whether this effect was due to hotter and drier growing conditions of summer restricting the translocation of C belowground is not known.

Distribution of ^{14}C by soil depth

The concentration of belowground ^{14}C in the surface 100 mm of soil (Fig. 4), which typically contains the bulk of root material, follows the pattern observed elsewhere for perennial grass species (Saggar *et al*. 1997). Soil C research and monitoring programs that sample only to a depth of 300 mm have been criticised for not sampling deep enough. In this study that sampled soil to a depth of 700 mm, the majority of C was allocated in the surface layer of the soil. This was particularly the case for kikuyu, in which the majority (90–94%) of the ^{14}C allocated belowground was found in the 0–300 mm layer at ~1 week following the last labelling event. The stratification between 0–100 mm and the layers below was less distinct for the Rhodes grass pasture. However, the 0–300 mm layer still accounted for 90% of the total ^{14}C belowground at 1 week after the end of labelling.

Distribution of ^{14}C in soil organic matter fractions

The recovery of the bulk of belowground ^{14}C initially within root materials (>2 mm in size) and the significant decline ($P < 0.05$) with time after labelling shown here for kikuyu (Figs 3 and 4) have been reported elsewhere for *Lolium perenne* (Saggar *et al*. 1997). In contrast, there was no decline in ^{14}C content in the root fraction of the Rhodes grass system during the 6 months following ^{14}C labelling (Figs 6 and 7). Just 142.8 mm of rain fell at the nearby weather station (Badgingarra Research Station 30°34′S, 115°54′E) during the period November 2011–May 2012 (Bureau of Meteorology 2012, website accessed 30 Nov. 2012), and the resulting low soil water contents could have restricted microbial decomposition of root material and smaller OM particles.

The decline in root ^{14}C for kikuyu was not initially accompanied by a significant increase in coarse-fraction ^{14}C for spring-labelled kikuyu (Fig. 3*a*) and summer-labelled kikuyu (Fig. 3*b*), although after 12 months, ^{14}C in the coarse SOM fraction was significantly ($P < 0.05$) higher than observed after 10 or 44 days for spring-labelled kikuyu (Fig. 3*a*). By contrast, there was no significant change in the proportion of ^{14}C in the coarse SOM fraction after the first sampling of the summer-labelled kikuyu (Fig. 3*b*). This difference in turnover of root OM to the coarse SOM fraction after 12 months, observed between spring- and summer-labelled kikuyu, is difficult to reconcile. Spring-labelled kikuyu was subjected to a long dry period ahead of the following winter–spring when a higher rate of breakdown of root material might be expected,

whereas root material in the summer-labelled kikuyu was younger during this period and less likely to have decomposed.

Another significant finding was the comparatively uniform recovery of ^{14}C in the fine SOM fraction through time for both grass types. The sequestering of ^{14}C into the SOM fine fraction within 7–10 days of the final labelling implies that photoassimilates released into the rhizosphere were readily used by the soil microbial biomass (SMB). Although SMB was not measured in this study, there is much evidence from controlled environment studies on the transfer of assimilated ^{14}C into SMB under *Lolium perenne* to support this hypothesis (Domanski *et al*. 2001). It is notable that the proportion of assimilated ^{14}C in the fine SOM fraction under kikuyu and Rhodes grass in this study was of similar magnitude to that reported previously for *Lolium perenne* and *Festuca arundinacea* (de Neergaard and Gorissen 2004; Sanaullah *et al*. 2012). The importance of rhizodeposition in C cycling in plant soil systems is widely accepted (Gregory 2006; Jones *et al*. 2009) and we propose that this process was responsible for the rapid allocation of ^{14}C into the SOM fine fraction following pulse labelling. Models of C flow in soil will need to better characterise C cycling occurring through rhizodeposition.

The lack of build-up of ^{14}C in the coarse and fine SOM fractions between 6 weeks and 12 months during a period of loss of ^{14}C from root material in the case of kikuyu raises further questions on the cycling of C under perennial pastures. There is a paucity of published information on the long-term fate of assimilated C in perennial-based pastures. None of the studies of C cycling under *Lolium perenne* described earlier (Saggar *et al*. 1997; Domanski *et al*. 2001; de Neergaard and Gorissen 2004) examined the fate of assimilated ^{14}C post 35 days. One explanation for the apparent lack of transfer of root C to coarse and fine fractions is that insufficient nutrients [nitrogen (N), phosphorus (P), and sulfur (S)] were present during root decomposition to grow the SMB. Research by Kirkby *et al*. (2011) has shown that conversion of OM to humus necessitates the immobilisation of N, P, and S. Under this scenario, microbial decomposition of C-rich root material primarily results in the production of CO_2, which leaves the soil system. Sanderman *et al*. (2013*b*) arrived at a similar conclusion after they used $\delta^{13}C$-derived SOM fractions under kikuyu pasture to validate the Roth C model. In that case, perennial-derived C was predicted to move through measured SOM fractions at a much faster rate than measured. By comparing output from Roth C for kikuyu grown on soils with different fertility status, they concluded that the WA soil used in this study was likely nutrient-limited. It is our understanding that both the Gnowellen and Badgingarra field sites were managed under minimal fertiliser strategies, thereby favouring a system that was C-rich and low in N, P and S. Research is needed to determine whether reductions in the C : N, C : P, and C : S ratios would assist to build stocks of coarse and fine OM in these soils.

Assimilation of C into SOM fractions

Because kikuyu has a spreading turf-grass habit, the density of kikuyu within the cores represented the typical uniform coverage of a kikuyu pasture, allowing realistic extrapolation of C sequestration estimates to a per hectare basis. We estimated a net C allocation per day remaining in the coarse + fine SOM fractions of 13.9 and 5.9 kg C ha^{-1} for the spring and summer labelling events, respectively (Table 3). Using the GrassGro model prediction of monthly and annual biomass for the region, we estimated 1052 kg C ha^{-1} (or ~1 t C ha^{-1}) was allocated to the coarse + fine SOM fractions at 0–700 mm depth in a year. If calculated only to 300 mm depth, this reduced to 978 kg C ha^{-1} in the coarse + fine SOM fractions. This is similar to estimates of C sequestration (0.9 t C ha^{-1}) under kikuyu (0–300 mm depth) using isotope ratios of $^{13}C/^{12}C$ to distinguish perennial grass C input from that of annual species (Sanderman *et al*. 2013*a*).

Calculations for the Rhodes grass pasture were much less certain than for kikuyu because of the uneven distribution and low density of grass clumps across the paddock under study, features that are typical of Rhodes grass-based pastures. Carbon sequestered into coarse and fine SOM fractions was estimated from measurements of dry matter production over a fixed time (114 days) and compared with our biomass measurements for 365 days, resulting in an estimate of 83 kg C ha^{-1} at 0–700 mm depth over 1 year, about one-tenth of the input under kikuyu. Estimates of C sequestration undertaken by Sanderman *et al*. (2013*a*) using isotope $^{13}C/^{12}C$ ratios also indicated very small gains in soil C below Rhodes grass systems following a transition from annual pasture systems.

Some of the benefits perceived by farmers as a result of planting perennial pasture were likely due to protection of organic C in the soil from significant wind erosion events (Nie *et al*. 2008). This seems particularly the case for the kikuyu pasture at Gnowellen. The soil type in this paddock was a deep, non-wetting sand, highly prone to topsoil losses from wind erosion events, which are common on the south coast of WA. Seeding with kikuyu has ensured good groundcover and hence stabilisation of the soil throughout the year. Hence, preservation of existing soil organic C together with some allocation of C belowground each year from photosynthesis have apparently resulted in improved soil stability and accrual of organic C in these systems.

Conclusion

Repeat pulse labelling of perennial grass pastures (kikuyu and Rhodes) with ^{14}C in the field in Western Australia indicated that >90% of belowground SOM C from photosynthesis was sequestered to 0–300 mm depth. Fine-fraction SOM (<50 µm) was detectable within 1 week after photoassimilation and remained stable for up to 6 months (Rhodes grass) and 12 months (kikuyu). Coarse SOM (50 µm–2 mm) was reasonably stable up to 12 months. Calculations of C assimilated into coarse + fine SOM under kikuyu were ~1 t C ha^{-1} $year^{-1}$ but estimates under Rhodes grass were about one-tenth of this.

Acknowledgments

This research was funded by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) and by CSIRO. The authors gratefully acknowledge Farmers Morgan and Debbie Sounness (Gnowellen, WA) and Aubrey and Lisa Panizza (Badgingarra) on whose properties the field experiments were conducted and for considerable assistance in setting up and decommissioning the trials. The authors are also most grateful to

Shayne Micin, Laurence Cross and Mar Alonso Fernandez for technical assistance.

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